Journal of Elementology

Quarterly Reports issued by
the Polish Society for Magnesium Research
and University of Warmia and Mazury in Olsztyn

Volume 14 Number 3 September 2009

Editorial Office
University of Warmia and Mazury
Michała Oczapowskiego 8, 10-719 Olsztyn, Poland, phone: +48 089 5233231
http:// www.uwm.edu.pl/jelementol

Webmaster: Sławomir Krzebietke

Publishing company is funded by Ministry of Science and Higher Education
and cooperation by University of Warmia and Mazury in Olsztyn

Science Citation Index Expanded (Sci Search®),
Journal Citation Reports/Science Edition

ISSN 1664-2296

Ark. wyd. 15,4; ark druk. 13,25; pap. offset. kl. III 80 g B-1
Druk: Zakład Poligraficzny UWM w Olsztynie

CONTENTS

ORIGINAL PAPERS

PAPER REVIEW

SPIS TREŚCI

PRACE ORYGINALNE

PRACA PRZEGLĄDOWA

J. Elementol. 2009, 14(3): 429–436

ORIGINAL PAPERS

HABITAT OCCURRENCE AND NUTRITION VALUE OF *ACHILLEA MILLEFOLIUM* L. IN GRASSLANDS

Jacek Alberski, Stanisław Grzegorczyk, Adam Kozikowski, Marzena Olszewska

Chair of Grassland
University of Warmia and Mazury

Abstract

In 1998-2000, in the area of Olsztyn Lake District, 33 plant communities on semi-natural grasslands with a high percentage of *Achillea millefolium* L. were analyzed. The analyzed objects were located on very light, light, medium and heavy mineral soils. The species composition was evaluated with Braun-Blanquet method. Soil samples were collected for chemicals analyses. In dry matter of *Achillea millefolium* L. the content of crude protein, crude fibre, crude ash and macroelements was determined. Meadow-pasture communities with high percentage of *Achillea millefolium* L. in sward are floristically very valuable, particularly those located on light and medium soils. Irrespective of soil type, *Achillea millefolium* L. was accompanied by *Poa pratensis, Dactylis glomerata, Festuca rubra, Trifolium repens, Vicia cracca, Heracleum sibiricum and Taraxacum officinale*. The heavy soils had a higher content of P, K, Mg, Ca and Na, whereas very light soils contained more humus. *Achillea millefolium* L. contained 110-121 $g \cdot kg^{-1}$ crude protein, 249--309 $g \cdot kg^{-1}$ crude fibre and 97-123 $g \cdot kg^{-1}$crude ash, much P, K and Ca irrespective of soil type and much Mg (3.1 $g \cdot kg^{-1}$) on very light soils.

Key words: habitat, soil category, nutrition value, *Achillea millefolium.*

dr inż. Jacek Alberski, Chair of Grassland, University of Warmia and Mazury, Poland, pl. Łódzki 1, 10-718 Olsztyn, e-mail: alberj@uwm.edu.pl

WARUNKI SIEDLISKOWE I WARTOŚĆ POKARMOWA *ACHILLEA MILLEFOLIUM* L. NA UŻYTKACH ZIELONYCH

Abstrakt

W latach 1998-2000, na terenie Pojezierza Olsztyńskiego, objęto badaniami 33 zbiorowiska roślinne na półnaturalnych użytkach zielonych ze znacznym udziałem *Achillea millefolium*. Badane obiekty były zlokalizowane na bardzo lekkich, lekkich, średnich i ciężkich glebach mineralnych. Skład gatunkowy runi wyceniono metodą Braun-Blanqueta. Pobrano próbki glebowe do analiz chemicznych. W materiale roślinnym *Achillea millefolium* określono zawartość białka ogólnego, włókna surowego, popiołu surowego oraz makro- i mikroelementów. Zbiorowiska łąkowo-pastwiskowe ze znacznym udziałem *Achillea millefolium* w runi są bardzo cenne florystycznie, szczególnie te zlokalizowane na glebach lekkich i średnich. Niezależnie od kategorii agronomicznej gleby, *Achillea millefolium* towarzyszyły: *Poa pratensis*, *Dactylis glomerata*, *Festuca rubra*, *Trifolium repens*, *Vicia cracca*, *Heracleum sibiricum* i *Taraxacum officinale*. Gleby ciężkie zawierały więcej P, K, Mg, Ca i Na, natomiast lekkie więcej próchnicy glebowej. *Achillea millefolium* L. zawierał: 110-121 $g \cdot kg^{-1}$ białka ogólnego, 249-309 $g \cdot kg^{-1}$ włókna surowego, 97-123 $g \cdot kg^{-1}$ popiołu surowego, duże ilości P, K i Ca niezależnie od kategorii agronomicznej gleby oraz więcej Mg (3,1 $g \cdot kg^{-1}$) na glebach bardzo lekkich.

Słowa kluczowe: siedlisko, kategoria gleby, wartość pokarmowa, *Achillea millefolium*.

INTRODUCTION

High fertility of soils, optimum moisture and rational utilization are conducive to the formation of communities which are floristically rich and at the same time stabilized in terms of the species (Kostuch 1995). Taking into account the economical, floristic and feed aspects, it is justifiable to use grasslands extensively, hence it is then possible to maintain communities in which meadow-pasture herbs have a significant share in the vegetation, becomes justifiable (Alberski 2004, Butkuviene, Butkute 2004, Lunnan 2004).

It is still an open question whether feed from extensive meadows, which is characterized by a lower content of protein and phosphorus but a higher level of fibre and lower digestibility, is of good feeding value (Falkowski 1996). In natural meadow-pasture communities, *Achillea millefolium* L. is the plant belonging to the group of the herbs and weeds which affects the biological value of feed (Kozłowski, Swędrzyński 1996, Trzaskoś et al. 2006).

The aim of this investigation was to monitor the occurrence of *Achillea millefolium* L. in grass communities and to indicate availability of this species for nutritional purposes through analyses of the nutrient content.

MATERIALS AND METHODS

The investigation was carried out on grasslands of Olsztyn Lake District in the years 1998-2000. In total, 33 meadow objects with a high percentage (above 5%) of *Achillea millefolium* in the vegetation, located on mineral soils, were selected. The species composition of the vegetation was estimated using Braun-Blanquet phytosociological method.

Soil samples were collected from every object. The structure of the soil was analyzed. In addition, the soil's pH, organic substance content and abundance in some macro- and microelements were determined. The content of crude protein, crude fibre, crude ash as well as macro- and microelements was examined in the plant material of *Achillea millefolium.*

The results of the experiment were assessed using the analysis of variations based on Tukey's test.

RESULTS

Achillea millefolium is a characteristic species of the *Molinio-Arrhenatheretea* class, which has a rich species diversity. According to many authors (TRZASKOŚ et al. 2006, GUDAITYTE, VENSKUTONIS 2007) milfoil appears in different habitats, such meadows, roadsides, forest meadows and abandoned fields. In 33 meadow objects covered by the study, in which *Achillea millefolium* was recorded, there were 117 plant species in total, most on light soils, similar to DEMOLDER (2007). Irrespective of the type of soil, feed-valuable species occurred most frequently. Among grasses, they were *Dactylis glomerata* L., *Festuca pratensis* L., *Festuca rubra* L.s.s., *Poa pratensis*, papilionaceus – *Trifolium repens, Vicia cracca,* herbs and weeds – *Heracleum sibiricum, Plantago lanceolata and Taraxacum officinale* (Table 1).

The analysed objects were located on soils belonging to different soil categories: 7 were on very light soils, 11 on light, 9 on medium and 6 on heavy soils. GUDAITYTE AND VENSKUTONIS (2007) qualified *Achillea millefolium* as a species of wide ecological amplitude. Irrespective of the agronomic category, all soils can be regarded as abundant in Mg and Zn, moderately abundant in K, Cu, Mn and Fe and low in P (*Border numbers for content estimation...* 1990).

Both a higher pH and higher abundance in the studied macro- and microelements were recorded in heavy soils. On the other hand, very light soils were characterized by a higher content of humus (4.25 $g \cdot kg^{-1}$) – Table 2. GRZEGORCZYK et al. (2004) ascertained higher abundance in humus on high moisture habitats.

The evaluation of *Achillea millefolium* dry matter demonstrated that this species accumulates similar contents of nutrients irrespective of the type of soil. The following mean values were determined in the analysed material: crude protein (116 $g \cdot kg^{-1}$), crude fibre (114 $g \cdot kg^{-1}$), Ca (14.1 $g \cdot kg^{-1}$), Na (0.8 $g \cdot kg^{-1}$), Cu (9.9 $mg \cdot kg^{-1}$), Fe (163.3 $mg \cdot kg^{-1}$) and Zn (33.4 $mg \cdot kg^{-1}$) – Table 3. *Achillea millefolium* in the sward of meadows improves the fodder value of the sward (TRZASKOŚ et al. 2006).

Table 1

The most frequent plant species in meadow with *Achillea millefolium*

Species	Soil category							
	very light		light		medium		heavy	
	C*	Cc**	C*	Cc**	C*	Cc**	C*	Cc**
1	2	3	4	5	6	7	8	9
Grasses								
Festuca pratensis Huds.	V	1680.0	III	410.0	III	85.6	III	85.0
Dactylis glomerata L.	V	1214.3	V	1455.5	V	2140.0	III	1251.7
Poa pratensis L.	VI	144.3	V	1778.2	V	2528.9	V	791.7
Festuca rubra L. s. s.	III	74.3	III	729.1	VI	114.4	VI	750.0
Phleum pratense L.	III	607.1	III	410.9	III	112.2	VI	86.7
Lolium perenne L.	III	72.9	II	47.3	II	56.7		
Deschampsia caespitosa (L.) P.Beauv.	III	38.6	I	23.6	II	28.9	V	631.7
Arrhenatherum elatius (L.) P. Beauv.	II	1071.4	I	341.8	I	1.1		
Holcus lanatus L.	II	71.4	III	432.7	I	27.8	V	128.3
Alopecurus pratensis L.	II	71.4	III	706.4	VI	891.1	VI	710.0
Anthoxanthum odoratum L.	I	535.7	III	410.0	II	83.3	VI	126.7
Avenula pubescens (Huds.) Dumort.	I	35.7	I	0.9	II	417.8		
Poa trivialis L.			II	364.5	I	1.1	I	1.7
Poa annua L.	I	35.7			I	1.1		
Festuca arundinacea Schreb.	I	1.4	I	22.7			I	1.7
Legumes								
Trifolium pratense L.	VI	750.0	I	204.5	III	750.0	II	126.7
Lotus corniculatus L.	III	1071.4	I	409.1	II	2.2	I	1041.7
Trifolium repens L.	III	752.9	III	615.5	VI	668.9	III	128.3
Vicia cracca L.	III	217.1	III	977.3	III	334.4	III	500.0

cont. Table 1

1	2	3	4	5	6	7	8	9
Medicago lupulina L.	III	217.1	I	0.9	I	83.3		
Vicia sepium L.	II	214.3	I	68.2	I	83.3	I	625.0
Trifolium dubium Sibth.	II	108.6	I	69.1	II	84.4		
Lathyrus pratensis L.	I	535.7	II	478.2	III	416.7	I	250.0
Lotus uliginosus Schk.			I	204.5	I	83.3	II	375.0
Trifolium hybridum L.			I	0.9	I	166.7	I	1.7
Herbs and weeds								
Achillea millefolium L.	V	928.6	V	1727.3	V	1333.3	V	1958.3
Taraxacum officinale F. H. Wigg.	V	1107.1	VI	840.9	V	807.8	VI	500.0
Heracleum sibiricum L.	III	1250.0	III	139.1	III	918.9	VI	251.7
Equisetum arvense L.	III	145.7	II	2.7	I	55.6		
Cirsium arvense (L.) Scop.	III	144.3	VI	435.5	III	113.3	II	333.3
Plantago lanceolata L.	II	72.9	VI	457.3	III	280.0	VI	251.7
Ranunculus repens L.	II	72.9	I	46.4	II	111.1	V	338.3
Alchemill vulgaris L. s.l.	II	72.9	II	137.3	II	56.7	III	791.7
Rumex acetosa L.	II	72.9	II	92.7	III	113.3	III	335.0
Stellaria graminea L.	II	2.9	II	91.8	III	58.9	I	1.7
Leontodon autumnalis L.	I	71.4	III	49.1	II	56.7		
Glechoma hederacea L.	I	1.4	I	0.9	I	55.6	I	1.7
Prunella vulgaris L.	I	1.4	I	45.5			I	1.7
Ranunculus acris L. s. s.	I	1.4	II	137.3	II	3.3	II	3.3
Cirsium oleraceum (L.) Scop.			I	0.9	II	222.2	I	250.0
Lychnis flos-cuculi L.			I	0.9	I	1.1	I	1.7

C – Constancy, **Cc – Cover coefficient

Table 2

Chemical properties of different soils

Specification	Soil category							
	very light		light		medium		heavy	
	Mv	min-max	Mv	min-max	Mv	min-max	Mv	min-max
pH_{KCl}	5.3	4.1-6.3	5.4	4.1-6.1	5.1	4.1-6.1	6.2	4.4-6.8
$g \cdot kg^{-1}$								
Humus	4.25	2.63-8.62	3.42	1.77-6.46	2.83	1.53-5.42	3.07	1.53-6.22
N	2.72	1.10-3.64	2.10	0.94-3.64	1.40	0.70-2.63	1.70	0.80-2.82
P	0.04	0.02-0.13	0.05	0.02-0.16	0.08	0.03-0.22	0.11	0.04-0.35
K	0.05	0.03-0.26	0.09	0.04-0.28	0.08	0.04-0.28	0.22	0.05-0.38
Mg	0.06	0.02-0.14	0.06	0.02-0.14	0.08	0.02-0.18	0.13	0.04-0.19
Ca	0.53	0.22-1.28	0.88	0.33-3.18	0.62	0.22-1.48	1.69	0.33-3.80
Na	0.02	0.01-0.05	0.02	0.01-0.05	0.02	0.01-0.05	0.03	0.01-0.05
$mg \cdot kg^{-1}$								
Cu	2.1	1.3-4.7	3.2	1.6-6.1	3.4	1.6-8.4	8.7	2.7-16.6
Mn	100.6	66.3-183.5	164.3	76.5-231.2	121.7	69.2-203.4	246.3	99.1-430.2
Zn	10.5	7.2-23.9	17.6	9.6-35.5	13.9	8.2-25.7	20.5	13.2-56.4
Fe	1673	988-2320	1689	1012-2340	1739	1043-2336	3096	1037-9034

Mv – mean value; min-max – minimum-maximum

Table 3

Content of the nutrients in *Achillea millefolium* (in DM)

Specification	Soil category							
	very light		light		medium		heavy	
	Mv	min-max	Mv	min-max	Mv	min-max	Mv	min-max
$g \cdot kg^{-1}$								
Total protein	110 *a*	89-121	121 *a*	106-131	118 *a*	102-128	111 *a*	102-121
Crude fibre	302 *a*	245-344	276 *a*	224-332	249 *a*	218-311	293 *a*	244-340
P	3.5 *a*	2.2-4.8	5.0 *b*	3.1-6.8	5.1 *b*	3.1-6.8	5.1 *b*	3.2-6.8
K	28.4 *a*	18.7-33.4	42.5 *b*	23.4-52.4	44.8 *b*	33.4-52.8	42.5 *b*	28.4-48.2
Mg	3.1 *b*	1.9-3.8	2.1 *a*	1.6-2.8	2.1 *a*	1.8-2.6	2.1 *a*	1.6-2.6
Ca	15.0 *a*	12.1-18.4	13.0 *a*	11.5-16.4	14.2 *a*	11.8-16.7	14.9 *a*	12.1-16.7
Na	0.7 *a*	0.2-0-1.3	0.9 *a*	0.3-1.5	0.9 *a*	0.2-1.6	0.8 *a*	0.3-1.6
$mg \cdot kg^{-1}$								
Cu	8.8 *a*	6.8-12.4	10.2 *a*	7.8-12.8	10.5 *a*	8.8-12.4	9.6 *a*	6.8-12.8
Mn	102.3 *ab*	48.3-178.2	150.8 *b*	65.1-244.8	165.3 *b*	91.5-362.1	50.4 *a*	31.4-206.1
Zn	36.7 *a*	28.3-58.1	36.3 *a*	26.4-56.3	32.5 *a*	24.1-56.8	25.5 *a*	21.1-43.7
Fe	169.2 *a*	83.2-231.4	151.1 *a*	98.5-214.2	141.3 *a*	46.6-221.6	211.5 *b*	96.4-387.1

a, ab, b – homogenous groups
Mv – mean value; min-max – minimum-maximum

Table 4

Significant coefficients of correlation between *Achillea millefolium* chemical composition and chemical properties of soil

Soil proper-ties	Elements content in plant									
	N	P	K	Mg	Ca	Na	Cu	Mn	Zn	Fe
pH								-0.633**		0.333*
Humus	0.325*	-0.380*		0.497**					0.345*	
N		-0.453**		0.538**						
P			0.327*			-0.387*				
K		0.311*						-0.372*		0.395*
Mg								-0.433**		
Ca								-0.480**		0.465**
Na								-0.354*		0.356*
Mn		0.399*						-0.334*		
Cu								-0.344*		
Zn		0.329*				0.326*				
Fe										

*significant at p=0.05
**significant at p=0.01

Achillea millefolium, irrespective of soil type, contained a lot of phosphorus and potassium as well as calcium, and little sodium and zinc. According to Marinas, Garcia-Gonzalez (2006) *Achillea millefolium* shows a high P and K content, especially in June. In plants from very light soils, significantly more Mg and significantly less P and K were determined, whereas significantly less Mn was observed in plants growing on heavy soils. Interesting data were obtained from the correlation between the influence of some chemical properties of soil and the content of nutrients in plants. In the examined species, a lower content of Mn with a higher pH and a higher abundance of soil in Ca and Mg was recorded. It was also found out that the growth of N content in the soil is followed by a decrease in the content of P and an increase in the content of Mg in plants (Table 4).

According to Michler, Arnold (1999) a high frequency of *Achillea millefolium* per site was positively correlated with phosphate, magnesium and manganese and negatively with carbonate and hydrogen ion concentration in soil.

CONCLUSION

1. Valuable species of grasses, *Papilionaceae* plants and other herbs occurred in large numbers and at high intensity in meadow-pasture communities with a high percentage of *Achillea millefolium* L. in vegetation.

2. Irrespective of the type of soil, the habitat of this species can be regarded as abundant in Mg ad Zn, moderately abundant in K, Cu, Mn and Fe and low in P.

3. In terms of nutritive value, *Achillea millefolium* is characterised by a high concentration of P, K and Ca.

REFERENCES

ALBERSKI J. 2004. *Cirsium oleraceum (L.) Scop. in grassland communieties of Olsztyn Lakeland.* Woda-Środowisko-Obszary Wiejskie, 4, 2b (12): 175-183. (in Polish).

Border numbers for content estimation of macro- and micronutrients in soils. 1990. IUNG Puławy. (in Polish)

BUTKUVIENE E., BUTKUTE R. 2004. *Effect of different surface improvement measures on yield and quality of pastures in Lithuania*. Grassld. Sci. Europe, 9: 556-558.

DEMOLDER H. 2007. *Floristic diversity and biomass production in former intensively used grasslands (Flanders).* Grassld. Sci. Europe, 12: 414-416.

FALKOWSKI M. 1996. *Changes in the attitude to the role of grasslands in the feed production and protection of natural environment in the light of recent world research.* Rocz. AR Poznań, 284, Rol., 47: 5-14. (in Polish).

GRZEGORCZYK S., GRABOWSKI K., ALBERSKI J. 2004. *The presence of legumes on natural grasslands of Olsztyn Lakeland* .Woda-Środowisko-Obszary Wiejskie, 4, 1 (10): 229-240. (in Polish).

GUDAITYTE O., VENSKUTONIS P.R. 2007. *Chemotypes of Achillea millefolium transferred from 14 different in Lithuania to the controlled environment*. Biochem. System. Ecol., 35 (9): 582-592.

KOSTUCH R. 1995. *The causes of floristic diversity occurrence on grassland ecosystems.* Ann. UMCS, Sec. E, 50: 23-32. (in Polish).

KOZŁOWSKI S., SWĘDRZYŃSKI A. 1996. *Herbaceous meadows in fodder and landscape aspects.* Zesz. Probl. Post. Nauk Rol., 442: 269-276. (in Polish).

LUNNAN T. 2004. *How does resowing of natural meadows affect yield, forage quality and botanical composition*. Grassld. Sci. Europe, 9: 550-552.

MARINAS A., GARCIA-GONZALEZ R. 2006. *Preliminary data on nutritional value of abundant species in supraforestal Pyrenean pastures*. Pirineos, 161: 85-110.

MICHLER B., ARNOLD C-G. 1999. *Predicting presences of proazulenes in the Achillea millefolium group*. J. Fol. Geobot., 34: 143-161.

TRZASKOŚ M., SZYDŁOWSKA J., STELMASZYK A. 2006. *Herbs in forest meadows communities in view of their utilization and landscape*. Ann. UMCS, Sec. E, 61: 319-331. (in Polish)

J. Elementol. 2009, 14(3): 437–447

LEVELS OF MICROELEMENTS (Cu, Zn, Fe) AND MACROELEMENTS (Mg, Ca) IN FRESHWATER FISH*

Ewa Brucka-Jastrzębska[1], Dorota Kawczuga[1], Monika Rajkowska[2], Mikołaj Protasowicki[2]

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

Abstract

The paper evaluates the effect of culture conditions and culture site on levels of certain microelements (Zn, Cu, Fe) and macroelements (Mg, Ca) in three species of freshwater fish: rainbow trout (*Oncorhynchus mykiss* Walbaum), common carp (*Cyprinus carpio* L.), and Siberian sturgeon (*Acipenser baeri* Brandt).

The study involved 90 individuals of freshwater fish aged from 6 to 12 months. Samples of blood, liver, kidney, gills, skin and muscles were collected from each fish and subjected to chemical assay of Mg, Ca, Zn, Cu, Fe with inductively coupled plasma-atomic emission spectrometry in a JY-24 Jobin Yvon apparatus.

The study revealed that culture site had statistically significant impact on levels of the examined elements among the three fish species. Mg content in kidney and skin was significantly higher in carp than in sturgeon. Similar regularities were observed for Ca content in skin and Zn content in kidney. Liver and kidney levels of Fe and Cu were significantly lower in carp than in rainbow trout. Of all the three examined fish species, rainbow trout had the highest skin levels of Ca and Mg, and the highest blood level of Fe.

The results indicate that culture site and culture conditions exerted significant influence on levels of macro- and microelements in freshwater fish.

Key words: freshwater fish, *Cyprinus carpio* L., *Oncorhynchus mykiss* Walbaum, *Acipenser baeri* Brandt, macroelements, microelements.

dr Ewa Brucka-Jastrzębska, Chair of Physiology, University of Szczecin, Piastów 40 B, Szczecin, ewabrucka@poczta.onet.pl

*The study has been financially supported by the State Committee for Scientific Research under the grant no. N 304 026 31/0814.

OCENA ZAWARTOŚCI MIKROELEMENTÓW (Cu, Zn, Fe) I MAKROELEMENTÓW (Mg, Ca) U RYB SŁODKOWODNYCH

Abstrakt

Celem pracy była ocena wpływu warunków i miejsca prowadzenia hodowli na zawartość mikroelementów (Zn, Cu, Fe) i makroelementów (Mg, Ca) w organizmach ryb słodkowodnych. Ocenie poddano trzy wybrane gatunki ryb: pstrąga tęczowego (*Oncorhynchus mykiss* Walbaum), karpia (*Cyprinus carpio* L.) i jesiotra syberyjskiego (*Acipenser baeri* Brandt).

Badaniu poddano 90 ryb słodkowodnych w okresie od 6. do 12. miesiąca życia. Z każdej ryby do analiz chemicznych pobrano próbki: krwi, wątroby, nerek, skrzeli, skóry i mięśni. Próbki poddano analizie na zawartość Mg, Ca, Zn, Cu, Fe, z użyciem emisyjnej spektrometrii atomowej w plazmie indukcyjnie sprzężonej (ICP-AES), w aparacie Jobin Yvon typ JY-24.

Wykazano, że miejsce hodowli ma statystycznie istotny wpływ na zawartości badanych pierwiastków u poszczególnych gatunków ryb słodkowodnych. Zawartość Mg w nerce i skórze karpi była istotnie wyższa niż u jesiotra. Podobne spostrzeżenia dotyczyły poziomu Ca w skórze i Zn w nerce. W przypadku Fe i Cu obserwowano niższą zawartość w wątrobie i nerkach karpi niż w tych samych narządach u pstrąga. Badania wykazały, że poziom Zn i Cu we wszystkich narządach przebadanych gatunków kształtował się na najniższym poziomie. Spośród badanych gatunków u pstrąga stwierdzono najwyższą zawartość Ca i Mg w skórze, a Fe we krwi.

Otrzymane wyniki pozwalają stwierdzić, że miejsce i warunki prowadzenia hodowli mają istotny wpływ na zawartość badanych makro- i mikroelementów w organizmach ryb słodkowodnych.

Słowa kluczowe: ryby słodkowodne, *Cyprinus carpio* L., *Oncorhynchus mykiss* Walbaum, *Acipenser baeri* Brandt, makroelementy, mikroelementy.

INTRODUCTION

In the growth and development of both terrestrial and aquatic animals, culture conditions play one of key roles. In the natural environment, many various chemicals occur. Most of them, however, do not penetrate in significant amounts into organisms despite being in direct contact with them. Organisms are dependent on the environment in which they live. During the evolution, countless relationships have been developed between organisms and their environment. When those relationships are disrupted by changed environmental conditions, diseases or even death of an organism may occur (JARA, CHODYNIECKI 1999).

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 xenobiotic and harmful substances that are able to impair life functions of organisms. Human activity exerts increasing pressure on the environment, which results in elevated pollution levels in aquatic and terrestrial ecosystems. Fish, living in the aquatic environment, are particularly exposed to anthropogenic impacts.

The aim of this study was to evaluate the effect of culture conditions and culture site on levels of certain microelements (Zn, Cu, Fe) and macroelements (Mg, Ca) in three species of freshwater fish: rainbow trout (*Oncorhynchus mykiss* Walbaum), common carp (*Cyprinus carpio* L.) and Siberian sturgeon (*Acipenser baeri* Brandt).

MATERIALS AND METHODS

The study involved 90 individuals of freshwater fish reared in commercial fish farms in West Pomeranian Province, Poland. The fish were represented by 30 individuals of each of the three species: rainbow trout, common carp and Siberian sturgeon, aged from 6 to 12 months, weighing from 189.5 to 315.4 g and measuring from 21.7 to 31.5 cm. The fish were fed Aller Aqua pelleted feeds (each species with an appropriate feed type). Table 1 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 blood, liver, kidney, gills, skin and muscles were collected from each fish. The collected material was stored at -20^{o}C.

Prior to analysis, 1-g subsamples of organs, weighed to the nearest 0.001 g, were mineralized wet in 3 cm^3 HNO_3 in a CEM MDS 2000 microwave oven. The solutions obtained were quantitatively transferred to polyethylene vials and brought up to 30 g with deionised water. Mg, Ca, Zn, Cu, Fe were determined with inductively coupled plasma atomic emission spectrometry (ICP-AES) in a JY-24 Jobin Yvon apparatus. Tissue concentrations of metals have been reported as mg kg^{-1} wet weight (mg kg^{-1} w.w.).

The results obtained were subjected to statistical treatment with the Statistica 6.0 software. Analyses of variance (ANOVA) was performed at the significance levels of $P = 0.05$ and $P = 0.01$.

RESULTS AND DISCUSSION

Intravital and *post mortem* examination showed no changes in fish behaviour or in their external and internal appearance. Comparison of water parameters (Table 1) revealed only slight differences among the three fish culture sites.

Table 1

Hydrochemical parameters from groups I and II culture sites in the direct vicinity of the Dolna Odra Power Station and from group III culture site 60-km distant from the power station

Water parameters	I	II	III	Statistically significant differences $P \leq 0.05$
	mean ± SD	mean ± SD	mean ± SD	
Temperature (°C)	14.80 ± 4.50	13.80 ± 3.40	13.20 ± 3.10	A
pH	7.88 ± 0.55	7.48 ± 0.95	7.61 ± 0.55	-
Dissolved oxygen (mg l^{-1})	7.81 ± 0.35	7.94 ± 0.55	7.44 ± 0.85	-
Oxygen saturation (%)	78.21 ± 2.50	79.51 ± 3.48	77.51 ± 3.148	-
Alkalinity (mmol l^{-1})	1.78 ± 0.84	1.68 ± 0.88	1.88 ± 0.58	-
Water hardness (mg l^{-1})	8.25 ± 1.08	7.19 ± 1.78	7.15 ± 1.18	A
ChOD (mg l^{-1})	1.67 ± 1.32	1.56 ± 1.12	1.66 ± 1.52	-
BOD_5 (mg l^{-1})	4.34 ± 1.22	4.61 ± 1.33	4.81 ± 1.72	A
NH_4-N (mg l^{-1})	1.18 ± 0.75	1.34 ± 0.48	1.37 ± 0.28	A
NO_3-N (mg l^{-1})	7.41± 1.05	6.11± 1.15	6.61± 1.45	A
NO_2-N (mg l^{-1})	0.68 ± 0.16	0.48 ± 0.36	0.48 ± 0.36	A
PO_4^{3}-P (mg l^{-1})	0.15 ± 0.07	0.14 ± 0.11	0.12 ± 0.11	-
Ca (mg l^{-1})	66.51 ± 4.25	59.51 ± 3.75	76.41 ± 3.77	A
Cd (mg l^{-1})	0.02 ± 0.01	0.01 ± 0.01	0.05 ± 0.01	-
Pb (mg l^{-1})	0.03 ± 0.05	0.03 ± 0.07	0.02 ± 0.05	-
Mg (mg l^{-1})	16.33 ± 4.05	15.23 ± 3.33	15.44 ± 4.17	A

A – statistically significant differences (ANOVA, test Duncan) in the water parameter among the three culture sites ($P \leq 0.05$);
common carp – I group; Siberian sturgeon – II group; rainbow trout – III group

The study revealed that average levels of microelements differed significantly among tissues and organs of the three examined fish species. Average iron content ranged from 3.1 to 54.7 mg kg^{-1} w.w. (Table 2). The highest iron levels were detected in the gills (54.7±5.7 mg kg^{-1} w.w.) and kidney (49.6±6.8 mg kg^{-1} w.w.) of Siberian sturgeon and in the kidney of rainbow trout (45.8±9.7 mg kg^{-1} w.w.). The lowest iron levels were found in the skin and muscles of all the examined fish (Table 2). Iron was distributed in fish bodies according to the following patterns of decreasing concentrations:

– common carp: gills> kidney> blood> liver> muscles> skin,
– rainbow trout: kidney> liver> gills> blood> muscles> skin,
– Siberian sturgeon: gills> kidney> liver> blood> skin> muscles.

Table 2

Microelement (Zn, Fe, Cu) levels in organs of three freshwater fish species

Bioelement	Tissue	Common carp *Cyprinus carpio* L.	Rainbow trout *Oncorhynchus mykiss* Rich	Siberian sturgeon *Acipenser baeri* Brandt
		mean ± SD	mean ± SD	mean ± SD
Fe ($mg\ kg^{-1}$ w.w.)	blood	27.1 ± 5.6	16.7 ±3.9	35.3 ± 3.9
	liver	23.6 ± 3.7	34.8 ± 4.1	37.3 ± 2.9
	kidney	33.6 ± 6.8	45.8 ± 9.7	49.6 ± 6.8
	gills	36.6 ± 6.4	28.9 ± 3.6	54.7 ± 5.7
	muscles	4.6 ± 1.1	6.2 ± 0.9	9.1 ± 1.2
	skin	3.1 ± 0.6	6.1 ± 0.7	11.5 ± 1.1
Cu ($mg\ kg^{-1}$ w.w.)	blood	0.8 ± 04	1.1 ± 0.9	3.8 ± 0.4
	liver	15.7 ± 17.6	19.2 ± 8.4	12.7 ± 5.6
	kidney	1.8 ± 0.2	3.3 ± 0.8	1.9 ± 0.3
	gills	3.1 ± 0.4	3.6 ± 0.9	3.4 ± 0.4
	muscles	0.4 ± 0.6	0.9 ± 0.3	0.7 ± 0.2
	skin	1.1 ± 0.4	1.1 ± 0.3	1.2 ± 0.6
Zn ($mg\ kg^{-1}$ w.w.)	blood	8.8 ± 04	4.1 ± 0.9	3.8 ± 0.4
	liver	50.7 ± 17.6	87.2 ± 8.4	98.7 ± 5.6
	kidney	19.3 ± 0.5	3.3 ± 0.8	3.3 ± 0.3
	gills	3.1 ± 0.4	3.6 ± 0.9	3.4 ± 0.4
	muscles	6.1 ± 17.6	8.1 ± 12.6	7.4 ± 11.6
	skin	52.1 ± 0.7	34.6 ± 0.6	51.9 ± 0.7

w.w. – wet weight, SD – standard deviation

Many authors reported considerably higher levels of this element in the muscles of freshwater and marine fish (Pujin 1990, Kargin 1996, Grosheva et al. 2000, Jurkiewicz-Karnakowska 2001).

Iron and its compounds are not toxic to fish, but disturbances in the mechanism that regulates its absorption often occur due to diseases or long-term exposure to high dietary iron levels. In such cases, iron is bound to proteins or as iron phosphate (haemosiderin), which is deposited in various tissues, mainly in liver. Both absorption and metabolic function of iron are dependent on influences of other elements. Especially Cd, Mn, Pb and Zn act antagonistically to iron. In the case of Cu, the relationship is very complex and often has a synergetic character, as Cu and Fe are involved in reduction-oxidation processes. Phosphorus reduces iron bioavailability because iron phosphates precipitate easily in various conditions (Kabata-Pendias, Pendias 1999).

Tissue levels of copper in the three fish species were comparable. Average tissue levels of copper ranged from 0.4 to 19.2 mg kg^{-1} w.w. The highest copper levels were found in the liver, while the lowest – in the muscles (Table 2). Copper was distributed in fish bodies according to the following patterns of decreasing concentrations:

– common carp and rainbow trout: liver> gills> kidney> skin> blood> muscles,
– Siberian sturgeon: liver> blood> gills> kidney> skin> muscles.

Copper participates in haematopoiesis, but high concentrations of this metal in combination with some other metals such as zinc, mercury, cadmium or lead, may produce anaemia in fish (DICK, DIXON 1985, EL-DOMIATY 1987, BANERJEE, HOMECHAUDHURI 1990, SINGH, REDDY 1990, VAN VUREN et al. 1994, KAZLAUSKIENË, VOSYLIENË 1995). SINGH and REDDY (1990) imply that long term exposure of fish to copper may produce anaemia caused by a disorder of kidney haematopoietic function. Worth noticing is that copper forms a synergetic system with iron (Cu-Fe), which advantageously influences the course of enzymatic processes (KABATA-PENDIAS, PENDIAS 1999). Copper also accompanies iron in all stages of cellular respiration. The metal forms a reductive-oxidative system, e.g., it conditions activity of oxidases (uricase, ascorbase, lysine oxidase, monoamine oxidases, and also cytochrome c oxidase, tyrosinase and porphobilinogen synthase), protecting the organism from adverse impact of reactive oxygen species (RFT/ROS).

Zinc levels in the examined fish varied from 3.1 to 98.7 mg kg^{-1} w.w. (Table 2). The highest zinc content (50.7 to 98.7 mg kg^{-1} w.w.) was detected in the liver, while the lowest in the gills (3.1 to 3.6 mg kg^{-1} w.w.). Zinc distribution in fish bodies followed the decreasing sequences:
– common carp: skin> liver> kidney> blood> muscles> gills,
– rainbow trout and Siberian sturgeon: liver> skin> muscles> blood> gills> kidney.

Zinc is weakly accumulated in fish tissues, as it is retained by the gills, where the metal is deposited in large amounts (JEZIERSKA, WITESKA 2001, WITESKA 2003). This may be explained by the fact that zinc penetrates to blood less easily than other metals (cadmium, nickel). Changes in zinc levels in the examined tissues resulted from its affinity to erythrocyte membranes (BARRON, ADELMAN 1984) and serum proteins (BETTGER et al. 1987) that participate in its transport. Zinc is transported mainly as zinc-albumin and zinc-macroglobulin complexes, and is excreted mostly in the faeces (70-80%). Zinc displays low toxicity to freshwater fish. Its adverse influence is mainly connected with secondary deficit of copper and does not produce any specific symptoms. Zinc absorption by animals is influenced by food quality and interactions among zinc and other elements. Metabolically significant antagonism occurs between Zn and Cd, as well as between Zn and Cu. Additionally, calcium and magnesium may reduce zinc absorption (KABATA-PENDIAS, PENDIAS 1999).

This study revealed that average levels of macroelements differed significantly among the three fish species examined. Average magnesium levels varied from 38.1 to 453.3 mg kg^{-1} w.w. Most magnesium was detected in the gills of Siberian sturgeon (453.3±14.5 mg kg^{-1} w.w.) and in the gills and muscles of common carp (346.1±17.6 and 256.1±15.2 mg kg^{-1} w.w., respectively; Table 3). On the contrary, the lowest magnesium level was found in the blood of rainbow trout (38.1±18.1 mg kg^{-1} w.w.). In all the three fish species, Mg distribution followed the same pattern of decreasing levels: gills> muscles> liver> kidney> skin> blood.

Calcium levels in the examined fish ranged from 72.2 to 4954.6 mg kg^{-1} w.w. In all the three species, the lowest calcium levels were recorded in the blood (72.2 to 96.8 mg kg^{-1} w.w.) and the highest in the gills (2214.6 to 4954.6 mg kg^{-1} w.w.; Table 3). Similarly as in the case of magnesium, calcium distribution followed the same pattern for all the three species, in decreasing order: gills> muscles> skin> liver> kidney> blood.

Table 3

Macroelement (Mg, Ca) levels in organs of three freshwater fish species

Bioelement	Tissue	Common carp *Cyprinus carpio* L.	Rainbow trout *Oncorhynchus mykiss* Rich	Siberian sturgeon *Acipenser baeri* Brandt
		mean ± SD	mean ± SD	mean ± SD
Mg (mg kg^{-1} w.w.)	blood	76.6 ± 16.1	38.1 ± 18.1	85.4 ± 11.4
	liver	139.0 ± 14.1	78.0 ± 13.1	157.6 ± 15.3
	kidney	96.3 ± 19.2	63.2 ± 13.6	117.3 ± 14.6
	gills	346.1 ± 17.6	158.1 ± 13.6	453.3 ± 14.5
	muscles	256.1 ± 15.2	122.1 ± 17.6	166.1 ± 17.6
	skin	96.1 ± 17.6	56.1 ± 10.6	113.1 ± 13.3
Ca (mg kg^{-1} w.w.)	blood	76.5 ± 8.1	72.2 ± 11.1	96.8 ± 12.8
	liver	118.2 ± 18.2	122.2 ± 13.3	197.1 ± 15.2
	kidney	103.9 ± 5.9	112.3 ± 25.4	153.2 ± 22.9
	gills	2214.6 ± 62.1	2989.6 ± 88.3	4954.6 ± 92.1
	muscles	277.3 ± 18.2	199.1 ± 14.4	246.8 ± 21.6
	skin	156.1 ± 13.5	186.5 ± 14.6	176.4 ±167.6

w.w. – wet weight, SD – standard deviation

This study focused on assessment of physiological condition of freshwater fish exemplified by common carp, rainbow trout and Siberian sturgeon, based on levels of certain microelements (iron, zinc and copper) and macroelements (magnesium and calcium) in their bodies. Levels of some bioelements in fish bodies depend on culture methods, water chemistry, and season of the year and feed quality. All these factors together influence physiological condition of fish, which can be disturbed by excess or deficiency of minerals. Excess or deficiency of minerals may seriously disturb biochemical processes and upset internal homeostasis, leading in consequence to various diseases. TACON (1992) reported that disorders occurred in organisms of various fish species due to deficiency or excess of micro- and macroelements which were caused by improper nutrition, avitaminosis or poisoning. It is therefore important to monitor levels of macro- and microelements in fish organisms.

Among the examined freshwater fish species, statistically significant differences in the levels of macro- and microelements were observed. The analyzed bioelements (Cu, Zn, Fe, Mg and Ca), which are regarded some of the most important macro- and microelements, were reported to accumulate in excess in disease conditions caused by bacterial and viral infections, as well as at increased activity of hepatocytes (POURAMAHAD 2000, LUSHCHAK et al. 2005). Levels of microelements recorded in this study were not high (Table 2, 3) and remained within the normal range for salmonids (*Salmonidae*) and cyprinids (*Cyprinidae*). For sturgeon family (*Acipenseridae*), an accurate normal range of macro- and microelements has not been determined.

In the Oder river basin within the West Pomeranian Province, cyprinid and salmonid fish are often reared in cooling water from a power plant, which are collected in a discharge canal and then disposed of to the Oder. Cooling water has nearly constant temperature all year round, which creates favourable conditions for all year round fish farming. Such activities have been undertaken for many years in cooling water from the Dolna Odra Power Station, West Pomerania. Cooling water discharged from the power plant contains various substances essential for proper functioning of ecosystems and also trace amounts of toxic chemicals, concentrations of which stay within the permissible limits (Rozporządzenie Ministerstwa OŚZNiL 1991, Raport WIOŚ 2003, JEZIERSKA, WITESKA 2001).

In all monitored rivers in the West Pomeranian Province, heavy metal concentrations, including cadmium, lead, mercury and nickel, do not exceed the limits (PROTASOWICKI, CHODYNIECKI 1988, Raport WIOŚ 2003). There are numerous reports on levels of macro- and microelements in organs and tissues of common carp of various age (DOBRZAŃSKI et al. 1996, KOŁACZ et al. 1996, MOORE, RAMAMOOTHY 1984, VIRK, KAUR 1999). On the contrary, there are no data on bioelement content in tissues of salmonids and sturgeons cultured in both cooling waters and fish ponds not supported by cooling waters.

Biological effect of a chemical depends on the following processes: absorption, biotransformation, accumulation and elimination of a compound. Biotransformation of xenobiotics may result in formation of metabolites that are less or more toxic, while accumulation means deposition of toxic substances or their metabolites in tissues. Elements present in water are regarded to be bound on the gill surface, which disturbs function of this organ. The amount of bioelements retained in fish body depends on many factors that condition the sorption efficiency. Some of them are: species, age, body weight and length, gender, season of the year and fishing ground (PROTASOWICKI, CHODYNIECKI 1988, LIANG, WONG 2000). Also the amount of bioelements accumulated in different tissues is varied. Interspecies differences derive mainly from different feeding habits (PROTASOWICKI 1991, LIANG, WONG 2000).

Within several minutes from absorption, most of bioelements find their way to heart, liver, kidney and brain. The second phase, when bioelements penetrate into muscles, skin and adipose tissue, is considerably slower, and balance in tissues is established within some half an hour to several hours. In distribution of various substances all over the organism, the circulatory system plays a key role. Substances are carried by blood to particular organs and tissues, and next removed from the body in the processes of excretion, which depends largely on the blood flow rate through the tissues. Blood physiological values in fish are considerably varied as they depend on individual variability, age, culture method, diet and season of the year. Blood parameters typical for healthy fish may vary in a wide range, therefore determination of adequate physiological reference values is much more difficult than for warm-blooded animals (ALLAN 1993, THOMAS et al. 1999). Literature reports indicate that the degree of metal accumulation and excretion in different organs is varied. According to SREEDEVI et al. (1992) common carp, exposed for 4 days to nickel dissolved in water in concentrations ranging from 20 to 70 mg dm^{-3}, accumulated most of the metal in the gills, and less in the liver, muscles and kidney. In contrast, a four-day-long experiment of RAY et al. (1990) on *Clarias batrachus* exposed to nickel revealed that accumulation of the metal in fish organs decreased in order: kidney > liver > gills > gut. Mercury concentrations in organs of bream caught in the Vistula River were the highest in the liver, gut, heart and gills (JEZIERSKA, WITESKA 2001).

This study revealed that culture site exerted statistically significant influence on bioelement levels in the examined fish species. Kidney and skin levels of magnesium were higher in common carp then in Siberian sturgeon. Similar regularities were observed for skin levels of calcium and kidney levels of zinc. Iron and copper levels in the liver and kidney were higher in common carp than rainbow trout. In all organs and tissues of common carp and rainbow trout, similar qualitative relationships were observed among the examined minerals. In all the three fish species, tissue levels of copper and zinc were the lowest. Rainbow trout had the highest skin levels of Ca and Mg, as well as blood level of Fe.

Concentrations of microelements (Zn, Fe, Cu) and macroelements (Mg, Ca) detected in this study in organs and tissues of common carp, rainbow trout and Siberian sturgeon seem to be within the normal physiological range reported for these fish species by other authors (BRUCKA-JASTRZĘBSKA, PROTASOWICKI 2004a, BRUCKA-JASTRZĘBSKA, PROTASOWICKI 2004b, VIRK, KAUR 1999).

CONCLUSIONS

1. The results indicate that culture site and culture conditions exerted significant influence on levels of macro- and microelements in freshwater fish.

2. Monitoring of bioelement levels in tissues of freshwater fish enables early observation of pathological changes in fish bodies. Disorders in the levels of bioelements appear very quickly and precede changes in fish behaviour and visible lesions.

REFERENCES

ALLAN P. 1993. *Effects of acute exposure to cadmium (II) chloride and lead (II) chloride on the hematological profile of Oreochromis aureus (Steindachner).* Comp. Biochem. Physiol., 105C, 213-217.

BANERJEE S., HOMECHAUDHURI S. 1990. *Hematological monitoring of a bio-indicator fish, Heteropneustes fossilis, on exposure to copper toxicity.* The Israeli J. Aquacult., 42: 46-51.

BARRON M., ADELMAN I. 1984. *Nucleic acid protein content, and growth of larval fish sublethally exposed to various toxicants.* J. Fish Aquat. Sci., 41: 141-150.

BETTGER W.J., SPRY D., COCKELL K., YOUNG CHO C., HILTON J. 1987. *The distribution of zinc and copper in plasma, erythrocytes and erythrocytes membranes of rainbow trout (Salmo gairdneri).* Comp. Biochem. Physiol., 87C: 445-451.

BRUCKA-JASTRZĘBSKA E., PROTASOWICKI M. 2004a. *Elimination dynamics of cadmium, administered by a single intraperitoneal injection in common carp Cyprinus carpio L.* Acta Ichthyol. Piscat., 34: 167-180.

BRUCKA-JASTRZĘBSKA E., PROTASOWICKI M. 2004b. *Elimination dynamics of nickel, administered by a single intraperitoneal injection in common carp Cyprinus carpio L.* Acta Ichthyol. Piscat., 34: 181-192.

DICK P.T., DIXON D.G. 1985. *Changes in circulating blood cell levels of rainbow trout, Salmo gairdneri Richardson, following acute and chronic exposure to copper.* J. Fish Biol. 26: 475-481.

DOBRZAŃSKI Z., KOŁACZ R., BODAK E. 1996. *Metale ciężkie w środowisku zwierząt [Heavy metals in animals' environment].* Med. Wet., 52(9): 570-574. [in Polish]

EL-DOMIATY N.A. 1987. *Stress response of juvenile Clarias lazera elicited by copper.* Comp. Biochem. Physiol., 88C: 259-262.

GROSHEVA E.I., VORONSKAYA G.N., PASTUKHOVE M.V. 2000. *Trace element biovailability in Lake Baikal.* Aquat. Ecosyst. Health Mange., 3: 229-234.

JARA Z., CHODYNIECKI A. 1999. *Ichtopatologia [Ichthyopathology].* Wyd. Akademii Rolniczej we Wrocławiu, 478.

JEZIERSKA B., WITESKA M. 2001. *Metal toxicity to fish.* Wyd. Akademii Podlaskiej, Siedlce.

JURKIEWICZ-KARNAKOWSKA E. 2001. *Heavy metals (Cu, Zn, Mn, Fe, Pb and Cd) in short food chains in the lowland dam reservoir (Zegrzyński Reservoir, Central Poland).* Ecohydrol. Hydrobiol., 1 (4), 449-456.

KABATA-PENDIAS A., PENDIAS H. 1999. *Biogeochemia pierwiastków śladowych [Biogeochemistry of trace elements].* Wyd. Nauk. PWN, Warszawa, 397. [in Polish]

KARGIN F. 1996. *Seasonal changes on levels of heavy metals in tissues of Mullus barbatus and Sparus aurata collected from Iskenderun gulf (Turkey).* Water, Air Soil Pollut., 90: 557-562.

KAZLAUSKIENĖ N., VOSYLIENĖ M.Z. 1995. *The effect of sublethal concentrations of copper on physiological indices of rainbow trout Salmo gairdneri.* [*In Russian*]. J. Ichthyol. 35: 412-416.

KOŁACZ R., DOBRZAŃSKI Z., BODAK E. 1996. *Bioakumulacja Cd, Pb i Hg w tkankach zwierząt [Bioaccumulation of Cd, Pb and Hg in animal tissues].* Med. Wet., 52 (11): 686-692. [in Polish]

LIANG Y., WONG M.H. 2000. *Reclamation of wastewater for polyculture of freshwater fish: bioassays using Chlorella and Gambusia.* Arch. Environ. Con. Toxicol., 39(4): 506-514.

LUSHCHAK V.I., BAGNYUKOVA T.V., LUSHCHAK O.V., STOREY J.M., STOREY K.B. 2005. *Hypoxia and recovery perturb free radical processes and antioxidant potential in common carp (Cyprinus carpio) tissues.* Int. J. Bioch. Cell Biol. 37, 1319-1330.

MOORE J., RAMAMOOTHY S. 1984. *Heavy metals in natural water.* Springer–Verlag, Berlin.

POURAMAHAD J., O'BRIEN, P.J. 2000. *A comparison of hepatocyte cytotoxic mechanisms for Cu^{2+} and Cd^{2+}.* Toxicology, 143: 263-273.

PROTASOWICKI M. 1991. *Long-term studies on heavy metals in aquatic organisms from the river Odra mouth area.* Acta Ichthyol. Piscat., 21 (Suppl.): 301–309.

PROTASOWICKI M., CHODYNIECKI A. 1988. *Bioakumulacja Cd, Pb, Cu i Zn w karpiu – Cyprinus carpio L. w zależności od stężenia w wodzie i czasu ekspozycji [Bioaccumulation of Cd, Pb, Cu and Zn in carp, Cyprinus carpio L., depending on concentration in water and exposition length]* . Zesz. Nauk. AR Szczecin, 133: 69-84. [in Polish]

PUJIN V., DJUKIĆ N., MALETIN S., OBRADOWIĆ S. 1990. *Content of heavy metals in some fish species in the section of the Danube flowing through Vojvodina.* Wat. Sci. Tech., 22(5): 79-86.

Raport o stanie środowiska w województwie zachodniopomorskim w latach 2002-2003 wydany przez Wojewódzki Inspektorat Ochrony Środowiska w Szczecinie. http://www.wios.szczecin.pl/bip/chapter_16002.asp

RAY D., BANERJEE K., CHATTERJEE M. 1990. *Bioaccumulation of nickel and vanadium in tissues of the Catfish claris batrachus.* J. Inorg. Biochem., 38: 169-173.

Rozporządzenie Ministerstwo Ochrony Środowiska Zasobów Naturalnych i Leśnictwa z dn. 5.11.1991 r. *W sprawie klasyfikacji wód oraz warunków, jakim powinny odpowiadać ścieki wprowadzane do wód lub ziemi*. Dz.U. 1991, 116, 503, 1579-1583.

SINGH H.S., REDDY T.V. 1990. *Effect of copper sulfate on, hematology, blood chemistry, and hepato-somatic index of an Indian catfish, Heteropneustes fossilis (Bloch), and its recovery.* Ecotoxicol. Environ. Saf., 20: 30-35.

SREEDEVI P., SIVARAMAKRISHNA B., SURESH A., RADHAKRISHNAIAH K. 1992. *Effect of nickel on some aspects of protein metabolism in the gill and kidney of the freshwater fish, Cyprinus carpio L.* Environ. Poll., 77(1): 59-63.

TACON A.G.J. 1992. *Nutritional fish pathology. Morphological signs of nutrient deficiency and toxicity in farmer fish.* FAO Fisheries Technical Paper 330.75pp.

THOMAS M.B., THOMAS W., HORNSTEIN T.W., HEDMAN S.C. 1999. *Seasonal leukocytes and erythrocyte counts in fathead minnows.* J. Fish Biol., 54: 1116-1178.

VAN VUREN J.H.J., VAN DR MERWE M., DU PREEZ H.H. 1994. *The effects of copper on the blood chemistry of Clarias gariepinus (Clariidae).* Ecotoxicol. Environ. Saf., 29: 187-199.

VIRK S., KAUR K. 1999. *Impact of mixture of nickel and chromium on the protein content of flesh and liver of Cyprinus carpio during spawning, and post-spawning phases.* Bull. Environ. Contam. Toxicol., 63: 499-502.

WITESKA M. 2003. *Wpływ metali (Pb, Cu, Cd i Zn) na parametry hematologiczne i morfologię krwi karpi [Effect of metals (Pb, Cu, Cd and Zn) on haematological parameters and blood morphology in carp].* Rozprawa naukowa nr 72. Wyd. Akademii Podlaskiej, Siedlce 2003. [in Polish]

J. Elementol. 2009, 14(3): 449–456

THE IMPACT OF MANURE, MUNICIPAL SEWAGE SLUDGE AND COMPOST PREPARED FROM MUNICIPAL SEWAGE SLUDGE ON CROP YIELD AND CONTENT OF Mn, Zn, Cu, Ni, Pb, Cd IN SPRING RAPE AND SPRING TRITICALE

Anna Iżewska

Chair of Environmental Chemistry
Western Pomeranian University of Technology

Abstract

The aim of the research was to estimate the impact of manure, municipal sewage sludge and compost prepared from municipal sewage sludge on crop yield and content of microelements (Mn, Zn, Cu, Ni) as well as Pb, and Cd in spring rape and spring triticale and also to specify the bioaccumulation indexes of microelements in test plants.

A pot experiment was set up in the Vegetation Hall of the University of Agriculture in Szczecin 2006. Manure, raw sewage sludge and compost prepared from sewage sludge with the GWDA method were used. The pot experiment was set up with the split plot method in three repetitions. Objects of the first factor were types of fertilizers (manure, municipal sewage sludge and compost prepared from sewage sludge) and objects of the second factor were the doses of individual fertilizers introduced to soil in conversion to the amount of incorporated nitrogen (85 and 170 kg $N \cdot ha^{-1}$ i.e. 0.26 and 0.52 g N per pot).

The results indicate that the best yields of spring rape seeds and spring triticale grains were obtained from the object fertilized with a double dose of sewage sludge. Fertilization with manure, sewage sludge and compost prepared from sewage sludge increased the content of microelements as well as Pb and Cd in seeds and grains of the test plants. These contents did not exceed permissible values for industrial plants. The calculated bioaccumulation indexes of microelements indicate that spring rape and spring triticale were accumulating moderate amounts of manganese and zinc.

Key words: manure, sewage sludge, compost prepared from sewage sludge, spring rape, spring triticale, bioaccumulation indexes.

dr inż. Anna Iżewska, Chair of Environmental Chemistry, Western Pomeranian University of Technology, ul. Słowackiego 17, 71-434 Szczecin, Poland, phone (091) 449 6337; e-mail: anna.izewska@zut.edu.pl

WPŁYW OBORNIKA, KOMUNALNEGO OSADU ŚCIEKOWEGO I KOMPOSTU Z NIEGO WYPRODUKOWANEGO NA WIELKOŚĆ PLONU I ZAWARTOŚĆ MIKROSKŁADNIKÓW Mn, Zn, Cu, Ni ORAZ Pb I Cd W RZEPAKU JARYM I PSZENŻYCIE JARYM

Abstrakt

Celem badań było określenie wpływu nawożenia gleb obornikiem, komunalnym osadem ściekowym i kompostem z niego wyprodukowanym na wielkość plonu i zawartość mikroskładników (Mn, Zn, Cu, Ni) oraz Pb i Cd. w rzepaku jarym i pszenżycie jarym oraz określenie wskaźników bioakumulacji tych pierwiastków w roślinach uprawianych.

W 2006 r. założono doświadczenie wazonowe na terenie hali wegetacyjnej AR w Szczecinie. Do badań użyto obornik, surowy osad ściekowy i wyprodukowany metodą GWDA kompost z osadów ściekowych.

Doświadczenie wazonowe założono metodą split plot w trzech powtórzeniach. Obiektami pierwszego czynnika były rodzaje nawozów (obornik, komunalny osad ściekowy, kompost z osadu ściekowego), a obiektami II czynnika – dawka poszczególnych nawozów wprowadzana do gleby w przeliczeniu na ilość wniesionego azotu (85 i 170 kg $N \cdot ha^{-1}$, tj. 0,26 i 0,52 g N na wazon). Uzyskane wyniki badań wskazują, że największy plon nasion rzepaku jarego i ziarna pszenżyta jarego zebrano z obiektów nawożonych podwojoną dawką osadu ściekowego. Nawożenie osadem ściekowym oraz kompostem spowodowało zwiększenie zawartości mikroskładników oraz Pb i Cd w nasionach oraz ziarnie roślin testowych. Zawartości te jednak nie przekroczyły dopuszczalnych wartości dla roślin przemysłowych. Obliczone wskaźniki bioakumulacji wskazują, że rzepak jary i pszenżyto jare w stopniu średnim akumulowały mangan i cynk.

Słowa kluczowe: obornik, osad ściekowy, kompost z osadu ściekowego, rzepak jary, pszenżyto jare, wskaźniki bioakumulacji.

INTRODUCTION

The amount of sewage sludge produced in Poland has been increasing in recent years.. In 2006 1,064.7 thousand ton d.m. of sewage sludge was produced, including 501.3 thousand ton d.m. of municipal sewage sludge.

Sewage sludge can be used for fertilization of plants if it is submitted to stabilization and hygienization processes. One of the stabilization processes is composting with organic materials. Composts from sewage sludge, like raw sewage sludge, are a rich source of organic substance and components. However, sewage sludge and composts produced from sewage sludge can be loaded with excessive amounts of heavy metals. Therefore, it is important to find the way of managing and utilizing sludge that will not threaten natural environment. One of the methods is phytoremediation, i.e. using the ability of plants to take up and accumulate polluted substances or to biodegrade them.

The aim of the research was to estimate the impact of manure, municipal sewage sludge and compost prepared from municipal sewage sludge on crop yield and content of microelements (Mn, Zn, Cu, Ni) as well as Pb and Cd in spring rape and spring triticale and also to specify the bioaccumulation indexes of microelements in the test plants.

MATERIAL AND METHODS

A pot experiment was set up in the Vegetation Hall of the University of Agriculture in Szczecin in 2006. Kick-Brauckmann's pots holding 9 dm^3 were filled with 9 kg of soil each. Manure, raw sewage sludge and compost prepared from sewage sludge with the GWDA method were tested. The chemical composition of the manure, raw sewage sludge and compost was presented in the earlier research (IŻEWSKA 2007).

In the experiment soil from the Agricultural Experimental Station in Lipnik belong to the University of Agriculture in Szczecin was used. It was taken from the level of Ap and represented the grain size distribution of clay slight dusty sand. Soil reaction was acid (pH in 1mol $KCl \cdot dm^{-3}$ – 5.13) and the content of available phosphorus, potassium and magnesium was average. The pot experiment was set up with the split plot method in three replications. The objects of the first factor were types of fertilizers (manure, municipal sewage sludge and compost prepared from sewage sludge) and the objects of the second factor were the doses of individual fertilizers to the soil in conversion to the amount of incorporated nitrogen (85 and 170 kg $N \cdot ha^{-1}$ i.e. 0.26 and 0.52 g N per pot). The objects with sole fertilization NPK received 0.18 g nitrogen per pot as the first dose and 0.36 g nitrogen as the second dose. In all the objects 0.12 g P per pot and 0.26 g K per pot were added. In the second year of the experiment, mineral fertilization was performed, including 0.30 g N per pot, 0.24 g P per pot and 0.72 g K per pot.

The test plant in the first year of the experiment was spring rape cv. Licosmos; in the second year it was spring triticale cv. Dalgety. Determination of microelements as well as Pb and Cd was accomplished with the AAS method after previous wet mineralization of the samples of plant material in a mixture of nitric acid (V) and perchloric acid (VII).

The bioaccumulation indexes were calculated as a ratio of the content of a given element in the plant to its content in the organic fertilizer (KABATA-PENDIAS et al. 1993). four-degree scale of the accumulation of heavy metals was obtained (MICHAŁOWSKI, GOŁAŚ, 2001).

RESULTS AND DISCUSSION

The study showed that fertilization of soil of manure, raw sewage sludge and compost prepared from sewage sludge at both fertilization levels had significant influence on the volume of yield of spring rape seeds (Table 1). An average yield of spring rape seeds was 11.6 g per pot, about 69.31% higher than the yield which was gathered from the control variant (Table 1).

Table 1

Seed yield of spring rape and grain yield of spring triticale (g from pot)

Specification	Spring rape			Spring triticale		
	dose I	dose II	mean	dose I	dose II	mean
Manure	10.07	11.32	10.70	20.00	23.60	21.80
Sewage sludge	12.32	15.20	13.76	22.50	28.00	25.30
Compost	10.89	11.63	11.26	24.40	20.80	22.60
NPK	11.68	10.16	10.92	25.50	23.40	24.50
Mean	11.68	12.08	11.66	23.10	24.00	23.60
Control	3.56			14.50		

$LSD_{0.01}$ for rape seeds I factor 1.88; II factor 0.99; IxII 1.96
$LSD_{0.01}$ for triticale grains I factor n.s; II factor n.s; IxII 5.19

The maximum yield of spring rape seeds was gathered from the objects with raw sewage sludge. It was considerably higher than the yield obtained from the remaining fertilization variants. The impact of manure, compost and NPK on yield did not differmuch. An average yield of seeds of spring rape after the first dose of fertilizer was 11.68 g per pot and was significantly lower than after the double dose.

Mineral fertilization as well introduction of manure, sewage sludge and compost from sewage sludge had non-significant influence on yield of spring triticale grain. The yield gathered from the control object was 14.50 g per pot, being about 9.1 g lower than the average yield obtained from the soils which were fertilized by any of the analyzed fertilizers. In the second year of the experiment, the maximum yield was likewise obtained after fertilization of soil with sewage sludge (25.30 g per pot) and the lowest yield occurred after application of manure (21.80 g from a pot). The best effect in terms of yield of spring triticale grain was obtained in the object where the higher dose of sewage sludge was used (28.00 g per pot). The grain yield from this object was significantly higher than the yield obtained from the soils, which were fertilized with manure and the double dose of compost.

Regarding the influence of fertilization on the content of Mn, Cu, Zn Ni as well as Pb and Cd, it was demonstrated that for both spring rape seeds (Table 2) and triticale grains (Table 3) the yields from fertilized objects were higher than from the control variant.

Sewage sludge used to fertilize spring rape caused increased content of zinc, copper, nickel and lead in seeds, but the increase was statistically proven only for nickel. As the dose of the fertilizer rose, the content of microelements as well as Pb and Cd in seeds of spring rape increased.

The experimental factors: the type of fertilizers (manure, sewage sludge, compost from sewage sludge) and the increasing doses, did not significantly

Table 2

Content of microelements as well as Pb and Cd in seeds of spring rape (mg·kg^{-1}d.m.)

Specification	Mn			Zn			Cu			Ni			Pb			Cd		
	1*	2*	$\overline{x}$	1*	2*	$\overline{x}$	1*	2*	$\overline{x}$	1*	2*	$\overline{x}$	1*	2*	$\overline{x}$	1*	2*	$\overline{x}$
Manure	53.3	51.5	52.4	28.1	27.7	27.9	3.62	3.95	3.78	2.72	3.09	2.90	4.29	4.25	4.27	0.142	0.188	0.165
Sewage sludge	49.5	49.8	49.6	35.7	48.1	41.9	3.82	4.38	4.10	3.86	3.99	3.76	4.70	4.66	4.68	0.132	0.128	0.130
Compost	56.8	41.3	49.0	31.4	32.2	31.8	2.78	4.18	3.48	2.86	2.58	2.72	3.33	4.31	3.82	0.198	0.230	0.214
NPK	45.6	55.0	50.3	28.6	37.8	33.2	4.22	4.15	4.18	3.63	3.94	3.78	5.08	5.26	5.17	0.162	0.198	0.180
Mean	51.3	49.4	50.3	30.9	36.5	33.7	3.61	4.16	3.89	3.27	3.32	3.29	4.35	4.62	4.48	0.159	0.186	0.172
Control		43.7			24.1			3.60			2.27			3.26			0.123	
$LSD_{0.01}$																		
I factor		n.s.			n.s.			n.s.			1.58			2.12			n.s.	
II factor		n.s.			n.s.			n.s.			n.s.			n.s.			n.s.	
IxII		n.s.			n.s.			n.s.			n.s.			n.s.			n.s.	

1*dose I;
2*dose II

Table 3

Content of microelements as well as Pb and Cd in grains of spring triticale ($mg \cdot kg^{-1}$d.m.)

Specification	Mn			Zn			Cu			Ni			Pb			Cd		
	1*	2*	$\overline{x}$	1*	2*	$\overline{x}$	1*	2*	$\overline{x}$	1*	2*	$\overline{x}$	1*	2*	$\overline{x}$	1*	2*	$\overline{x}$
Manure	46.38	53.67	50.03	32.4	37.9	35.2	4.69	5.16	4.93	0.194	0.243	0.219	0.46	0.42	0.44	0.102	0.100	0.101
Sewage sludge	53.67	67.94	60.81	34.6	41.1	37.9	5.95	5.34	5.65	0.244	0.236	0.240	0.55	0.41	0.48	0.104	0.092	0.098
Compost	55.89	73.30	64.60	38.6	37.8	38.2	5.09	6.37	5.73	0.155	0.258	0.207	0.35	0.56	0.46	0.107	0,122	0.115
NPK	76.11	75.74	75.93	35.2	32.4	33.8	6.40	5.12	5.76	0.227	0.258	0.256	0.51	0.52	0.52	0.112	0.107	0.109
Mean	58.01	67.66	62.84	35.2	37.3	36.3	5.53	5.50	5.52	0.218	0.256	0.231	0.47	0.48	0.48	0.106	0.105	0.106
Control	42.24			28.7			4.99			0.114			0.47			0.087		
$LSD_{0.01}$ I factor II factor IxII	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.			n.s. n.s. n.s.		

1*dose I;
2*dose II

Table 4

Average bioaccumulation indexes of microelements as well as Pb and Cd in seeds of spring rape and in grains of spring triticale

Specification	Mn		Zn		Cu		Ni		Pb		Cd	
	rape	triticale	rape	triticale	rape	triticale	rape	triticale	rape	triticale	rape	triticale
Manure	0.600	0.573	0.252	0.318	0.150	0.198	0.268	0.020	0.178	0.018	0.157	0.096
Sewage sludge	0.127	0.155	0.104	0.095	0.028	0.039	0.114	0.007	0.080	0.008	0.032	0.025
Compost	0.108	0.143	0.099	0.119	0.033	0.055	0.124	0.010	0.055	0.007	0.044	0.024
Mean	0.278	0.290	0.152	0.178	0.070	0.097	0.169	0.012	0.104	0.011	0.078	0.048

influence the content of microelements or Pd and Cd in grain of spring triticale (Table 3). The soil used for the experiment was acid in reaction (pH_{KCl} 5,13), which raised the availability of heavy metals in the soil (KABATA-PENDIAS, PENDIAS 1999). In the author's own research, despite the double dose of the fertilizers, the content of microelements in spring rape seeds and spring triticale grain increased marginally and did not deviate from the values which are given for these species in the literature (KABATA-PENDIAS et al. 1993). According to BARAN et al. (1996), FLIS-BUJAK et al. (1996), BARAN et al. (1998), Gorlach and Gambuś (1999) organic substance added to soil caused increasing sorption capacity of soil. As a result, the amounts of available forms of these elements were enlarged. The impact of organic substance raised complexing properties of soil and caused formation of metal-organic bonds, which reduced assimilation of the microelements.

The analysis of the average bioaccumulation factors of microelements as well as Pb and Cd in rape seeds and triticale grain (Table 4) showed that only the bioaccumulation index for manganese and zinc was on an average level, likewise the indexes for nickel and lead in spring rape seeds. The remaining indexes were on a low level. Similar dependences were given by FILIPEK-MAZUR et al. 2002, MICHAŁOWSKI and Gołaś 2001.

CONCLUSIONS

1. The best effects in respect to the yield of spring rape seeds of and spring triticale grain were received from the object with the double dose of sewage sludge.

2. Fertilization with sewage sludge and sewage sludge compost increased the content of microelements as well as Pb and Cd in seeds and grains of the test plants. The content of the heavy metals did not exceed permissible values for industrial plants.

3. The computed bioaccumulation indexes showed that spring rape and spring triticale accumulated manganese and zinc to an average degree.

REFERENCES

BARAN S., FLIS-BUJAK M., TURSKI R., ŻUKOWSKA G. 1996. *Zmiany właściwości fizyko-chemicznych gleby użyźnianej osadem ściekowym Modifications of physicochemical properties of soils fertilized with sewage sludge].* Roczn. Glebozn., 47(3/4): 123-130. [in Polish].

BARAN S., FLIS-BUJAK M., ŻUKOWSKA G., KWIECIEŃ J., PIETRASIK W., KĘPCZYNSKI A. 1998. *Zmiany fizykochemicznych właściwości gleby lekkiej użyźnianej osadem ściekowym i wermikompostem osadowym [Changes of he physicochemical properties of light soil fertilized with sewage sludge and sludge vermicompost].* Zesz. Prob. Post. Nauk Rol., 456: 515--523. [in Polish].

FILIPEK-MAZUR B., MAZUR K., GONDEK K. 2002. *Zawartość metali ciężkich w glebie jako efekt nawożenia osadami pochodzenia garbarskimi i ich kompostami [Content of heavy metals in soil as a result of fertilization with sludge derived from the tanning industry and composts made from such sludge].* Zesz. Prob. Post. Nauk Rol., 467 (2): 489-497. [in Polish].

FLIS-BUJAK M., BARAN S., ŻUKOWSKA G. 1996. *Właściwości materii organicznej wybranych odpadów o charakterze nawozowym [Properties of organic matter of some waste products potentially used as fertilizers].* Zesz. Prob. Post. Nauk Rol., 437: 147-153. [in Polish]

GORLACH E., GAMBUŚ F. 1999. *Wpływ osadów ściekowych na zawartość metali ciężkich w glebie i roślinach oraz ich przemieszczanie się w profilu glebowym [Effect of sewage sludge on content of heavy metals in soil and plants as well as translocation of heavy metals in the soil profile].* Zesz. Prob. Post. Nauk Rol., 467: 505-511. [in Polish]

IŻEWSKA A. 2007. *Wpływ nawożenia obornikiem, osadem ściekowym i kompostem z osadów ściekowych na właściwości gleby [Effect of fertilization with manure, sewage sludge and compost from sewage sludge on soil properties].* Zesz. Prob. Post. Nauk Rol., 518: 85-82. [in Polish].

KABATA-PENDIAS A., MOTOWICKA-TERELAK H., PIOTROWSKA M., TERELAK T., WITEK T. 1993. *Ocena stopnia zanieczyszczenia gleb i roślin metalami ciężkimi i siarką [Assessment of the degree of contamination of soil and plants with heavy metals and sulphur].* IUNG, Puławy, 53. [in Polish].

KABATA-PENDIAS A., PENDIAS H. 1999. *Biogeochemia pierwiastków śladowych [Biogeochemistry of trace elements].* PWN, Warszawa, ss. 363. [in Polish].

MICHAŁOWSKI M., GOŁAŚ J. 2001. *Zawartość wybranych metali ciężkich w organach wierzby jako wskaźnik wykorzystania jej w utylizacji osadów ściekowych [Content of heavy metals in willow organs as an indicator of using willow for utilization of sewage sludge].* Zesz. Prob. Post. Nauk Rol., 477: 411-419. [in Polish].

J. Elementol. 2009, 14(3): 457–466

EVALUATION OF IMPACT OF FERTILIZATION WITH MANURE, MUNICIPAL SEWAGE SLUDGE AND COMPOST PREPARED FROM SEWAGE SLUDGE ON CONTENT OF Mn, Zn, Cu, AND Pb, Cd IN LIGHT SOIL

Anna Iżewska, Edward Krzywy, Czesław Wołoszyk

Chair of Environmental Chemistry
Western Pomeranian University of Technology

Abstract

The aim of the research was to estimate the impact of fertilizing with manure, sewage sludge and compost prepared from sewage sludge on the content of total and soluble forms of Mn, Zn, Cu as well as Pb and Cd in light soil.

A pot experiment was set up with the split plot method in 2006. The first factor objects were types of fertilizers: manure, municipal sewage sludge, compost prepared from sewage sludge. The second factor consisted of doses of manure and organic fertilizers incorporated into the soil, expressed as amounts of nitrogen (0.26 and 0.52 g N per pot). In the second year mineral fertilizing was applied in a dose of 0.30 g $N \cdot pot^{-1}$ and 0.72 g $K \cdot pot^{-1}$. Soil used in the experiment was taken from Ap level and had grain size distribution of light loamy sand. The soil was acidic (pH in 1 mol $KCl \cdot dm^{-3}$ – 5.13) and moderately abundant in available phosphorus, potassium and magnesium. The content of microelements in soil before the experiment was below the permissible concentrations given in the Ordinance of the Minister of Environment of 9.09.2002 on quality standards of soils and ground (Journal of Law, 2002, no. 165 item 1359). As regards the contamination of soil with Cu, Mn, Ni, Pb, Zn, the content of these heavy metals, according to IUNG classification, was 0, which stands for natural content, although the content of Cd was raised up to I^{o}.

It has been found out that fertilizing with manure, sewage sludge and compost prepared from sewage sludge increased, in direct and successive effect, the content of total and soluble in 1 mol $HCl \cdot dm^{-3}$ forms of microelements. In the case of soil fertilized with organic fertilizers, first degree contamination with cadmium and nickel occurred (exceeding na-

---

dr inż. Anna Iżewska, Chair of Environmental Chemistry, Western Pomeranian University of Technology, ul. Słowackiego 17, 71-434 Szczecin, Poland, phone (091) 449 6337; e-mail: anna.izewska@zut.edu.pl

tural content in soil). The application of manure and sewage sludge, in turn, raised the content of lead.

On the basis of the mean share of soluble forms in the total content in soil from objects fertilized with organic fertilizers, the microelements could be ranked as follows: Cu > Pb > Ni > Cd > Mn > Zn in the first year of the research, and Cd > Pb > Cu > Mn > Zn > Ni in the second year.

Key words: manure, sewage sludge, sewage sludge compost , light soil.

OCENA WPŁYWU NAWOŻENIA OBORNIKIEM, KOMUNALNYM OSADEM ŚCIEKOWYM I KOMPOSTEM Z OSADU ŚCIEKOWEGO NA ZAWARTOŚĆ Mn, Zn, Cu, ORAZ Pb I Cd W GLEBIE LEKKIEJ

Abstrakt

Celem badań była ocena wpływu nawożenia obornikiem, komunalnym osadem ściekowym i kompostem z osadu ściekowego na zawartość form ogólnych i rozpuszczalnych: Mn, Zn, Cu, oraz Pb i Cd w glebie lekkiej.

W 2006 r. założono metodą split plot doświadczenie wazonowe. Obiektami pierwszego czynnika były rodzaje nawozów: obornik, komunalny osad ściekowy, kompost z niego wyprodukowany, obiektami II czynnika – dawki obornika i nawozów organicznych zastosowane do gleby w przeliczeniu na ilość wniesionego azotu (0,26 i 0,52 g N na wazon). W drugim roku badań zastosowano nawożenie mineralne w dawce 0,30 g N na wazon, 0,24 g P na wazon oraz 0,72 g K na wazon. Gleba użyta w doświadczeniu pobrana została z warstwy Ap i miała skład granulometryczny piasku gliniastego lekkiego pylastego. Odczyn gleby był kwaśny (pH w 1 mol $KCl \cdot dm^{-3}$ – 5,13), zawartość fosforu, potasu i magnezu przyswajalnego była średnia. Zawartość mikroskładników w glebie przed założeniem doświadczenia była poniżej wartości dopuszczalnych stężeń w glebie podanych w RMŚ z dnia 9 września 2002 r. w sprawie standardów jakości gleby oraz standardów jakości ziemi (DzU z 2002 r. nr 165 poz. 1359). Pod względem zawartości Mn, Zn, Cu, Ni, Pb stopień zanieczyszczenia gleb tymi pierwiastkami według klasyfikacji IUNG wynosił 0, co oznacza zawartość naturalną, natomiast zawartość Cd była podwyższona i stanowiła I^o.

W badaniach stwierdzono, że po nawożeniu obornikiem, osadem ściekowym i kompostem z osadu ściekowego zwiększyła się w glebie – w działaniu bezpośrednim i następczym – zawartość form ogólnych i rozpuszczalnych w 1 mol $KCl \cdot dm^{-3}$ mikroskładników oraz Pb i Cd. W przypadku nawożenia gleby nawozami organicznymi nastąpiło zanieczyszczenie I stopnia kadmem i niklem (przekroczenie naturalnej zawartości w glebie), natomiast po zastosowaniu obornika i osadu ściekowego nastąpił wzrost zawartości ołowiu.

Na podstawie średniego udziału formy rozpuszczalnej w formie ogólnej z obiektów nawożonych nawozami organicznymi pierwiastki można było uszeregować następująco: I rok badań – Cu > Pb > Ni > Cd > Mn > Zn, II rok badań – Cd > Pb > Cu>Mn > Zn > Ni.

Słowa kluczowe: obornik, osad ściekowy, kompost z osadu ściekowego, gleba lekka.

INTRODUCTION

Agricultural use of sewage sludge and composts prepared from sewage sludge, in accord with all binding laws connected with environmental protection, is one of the methods of recycling. Rational fertilizing with waste and composts increases organic substance in light soil as well as the content of macro- and microelements. Every batch of sewage sludge intended to be used in agriculture must fulfill qualitative standards such as the maximum permissible amounts of trace elements Cd, Pb, Cu, Zn, Ni, Hg, Cr and counts of live intestinal parasite eggs and *Salmonella* bacteria (Rozporządzenie Ministra Środowiska w sprawie komunalnych osadów ściekowych DzU z 2002 r. nr 134 poz. 1140).

The aim of the research was to estimate the impact of fertilizing with manure, sewage sludge and sewage sludge compost on the content of total and soluble forms in 1 mol $HCl \cdot dm^{-3}$ of Mn, Zn, Cu, Pb and Cd in light soil.

MATERIAL AND METHODS

In 2006, a pot experiment was set up with the split plot method in a greenhouse at the University of Agriculture in Szczecin. The capacity of the pots was 9 dm^3 of soil. The first factor objects were types of fertilizers: manure, municipal sewage sludge and compost prepared from sewage sludge. The second factor objects were doses of manure and organic fertilizers incorporated into the soil expressed as amounts of nitrogen (0.26 and 0.52 g N per pot). The objects fertilized exclusively with NPK received 0.18 g of nitrogen (the first dose) and 0.36 g of nitrogen per pot (the second dose). All of the fertilized objects received 0.12 g P and 0.26 g K per pot. In the second year, mineral fertilization consisted of 0.30 g $N \cdot pot^{-1}$, 0.24 g $P \cdot pot^{-1}$ and 0.72 g $K \cdot pot^{-1}$. The soil reaction was was acid (pH in 1 mol $KCl \cdot dm^{-3}$ – 5.13) and the content of available phosphorus, potassium and magnesium in soil was average. Table 1 shows the content of microelements as well as Pb and Cd in the soil before the experiment was set up. The test plant in the first year of the experiment was spring rape and in the second one – spring triticale.

The content of microelements in soil before setting up the experiment was below the value of permissible concentration given in the Ordinance of the Minister of Environment of 9.09.2002 on quality standards of soils and ground (Journal of Law, 2002, no. 165 item 1359) As regards the contamination of the soil with Cu, Mn, Ni, Pb, Zn, the content of these metals, according to the IUNG classification, was 0, which stands for natural content, although the content of Cd was raised up to I^o.

RESULTS AND DISCUSSION

The content of microelements as well as Pb and Cd in soil after the harvest of spring rape increased on the objects fertilized with manure and organic fertilizers, in comparison with the initial values (Tables 1 and 2). In soil of the objects with organic and mineral fertilizers applied, in comparison with the control, manganese rose by 19.40%, zinc by 15.70%, copper by 23.89%, nickel by 29.27%, lead by 41.91% and cadmium by 76.75%. As a result of fertilization with sewage sludge, the content of manganese, lead and cadmium increased most profoundly, whereas the application of manure increased the content of zinc and copper (Table 2).

Table 1

Content of some microelements in soil before the experiment

Total content ($mg \cdot kg^{-1}$ d.m.)					
Mn	Zn	Cu	Ni	Pb	Cd
225.1	26.1	6.30	8.25	19.8	0.42
Soluble forms ($mg \cdot kg^{-1}$ d.m.)					
68.2	4.15	1.92	2.30	8.75	0.12

In the second year of the experiment, the total content of microelements as well as Pb and Cd in soil after the harvest of spring triticale was likewise higher than the initial values (Table 3). In the successive impact, the highest mean content of manganese, lead and cadmium was in soil fertilized with sewage sludge, while that of copper and nickel was the highest after the application of compost.

Having analyzed the content of soluble forms in 1 mol $HCl \cdot dm^{-3}$ in soil after the harvest of spring rape, it was stated that, soil fertilized with sewage sludge compost contained the lowest content of soluble forms of manganese (65.9 $mg \cdot kg^{-1}$), zinc (4.76 $mg \cdot kg^{-1}$) and copper (2.28 $mg \cdot kg^{-1}$). Fertilizing with manure, however, enhanced solubility in 1 mol $HCl \cdot dm^{-3}$ of manganese (66.4 $mg \cdot kg^{-1}$) and zinc (8.38 $mg \cdot kg^{-1}$), copper (5.18 $mg \cdot kg^{-1}$) and cadmium (0.216 $mg \cdot kg^{-1}$, Table 4).

The data presented in Table 5 show that the experimental factors, i.e. the type of fertilizers (manure, sewage sludge and compost prepared from sewage sludge) and their doses, had no influence on shaping the content of forms soluble in 1 mol $HCl \cdot dm^{-3}$ of manganese, zinc, copper, nickel and cadmium in soil after the harvest of spring triticale. The content of lead in soil fertilized with manure was statistically significantly higher in comparison with its content in soil taken from the other fertilizing objects.

Table 2

Content of total forms of microelements in soil after the harvest of spring rape ($mg \cdot kg^{-1}$ d.m.)

Specification	Mn			Zn			Cu			Ni			Pb			Cd		
	1*	2*	$\overline{x}$	1*	2*	$\overline{x}$	1*	2*	$\overline{x}$	1*	2*	$\overline{x}$	1*	2*	$\overline{x}$	1*	2*	$\overline{x}$
Manure	263.3	283.4	273.4	39.3	31.9	35.6	8.95	9.35	9.15	11.30	10.65	10.98	32.9	30.5	31.7	0.765	0.783	0.774
Sewage sludge	271.2	299.6	285.4	33.2	30.5	31.9	8.22	8.55	8.38	9.95	11.98	10.96	59.1	57.8	58.4	0.785	0.832	0.809
Compost	264.1	277.6	270.8	28.8	27.8	28.3	6.52	7.08	6.80	10.94	12.58	11.76	25.6	25.4	25.5	0.568	0.585	0.577
NPK	260.5	265.7	263.1	28.4	30.3	29.3	6.75	9.20	7.98	12.46	12.76	12.61	23.2	22.2	22.7	0.452	0.455	0.454
Mean	264.8	281.6	273.2	32.4	30.1	31.2	7.61	8.54	8.08	11.16	11.99	11.58	35.2	34.0	34.6	0.643	0.664	0.654
Control	220.2			26.3			6.15			8.19			20.1			0.370		
$LSD_{0.01}$																		
I factor	n.s.			n.s.			n.s.			n.s.			7.777			0.142		
II factor	n.s.			n.s.			n.s.			n.s.			n.s.			n.s.		
IxII	n.s.			n.s.			n.s.			n.s.			n.s.			n.s.		

1*dose I;
2*dose II

Table 3

Content of total forms of microelements in soil after the harvest of spring triticale ($mg \cdot kg^{-1}$ d.m.)

Specification	Mn			Zn			Cu			Ni			Pb			Cd		
	1*	2*	$\overline{x}$	1*	2*	$\overline{x}$	1*	2*	$\overline{x}$	1*	2*	$\overline{x}$	1*	2*	$\overline{x}$	1*	2*	$\overline{x}$
Manure	261.2	281.1	271.2	36.85	29.52	33.18	8.88	8.50	8.69	11.58	9.46	10.52	32.35	29.29	30.82	0.735	0.759	0.747
Sewage sludge	269.0	295.6	282.3	29.88	28.35	29.11	8.88	8.31	8.60	9.53	11.19	10.36	49.89	56.89	53.39	0.732	0.814	0.773
Compost	263.5	276.1	269.8	27.42	28.91	28.16	6.11	7.89	7.00	11.12	11.84	11.48	24.90	25.38	25.14	0.513	0.514	0.514
NPK	258.3	269.5	263.9	28.07	29.51	28.79	6.91	9.18	8.04	11.94	11.02	11.48	22.90	22.20	22.55	0.416	0.414	0.415
Mean	263.0	280.6	271.8	30.55	29.07	29.81	7.70	8.47	8.08	11.04	10.88	10.96	32.51	33.44	32.97	0.599	0.625	0.612
Control	219.7			26.75			6.22			5.49			19.72			0.314		
$LSD_{0.01}$																		
I factor	n.s.			n.s.			n.s.			1.56			n.s.			n.s.		
II factor	n.s.			n.s.			n.s.			n.s.			n.s.			n.s.		
IxII	n.s.			n.s.			n.s.			n.s.			n.s.			n.s.		

1*dose I; 2*dose II

Table 4

Content of forms soluble in 1 mol $HCl \cdot dm^{-3}$ of microelements after the harvest of spring rape ($mg \cdot kg^{-1}$d.m.)

Specification	Mn			Zn			Cu			Ni			Pb			Cd		
	1*	2*	$\overline{x}$	1*	2*	$\overline{x}$	1*	2*	$\overline{x}$	1*	2*	$\overline{x}$	1*	2*	$\overline{x}$	1*	2*	$\overline{x}$
Manure	66.7	66.0	66.4	8.28	8.48	8.38	4.85	5.50	5.18	2.53	2.81	2.67	9.58	12.81	11.20	0.207	0.224	0.216
Sewage sludge	65.1	67.0	66.0	6.22	6.82	6.52	3.71	3.31	3.51	3.64	3.66	3.65	12.73	15.39	14.06	0.171	0.202	0.187
Compost	65.2	66.6	65.9	4.50	5.03	4.76	2.26	2.31	2.28	2.37	4.26	3.32	9.78	9.23	9.50	0.146	0.184	0.165
NPK	66.8	66.4	66.6	4.70	8.48	6.59	2.14	2.41	2.28	4.78	5.33	5.06	9.06	8.53	8.80	0.119	0.124	0.122
Mean	66.0	66.5	66.2	5.92	7.20	6.56	3.24	3.38	3.31	3.33	4.02	3.68	10.29	11.49	10.89	0.161	0.184	0.173
Control		64.3			4.07			1.88			2.15			8.22			0.098	
$LSD_{0.01}$																		
I factor		n.s.			n.s.			2.13			1.56			n.s.			n.s.	
II factor		n.s.			n.s.			n.s.			n.s.			n.s.			n.s.	
IxII		n.s.			n.s.			n.s.			n.s.			n.s.			n.s.	

1*dose I;
2*dose II

Table 5

Content of forms soluble in 1 mol HCl $\cdot$ dm^{-3} of microelements in soil after the harvest of spring triticale (mg $\cdot$ kg^{-1} d.m.)

Specification	Mn			Zn			Cu			Ni			Pb			Cd		
	1*	2*	$\bar{x}$	1*	2*	$\bar{x}$	1*	2*	$\bar{x}$	1*	2*	$\bar{x}$	1*	2*	$\bar{x}$	1*	2*	$\bar{x}$
Manure	110.2	111.7	111.0	6.14	7.72	6.93	2.99	4.82	3.90	1.38	1.99	1.68	14.97	15.09	15.03	0.432	0.301	0.367
Sewage sludge	105.5	98.0	101.8	6.67	7.69	7.18	3.05	3.34	3.20	1.33	1.63	1.48	11.14	11.82	11.48	0.228	0.268	0.248
Compost	92.6	95.8	94.2	5.71	7.44	6.58	2.57	2.81	2.69	1.55	1.70	1.63	10.38	10.84	10.61	0.257	0.255	0.256
NPK	96.7	101.9	99.3	6.67	6.48	6.58	2.55	2.50	2.53	1.49	1.49	1.49	10.53	10.16	10.34	0.274	0.270	0.272
Mean	101.2	101.8	101.6	6.30	7.33	6.82	2.79	3.37	3.08	1.44	1.70	1.57	11.75	11.98	11.86	0.300	0.274	0.286
Control	92.8			6.18			2.52			1.18			10.36			0.262		
$LSD_{0.01}$ I factor II factor IxII	n.s. n.s. n.s.			n.s. n.s. n.s.			n.s. n.s. n.s.			n.s. n.s. n.s.			2.39 n.s. n.s.			n.s. n.s. n.s.		

1*dose I;
2*dose II

Table 6

Mean share of forms soluble in 1 M HCl in the total form (total form=100%)

Specification	Mn		Zn		Cu		Ni		Pb		Cd	
	rape	triticale	rape	triticale	rape	triticale	rape	triticale	rape	triticale	rape	triticale
Manure	24.29	40.96	23.54	20.89	56.61	44.88	24.32	15.97	35.33	48.77	27.91	57.83
Sewage sludge	23.12	36.06	20.44	24.66	41.88	37.21	33.30	13.92	24.08	21.50	23.11	32.08
Compost	24.34	34.91	16.82	23.37	48.01	38.43	28.23	14.11	37.25	42.20	28.60	49.80
NPK	25.31	37.63	22.41	22.86	28.57	31.34	40.13	12.98	38.77	45.85	26.87	65.54
Mean	24.26	37.39	20.80	22.94	43.77	37.97	31.50	14.24	33.86	39.58	26.62	51.31

On the basis of the mean share of forms soluble in 1 mol $HCl \cdot dm^{-3}$ in the total content, it was stated that in the two-year period availability of manganese, zinc, lead and cadmium increased and that of copper and nickel decreased (Table 6).

Increase in the total and soluble forms of manganese, zinc, copper and nickel in soil was reported by IŻEWSKA et al. (2006), who tested sewage sludge and composts. JAKUBUS (2006) and JAMALI et al. (2008) also demonstrated increase in the total content of manganese, zinc, copper in soil after application of sewage sludge. However, CZYŻYK, KOZDRAŚ (2004) confirmed loss of soluble nickel in soil after application of composts prepared from sewage sludge.

The content of microelements as well as Pb and Cd in soil after two years of the experiment testing application of manure, sewage sludge, compost prepared from sewage sludge and NPK did not exceed the values of permissible concentrations given in the Ordinance of the Minister of Environment of 9.09.2002 on quality standards of soil and quality standards of ground (Journal of Law, 2002, no. 165 item 1359).

CONCLUSIONS

1. After fertilization with manure, sewage sludge and compost prepared from sewage sludge, the content of total content and forms soluble in 1 $mol \cdot dm^{-3}$ HCl of microelements as well as Pb and Cd under direct and successive impact increased in comparison with the initial content.

2. Fertilizing with organic fertilizers caused first degree contamination of soil with nickel and cadmium (exceeding their natural content in soil).

3. On the basis of the mean share of forms soluble in 1 mol $HCl \cdot dm^{-3}$ in the total content in soils taken from objects fertilized with organic fertilizers, the elements could be ranked as follows: Cu > Pb > Ni > Cd > Mn > Zn in the first year and Cd > Pb > Cu>Mn > Zn > Ni in the second year of the research..

REFERENCES

CZYŻYK F., KOZDRAŚ M. 2004. *Zawartość mikroelementów w glebie lekkiej nawożonej kompostami z wiejskich osadów ściekowych [Content of micronutrients in light soil fertilized with rural sewage sludge composts].* Zesz. Prob. Post. Nauk Rol., 502: 737-744. [in Polish]

IŻEWSKA A., KRZYWY E., WOŁOSZYK CZ., BALCER K. 2006. *Zawartość metali ciężkich w glebie lekkiej w trzecim roku po zastosowaniu osadu ściekowego i kompostów wyprodukowanych z osadu ściekowego [Content of heavy ,etals in light soil three years after application of sewage sludge and sewage sludge composts].* Zesz. Prob. Post. Nauk Rol., 512: 173-181. [in Polish]

JAKUBUS M. 2006. *Wpływ wieloletniego stosowania osadu ściekowego na zmiany wybranych właściwości chemicznych gleby [Effect of long-term application of sewage sludge on modification of some chemical properties of soil].* Zesz. Prob. Post. Nauk Rol., 512: 209--219. [in Polish]

JAMALI M.K., KAZI T.G., ARAIN M.B., AFRIDI H.I., JALBANI N., KANDHRO G. A., SHAH A., Q., BAIG J. 2008. *Heavy metal accumulation in different varieties of wheat (Triticum aestivim L.)grown in soil amended with domestic sewage sludge.* J. Hazard. Mater. Doi 10.1016/ j.hazmat.2008.09.056.

Rozporządzenie Ministra Środowiska 2002. z dnia 01.08. 2002 roku *w sprawie komunalnych osadów ściekowych* (Dz U z 2002 r. nr 134 poz. 1140). Ordinance of Minister of Environment of 1st August 2002 on communal sewage sludge (Journal of Law, 2002, no 134, item 1140). [in Polish]

Rozporządzenie Ministra Środowiska 2002. z dnia 09.09.2002 roku *w sprawie standardów jakości gleby oraz standardów jakości ziemi* (DzU z 2002 r. nr 165 poz. 1359). Ordinance of the Minister of Environment of 9th September 2002 on soil and ground quality standards (Journal of Law, 2002, no 165, item 1359). [in Polish]

J. Elementol. 2009, 14(3): 467–476

THE ROLE OF STORAGE RESERVOIRS IN REDUCING CALCIUM MIGRATION FROM AGRICULTURAL CATCHMENTS*

Józef Koc, Marcin Duda

Chair of Land Improvement and Environmental Management
University of Warmia and Mazury in Olsztyn

Abstract

This study was conducted on a storage reservoir situated in a valley, in the lower course of the Sząbruk stream in north-eastern Poland, the Olsztyn Lakeland mesoregion. The catchment area of the Sząbruk stream consists of an agricultural and an afforested part. A storage reservoir is found in the lower part of the Sząbruk stream valley. The reservoir was built 25 years ago. It is enclosed by a dike and equipped with an outlet box. Outflows from the reservoir pass through the terminal segment of the Sząbruk stream to Lake Wulpińskie.

The results of the experiment indicate that the calcium content of water evacuated from the catchment was determined by the type and intensity of catchment use, ranging from 22.3 mg $Ca \cdot dm^{-3}$ to 178 mg $Ca \cdot dm^{-3}$. The highest calcium concentrations, 113 mg $Ca \cdot dm^{-3}$ on average, were noted in the agricultural catchment connected to a drainage network; lower levels, 78.7 mg $Ca \cdot dm^{-3}$, were found in farming areas drained via ditches, while the lowest Ca content in water, 38.7 mg $Ca \cdot dm^{-3}$ on average, was determined in outflows from afforested catchments. Calcium concentrations were lower during the growing season in all studied catchment types. The calcium load discharged from the catchment dependnded on the catchment management. The highest calcium loss per area unit was observed in the drained agricultural catchment (76.6 kg $Ca \cdot ha^{-1} \cdot year^{-1}$), followed by the catchment drained via ditches (56.3 $Ca \cdot ha^{-1} \cdot year^{-1}$) and the afforested catchment (31.8 $Ca \cdot ha^{-1} \cdot year^{-1}$). Despite the inflow of calcium-rich drainage water, calcium concentrations decreased by 11%, from 56.8 mg $Ca \cdot dm^{-3}$ to 50.3 mg $Ca \cdot dm^{-3}$, after the stream's waters passed through the storage reservoir. An increase in Ca levels was noted in the girdling ditch. The flow of water through the ditch minimizes sedimentation, and higher quantities of Ca were supplied with drainage water. The reservoir accumulated 242.4 kg Ca per ha in the course of one year, mostly in the growing season (83%). The above indicates high involvement of biological processes. The reservoir fulfilled the role of a barrier inhibiting calcium loss from the catchment.

prof. dr hab. Józef Koc, Chair of Land Reclamation and Management, University of Warmia and Mazury in Olsztyn, pl. Łódzki 2, 10-719 Olsztyn, phone: 089 523 36 48, e-mail: katemel@uwm.edu.pl

*Supported by the European Union within the European Social Fund.

Key words: storage reservoir, agricultural catchment, calcium.

ZNACZENIE ZBIORNIKA RETENCYJNEGO W OGRANICZENIU MIGRACJI WAPNIA ZE ZLEWNI ROLNICZEJ

Abstrakt

Badania prowadzono na zbiorniku retencyjnym położonym w dolinie końcowego biegu strugi Sząbruk położonej w północno-wschodniej Polsce, w mezoregionie Pojezierza Olsztyńskiego. Zlewnia strugi Sząbruk składa się z części leśnej i rolniczej. W dolnej części doliny strugi Sząbruk jest położony zbiornik retencyjny wykonany przed 25 laty, zamknięty groblą i mnichem. Odpływy ze zbiornika kierowane są końcowym odcinkiem strugi Sząbruk do Jeziora Wulpińskiego.

W wyniku badań stwierdzono, że stężenie wapnia w wodzie odpływającej ze zlewni zależało od sposobu i intensyfikacji użytkowania i mieściło się w granicach od 22,3 mg $Ca \cdot dm^{-3}$ do 178 mg $Ca \cdot dm^{-3}$. Najwyższe stężenie, średnio 113 mg $Ca \cdot dm^{-3}$, wystąpiło w wodach zlewni rolniczych odwadnianych siecią drenarską, niższe, średnio 78,7 mg $Ca \cdot dm^{-3}$, w wodach z terenów rolniczych odwadnianych rowami, a najniższe, średnio 39,7 mg $Ca \cdot dm^{-3}$, w wodach ze zlewni leśnej. We wszystkich zlewniach cząstkowych mniejsze stężenia wapnia, stwierdzono w okresie wegetacyjnym niż poza nim. Ładunek wapnia odprowadzany z obszaru zlewni był uzależniony od sposobu jej zagospodarowania. Największy odpływ wapnia z jednostki powierzchni stwierdzono w zlewni rolniczej zdrenowanej (76,6 kg $Ca \cdot ha^{-1} \cdot rok^{-1}$), mniejszy ze zlewni odwadnianej rowami (56,3 kg $Ca \cdot ha^{-1} \cdot rok^{-1}$) i najmniejszy ze zlewni leśnej (31,8 kg $Ca \cdot ha^{-1} \cdot rok^{-1}$). W wyniku przepływu wody przez zbiornik retencyjny następowało zmniejszenie w niej stężenia wapnia o 11%, z 56,8 mg $Ca \cdot dm^{-3}$ do 50,3 mg $Ca \cdot dm^{-3}$, pomimo zasilania zasobnymi w wapń wodami drenarskimi. W wodach przepływających rowem opaskowym stwierdzono wzrost stężeń Ca, gdyż w czasie przepływu rowem procesy sedymentacji są dużo mniejsze, a o wzroście stężeń zadecydowało zasilanie wodami drenarskimi. W ciągu roku w misie zbiornika zostało zakumulowane 242,4 kg Ca na 1 ha jego powierzchni, z czego większość (83%) w okresie wegetacyjnym. Świadczy to o istotnym udziale procesów biologicznych. Zbiornik retencyjny pełnił funkcję bariery zatrzymującej odpływ związków wapnia ze zlewni.

Słowa kluczowe: zbiornik retencyjny, zlewnia rolnicza, wapń.

INTRODUCTION

Calcium ions are the predominant ions in water migrating from agricultural catchment areas. For farming needs, the calcium content of soil has to be regularly supplemented through the use of calcium fertilizers. Surface waters contain high levels of calcium due to the leaching of this element from soil, which is a natural and unavoidable process. It results from the open circulation of substances in agricultural ecosystems and their environmental dispersion at every level of the trophic and geochemical cycles. In addition to intensive farming practices, calcium leaching is also supported by acid precipitation, which leads to soil acidification (Bowszys et al. 2005). The problem of calcium concentrations in water should be viewed from a different perspective than typical contamination or eutrophication. Calcium significantly affects the physical and chemical properties of water, including its pH (environmental alkalinization), carbonate (carbonate precipitation), sulfate and phosphate concentrations, as well as the coagulation of colloids. As a nutrient, calcium also has a profound effect on hydrobiological processes. Calcium content in water from agricultural catchment areas has been investigated by few researchers, although calcium concentrations contribute to biological, physical and chemical processes that determine the characteristic properties of water and bottom sediments in water bodies. Calcium levels in water are determined mainly by the volume of calcium load evacuated from the catchment area as well as fixation and sedimentation processes. Calcium is precipitated at the bottom of water bodies in the form of carbonates and organic residues as calciferous gyttia deposits (FÖLLMI 1989, KRAJEWSKI 1984). By fixing other elements, including heavy metal colloids, precipitated calcium compounds retain various pollutants.

The objective of this study was to investigate the role of storage reservoirs as barriers reducing the pollution of water evacuated from agricultural catchments, their effect on the migration of calcium compounds from agricultural catchments to surface waters, and their contribution to changes in calcium concentrations and accumulation in the environment.

MATERIALS AND METHODS

The role of a storage reservoir in calcium migration from an agricultural catchment area was analyzed during the hydrological years 2005/2006 and 2006/2007. The investigated storage reservoir is situated in a valley, in the lower course of the Sząbruk stream in north-eastern Poland, in the Masurian Lakeland macroregion and in the Olsztyn Lakeland mesoregion, 10 km south-west of Olsztyn. The catchment area of the Sząbruk stream consists of an agricultural and an afforested part. The afforested part of an area of 4.4 km^2 occupies 33% of the total catchment area of 13.2 km^2 (Figure 1). The storage reservoir of an area of 24.8 ha and a maximum depth of 1.5 m is found in the lower part of the Sząbruk stream valley. It is enclosed by a dike and equipped with an outlet box. The reservoir was built in the 1980s as a fish farming pond, but currently is not used for production purposes. In the western part, the reservoir is enclosed by a girdling ditch which regulates water flow by evacuating excess water to the Sząbruk stream. Outflows from the reservoir pass through the terminal segment of the Sząbruk stream to Lake Wulpińskie. The experiment covered the Sząbruk stream and the inflows and outflows of the storage reservoir (Figure 1).

Water flow rates in the Sząbruk stream were measured below the afforested catchment, at the reservoir inflow (outflow from the agricultural catch-

Fig. 1. Map of Sząbruk stream catchment area: 1 – Sząbruk stream below the afforested catchment, 2 – Sząbruk stream above the storage reservoir, 3 – inflow from the drained catchment to the storage reservoir, 4 – inflow from the drained catchment to the girdling ditch, 5 – outflow from the storage reservoir, 6 – outflow from the girdling ditch, 7 – inflow to Lake Wulpińskie

ment, inflow to the storage reservoir and to the girdling ditch), at the drainage outflow to the girdling ditch, at the drainage outflow to the storage reservoir, at the outflow from the reservoir, at the outflow from the girdling ditch and at the inflow to Lake Wulpińskie. Flow measurements were performed weekly with the use of a VALEPORT electromagnetic flow meter. The volumetric method was applied at low flow levels (below 2 $dm^3 \cdot s^{-1}$). Water samples for physical and chemical analyses were collected every two months at the flow measurement points, and Ca^{2+} levels were determined by atomic absorption spectrophotometry in line with the generally observed standards (HERMANOWICZ et al. 1999).

The calcium load evacuated from the catchment, supplied to and discharged from the reservoir, was calculated by summing up the product of average monthly flows and the corresponding calcium concentrations.

RESULTS AND DISCUSSION

The results of the study showed significant variations in calcium concentrations in different segments of the Sząbruk stream, in the inflows and outflows from the storage reservoir and the girdling ditch, and in the inflows to Lake Wulpińskie, subject to the type of catchment area (Table 1). The lowest calcium levels were noted in the Sząbruk stream below the afforested catchment, which is attributable to the continued leaching of calcium from the afforested catchment and insufficient calcium supplementation from precipitation and weathering. Higher calcium levels were determined in stream inflows from the agricultural catchment, at 78.7 mg $Ca \cdot dm^{-3}$ on average. The above increased the average Ca content of stream outflows from the combined afforested and agricultural catchments to 56.8 mg $Ca \cdot dm^{-3}$. High calcium concentrations, at 113 mg $Ca \cdot dm^{-3}$ on average, were noted in outflows from drained catchments. Higher calcium content of outflows from arable land could be attributed to the use of mineral and organic fertilizers (SAPEK 1996). Fertilizer components participate in exchange processes in the soil, and excess nutrients not absorbed by plants are transferred deeper into the soil and ground waters. The movement of calcium from the soil solution to ground and drainage waters is the main cause of soil depletion. Adequate calcium levels have to be maintained to ensure the soil's optimal properties. Calcium concentrations decreased as a result of water flow through the storage reservoir – by 11% on the annual basis and by 24% in the growing period. The observed calcium content of outflows from the storage reservoir was similar to calcium concentrations in outflows from small, mid-field ponds (54.7 mg $Ca \cdot dm^{-3}$ and 55.4 mg $Ca \cdot dm^{-3}$ on average respectively) on brown and black soils of the Masurian Lakeland (CYMES, SZYMCZYK 2005), and to calcium levels in the outflows from Lake Ardung (60.5 mg $Ca \cdot dm^{-3}$) noted

Table 1

Average calcium concentrations at the investigated sites (n=12)

Specification	Ca ($mg \cdot dm^{-3}$)					
	hydrological years 2005/2006 and 2006/2007		non-growing period		growing period	
	average	range	average	range	average	range
Sząbruk stream below the afforested catchment	39.7	22.3-49.2	36.5	22.3-49.2	42.8	22.3-49.2
Sząbruk stream above the storage reservoir	56.8	43.4-72.4	56.1	43.4-72.4	57.5	43.4-72.4
Inflows from the drained catchment to the storage reservoir	112.3	81.0-166.0	120.8	81.0-166.0	102.2	81.0-166.0
Inflow from the drained catchment to the girdling ditch	114.5	86.8-178.0	112.1	86.8-178.0	117.0	86.8-178.0
Outflows from the storage reservoir	50.3	34.8-67.5	57.0	34.8-67.5	43.6	34.8-67.5
Outflows from the girdling ditch	88.6	65.2-130.0	90.9	65.2-130.0	86.3	65.2-130.0
Inflows to Lake Wulpińskie	75.0	62.8-126.0	77.3	62.8-126.0	72.3	62.8-126.0

in a study carried out in the Olsztyn Lakeland (Koc et al. 2006). A comparison of Ca concentrations in the Sząbruk stream after passage through the storage reservoir and the girdling ditch showed a greater decrease in the calcium content of reservoir water than of stream water. Once the outflows from the storage reservoir and the girdling ditch were mixed, the average calcium load supplied to Lake Wulpińskie reached 75.0 mg $Ca \cdot dm^{-3}$.

An annual calcium balance was developed for different parts of the Sząbruk stream's catchment area (Table 2). The results of the study indicate that along a section stretching from the stream's source to a point situated above the storage reservoir, Sząbruk's waters carried 34.8 t $Ca \cdot year^{-1}$. The outflows from the afforested catchment carried 13.7 t $Ca \cdot year^{-1}$. The above findings supported the conclusion that the Sząbruk stream collected 21.1 t $Ca \cdot year^{-1}$ during its passage through the agricultural catchment. Annual calcium outflows per hectare of the catchment area increased after the stream's waters passed from woodland areas to agricultural areas. The calcium loss from afforested areas reached 31.8 kg $Ca \cdot year^{-1}$, and it was higher than the amount of calcium evacuated from the catchment above the storage reservoir (36.4 kg $Ca \cdot ha^{-1} \cdot year^{-1}$). The average Ca outflow from arable land drained via a ditch network was 49.2 kg $Ca \cdot ha^{-1} \cdot year^{-1}$. The calcium loss from drained catchments reached 76.0 kg $Ca \cdot ha^{-1} \cdot year^{-1}$.

Table 2

Calcium load at various points of the catchment

Specification	Total Ca load (kg·year^{-1})			Ca load (kg·ha^{-1}·year^{-1})
	hydrological years 2005/2006 and 2006/2007	non-growing period	growing period	hydrological years 2005/2006 and 2006/2007
Sząbruk stream below the afforested catchment	13 657	5022	8634	31.8
Sząbruk stream above the storage reservoir	34 762	137 230	21 032	36.4
Inflows from the Sząbruk stream to the storage reservoir	23 418	9183	14 236	-
Inflows from the Sząbruk stream to the girdling ditch	11 433	4637	6796	-
Inflows from the drained catchment to the reservoir (agricultural catchment)	2719	1163	1556	75.5
Inflows from the drained catchment to the girdling ditch (agricultural catchment)	5483	1523	3960	76.6
Outflows from the storage reservoir	20 124	9330	10 794	-
Outflows from the girdling ditch	19 470	9027	10 444	-
Inflows to Lake Wulpińskie	39 595	18 357	21 238	38.8

The Sząbruk stream was divided above the storage reservoir, as a result of which part of its waters were fed directly to the reservoir, while the remaining flow was evacuated via the girdling ditch. According to the annual balance, the storage reservoir accumulated 31.8 kg Ca·ha^{-1}·year^{-1} (Table 3). A decrease in the calcium load could be attributed to the formation of insoluble or sparingly soluble calcium compounds as well as to sedimentation (carbonates, sulfates and phosphates) (Föllmi 1989, Krajewski 1984). Calcium was accumulated mainly in the growing period (83% of annual accumulation) when the calcium load in water flowing through the storage reservoir was clearly lower. The reservoir accumulated 1.014 kg Ca·ha^{-1}·year^{-1}. The above suggests that calcium binds with the phosphate form released during the decomposition of fresh organic matter and deposits in the warm season of the year, and that it forms insoluble calcium carbonate that binds with hydrated excess carbon dioxide. Calcium could also be

Table 3

Calcium balance in the inflows from the Sząbruk stream catchment to the lake

Specification		Ca load ($kg \cdot year^{-1}$)			
		stream and girdling ditch	drainage network	inflow to Lake Wulpińskie	difference (Ca reduction in the reservoir)
Hydrological year	excluding the reservoir	42 889	2719	45 608	6012
	including the reservoir	42 889	2719	39 596	
Non-growing season	excluding the reservoir	18 210	1163	19 373	1014
	including the reservoir	18 210	1163	18 359	
Growing season	excluding the reservoir	24 679	1556	26 235	4998

absorbed by the reservoir's vegetation in amounts reaching 16.6 kg $Ca \cdot ha^{-1} \cdot year^{-1}$ (Koc, Szyperek 2003). On average, the reservoir accumulated 242.9 kg Ca per ha, including 201.5 kg (83%) in the growing season and only 40.9 kg (17%) in the winter. A much higher drop in calcium concentrations and loads in the reservoir over the growing period is indicative of intense biological processes during which the investigated element is fixed and accumulated in bottom deposits. The accumulation of calcium compounds in bottom deposits was also noted during a previous study of the storage reservoir in Sząbruk (Koc, Skonieczek 2006). The authors found that the calcium content of a dry deposit sample collected in the terminal segment of the reservoir was 2.5% at a depth of up to 10 cm and 9.3% at a depth of 10 to 20 cm. Different results were reported in respect of calcium concentrations and loads in the girdling ditch parallel to the analyzed reservoir. Ditch water was additionally supplied with calcium by inflows from the drainage network (5.3 t $Ca \cdot year^{-1}$) and by percolation. For this reason, calcium accumulation levels were measured in both the reservoir and the girdling ditch. A total of 45.6 t $Ca \cdot year^{-1}$ was discharged to the catchment, of which 39.6 t $Ca \cdot year^{-1}$ was evacuated from the girdling ditch and the storage reservoir to Lake Wulpińskie. The average calcium load discharged from the entire catchment area to the lake decreased by 13%, from 44.7 kg $Ca \cdot ha^{-1} \cdot year^{-1}$ to 38.8 kg $Ca \cdot ha^{-1} \cdot year^{-1}$.

CONCLUSIONS

1. The calcium content of water evacuated from the catchment was determined by the type and intensity of catchment use. The highest calcium outflows per area unit were noted in the agricultural catchment connected to a drainage network, lower concentrations were found in the catchment drained via ditches, while the lowest Ca content of water was determined in the outflows from afforested catchments. Calcium concentrations were lower during the growing season in all studied catchment types.

2. Calcium concentrations decreased by 11%, from 56.8 mg $Ca \cdot dm^{-3}$ to 50.3 mg $Ca \cdot dm^{-3}$, following the passage of the stream's waters through the storage reservoir. An increase in Ca levels was noted in the water flowing through the girdling ditch.

3. The reservoir accumulated 242.4 kg Ca per ha in the course of one year, mostly in the growing season (83%). The above indicates high involvement of biological processes. The reservoir fulfilled the role of a barrier inhibiting calcium loss from the catchment.

REFERENCES

Borowiec S., Pieńkowski P. 1993. *Oddziaływanie geochemiczne i antropogeniczne na chemizm wód obszarów rolniczo-leśnych Pomorza Zachodniego [The geochemical and anthropogenic impact of agricultural and afforested areas in Western Pomerania].* Geoekosystem obszarów nizinnych, Ossolineum, Wrocław, 19-23. [in Polish]

Bowszys T., Ruszkowski K., Bobrzecka D., Wierzbowska J. 2005. *Wpływ wapnowania i nawozów wieloskładnikowych na odczyn i zawartość niektórych metali ciężkich w glebie [Effect of liming and multicomponent fertilizers on reaction and content of some heavy metals in soil].* J. Elementol., 10(1): 33-40. [in Polish]

Cymes I., Szymczyk S. 2005. *Wpływ użytkowania i typu gleby na zawartość sodu, wapnia i magnezu w wodzie śródpolnych małych zbiorników [Effect of land use and type on the content of sodium, calcium and magnesium in water of small mid-field water bodies].* J. Elementol., 10(2): 249-257. [in Polish]

Föllmi K. B. 1989. *Evolution of the Mid-Cretaceous Triad. Platform carbonates, Phosphatic sediments, and pelagic Carbonates along the Northern Tethys Margin.* Lec. Notes Earth Sci., 23: 1-153.

Haycock N.E., Pinay G., Burt T.P., Goulding K-W.T. 1996. *Buffer zones: Currcnt concems and future directions.* In*: Buffer zones: Quest Environmcntal. Hertfordshire,* UK, 305-312.

Hermanowicz W., Dojlido J., Dożańska W., Koziorowski B., Zerbe J. 1999. *Fizyczno-chemiczne badanie wody i ścieków [Physicochemical examination of water and sewage].* Arkady, Warszawa, ss. 556. [in Polish}

Koc J., Koc-Jurczuk J., Sidoruk M. 2006. *Effect of renaturization of a lake on the quality of its waters: a case study of Ardung Lake in the capture basin of the Łyna river.* Pol. J. Environ. Stud., 15(5D): 586-591.

Koc J., Skonieczek P. 2006. *Concentration of trace elements in bottom sediments of a stream, a pond and a lake loaded with runoffs from an agricultural catchment.* Pol. J. Environ. Stud., 15 (2a): 356-363.

KOC J., SZYPEREK U. 2003. *Znaczenie szaty roślinnej zlewni i przybrzeżnych pasów roślinności w ochronie śródpolnych oczek wodnych [Role of plant cover in a catchment and shoreline plant belts in protecting mid-field water ponds].* Wiad. Mel. Łąk., 2: 73-75. [in Polish]

KRAJEWSKI K.P, 1984. *Early diagenetic phosphate cements in the Albian condensed glauconite limestone of the Tatra Mountains, Western Carpathians*. Sedimentology, 31: 443–470.

SAPEK A. 1996. *Udział rolnictwa w zanieczyszczeniu wody składnikami nawozowymi [Contribution of agriculture in contaminating water with fertilizer components].* Zesz. Eduk., 1: 9-33, Wyd. IMUZ, Falenty. [in Polish]

SZYMCZYK S., CYMES I. 2005. *Wpływ ilości opadów i sposobu użytkowania terenu na odpływ sodu wapnia i magnezu siecią drenarską z gleb ciężkich [Effect of precipitation volumes and land use type on outflow of calcium and magnesium through a drainage network from heavy soils].* J. Elementol., 10(2): 395-401. [in Polish]

J. Elementol. 2009, 14(3): 477–487

EFFECT OF HERBICIDES ON THE COURSE OF AMMONIFICATION IN SOIL

Jan Kucharski, Małgorzata Baćmaga, Jadwiga Wyszkowska

Chair of Microbiology
University of Warmia and Mazury in Olsztyn

Abstract

A laboratory experiment has been performed to determine the effect of soil pollution with the herbicides: Harpun 500 SC, Faworyt 300 SL, Akord 180 OF and Mocarz 75 WG on the course of ammonification. The soil material for the experiment consisted of loamy sand of pH 6.5.

The experiment comprised five replications. Soil samples in particular objects were polluted with the herbicides at rates corresponding to the dose recommended by the manufacturer: 0 – control, 1 – a dose recommended by the producer, and the rates 50-, 100-, 150- and 200-fold higher than the recommended dose. Next, nitrogen was introduced to soil in the form of L-aspartic acid, DL-alanine, L-arginine and urea in the amounts of 0 and 300 mg N kg^{-1} soil. Having been thoroughly mixed with the additional substances, the soil was brought to moisture equal 60% capillary water capacity and incubated for 12, 24, 36 and 48 hours at 25°C.

The study has demonstrated that the course of ammonification depended on the type and rate of a herbicide added to soil, type of an organic compound undergoing ammonification and duration of the trial. L-arginine was ammonified most rapidly, while ammonification of L-aspartic acid lasted the longest. Among the tested herbicides, the strongest inhibitory effect on ammonification process was produced by Mocarz 75 WG, which continued to exert negative influence on mineralisation of organic substances for 36 hours. The other preparations did not have such a considerable effect on the quantities of ammonified nitrogen.

Key words: soil pollution, herbicides, ammonification, amino acids.

prof. dr hab. Jan Kucharski, Chair of Microbiology, University of Warmia and Mazury, pl. Łódzki 3, 10-727 Olsztyn, Poland, e-mail: jan.kucharski@uwm.edu.pl

WPŁYW HERBICYDÓW NA PRZEBIEG PROCESU AMONIFIKACJI W GLEBIE

Abstrakt

W doświadczeniu laboratoryjnym określono wpływ zanieczyszczenia gleby herbicydami: Harpun 500 SC, Faworyt 300 SL, Akord 180 OF i Mocarz 75 WG na przebieg procesu amonifikacji. Materiałem glebowym użytym w badaniach był piasek gliniasty o pH 6,5.

Doświadczenie przeprowadzono w pięciu powtórzeniach. Próbki glebowe w odpowiednich obiektach zanieczyszczono herbicydami w dawkach wyrażonych jako wielokrotność dawki zalecanej przez producenta: 0 – kontrola, 1 – dawka zalecana przez producenta, dawki 50-, 100-, 150- i 200-krotnie większa od zalecanej przez producenta. Następnie do gleby wprowadzono azot w postaci kwasu L-asparaginowego, DL-alaniny, L-argininy i mocznika w ilości 0 i 300 mg $N \cdot kg^{-1}$ gleby. Po dokładnym wymieszaniu glebę doprowadzono do wilgotności 60% kapilarnej pojemności wodnej i w takim stanie inkubowano próbki glebowe przez 12, 24, 36 i 48 h w temp. 25°C.

Wykazano, że przebieg procesu amonifikacji determinowany był rodzajem i dawką herbicydu, rodzajem amonifikowanego związku organicznego i czasem trwania doświadczenia. Najszybciej amonifikacji ulegała L-arginina, natomiast najwolniej kwas L-asparaginowy. Największy inhibicyjny wpływ na proces amonifikacji spośród wszystkich testowanych środków chwastobójczych wywierał Mocarz 75 WG. Jego negatywne oddziaływanie na mineralizację związków organicznych utrzymywało się przez 36 h. Pozostałe preparaty nie wpływały tak jednoznacznie na ilość zamonifikowanego azotu.

Słowa kluczowe: zanieczyszczenie gleby, herbicydy, amonifikacja, aminokwasy.

INTRODUCTION

In farming, herbicides are used to control weeds and improve the quality of agricultural produce. However, these preparations can also limit the uptake of nitrogen by crops and microorganisms, as well as modify mineralisation of organic compounds present in soil (Kara et al. 2004). Transformation of these compounds could serve as a principal index when evaluating changes triggered by various xenobiotics, including plant protection chemicals (Kostov, Cleemput 2001, Pietril, Brookes 2008). The process of mineralisation of organic nitrogen forms is highly sensitive to soil pollution with biocides, which can therefore partly or completely contribute to the inhibition of ammonification. The effect produced by these substances depends mainly on applied doses and biodegradability of a given chemical (Martens, Bremner 1994).

Mineralisation of organic compounds is one of the basic processes which condition the amounts of nitrogen easily available to plants and soil microorganisms (Bonde et al. 2001, Castaldi et al. 2009, Kucharski et al. 2004, Qian, Cai 2007, Wyszkowska et al. 2006). The rate of this process is dependent on the soil reaction (pH), temperature, soil moisture, content of organic substances and soil biological activity (Bottomley et al. 2004, Brierley et al. 2001, Krave et al. 2002, Kucharski 1997, Sierra 2006, Zhu, Carreiro 1999).

Nitrogen occurring in soil in the organic form is broken down to ammonia or NH_4^+ ions (Barabasz et al. 2002). The decomposition of organic forms of nitrogen such as proteins, amides and amino acids is run by various microorganisms (Barabasz et al. 2002, Kostov, Cleemput 2001, Lina, Brookes 1999), which are all highly sensitive to effects produced by plant protection chemicals (Kucharski, Wyszkowska 2008). During mineralisation, organic compounds can be decomposed by bacteria and fungi (Barabasz et al. 2002, Lina, Brookes 1999).

The objective of the present study has been to determine the effect of soil pollution with the newest generation herbicides, such as Harpun 500 SL, Faworyt 300 SL, Akord 180 OF and Mocarz 75 WG, on the course of ammonification.

MATERIAL AND METHODS

A laboratory experiment was established on soil material collected from the arable-humus soil horizon. Under natural conditions, this was proper brown soil classified as loamy sand according to its granulometric composition. The soil properties are presented in Table 1.

For the purpose of the experiment, 100 cm^3 beakers were filled with 50 g air dried soil mass, sieved through a 1 mm mesh sieve. All the experimental objects had five replicates. Soil samples were polluted with the herbicides Harpun 500 SL, Faworyt 300 SL, Akord 180 OF and Mocarz 75 WG (factor I) in rates corresponding to multiples of the dose recommended by the manufacturer: 0 – control, 1 – recommended dose, 50- 100-, 150- and 200-fold higher than the recommended dose (factor II).

The active substances in the herbicides were: isoproturon – a compound belonging to the class of urea derivatives (500 g dm^{-3}) in Harpun 500 SC, chlopyralid – a compound in the form of monoethanolamine salt (300 g dm^{-3}) in Faworyt 300 SL, fenmedifan, desmedifan – compounds belonging to phenyl carbaminians (each in the amount of 60 g dm^{-3}) and ethofumesate – a ompound belonging to benzofuran derivatives in Akord 180 OF and tritosulfuron – a compound belonging to sulfonylurea derivatives (25%) and dicamba – a benzoic acid derivative (50%) in Mocarz 75 WG.

Once the herbicides had been applied, nitrogen was introduced to soil in the form of the following organic compounds (factor III): L-aspartic acid, DL-alanine, L-arginine and urea, in the amounts of 0 and 300 mg N kg^{-1} soil (factor IV). Whole portions of soil were thoroughly mixed and brought to the soil moisture level corresponding to 60% capillary water capacity. Soil samples were incubated in a thermostat at temperature of 25°C for 12, 24, 36 and 48 hours (factor V).

The concentration of mineral nitrogen forms was determined using an Orion 720A ionometer. Aqueous solution of 0.03 mol acetic acid was used for extraction of N-NH_4^+ and N-NO_3^-. The extractor to soil ratio was 10 : 1. Levels of ions were measured using ion-selective electrodes. NH_4^+ ions were determined with an ammonium electrode (type 9300BNWP) whereas NO_3^- ions were determined with a nitrate electrode (type 9300BN). The intensity of ammonification was determined at appropriate hours according to the concentrations of N-NH_4^+ and N-NO_3^- ions from the formula (WYSZKOWSKA 2002):

$$N = \left(\frac{N_1 - N_0}{D} \right) \cdot 100\%$$

where:

N – % ammonified nitrogen;

N_1 – content of N-NH_4^+ and N-NO_3^- in the analysed object, in mg;

N_0 – content of N-NH_4^+ and N-NO_3^- in the control object, in mg;

D – rate of nitrogen N kg^{-1} soil.

The results were verified statistically using Duncan's multiple range test. All statistical computations were run with the software Statistica (Statsoft, Inc. 2006).

Table 1

Some physicochemical properties of soil used in the experiment

Soil type	Granulometric compostion soil grains diameter (mm)			pH_{KCl}	Hh	S	Corg ($g\ kg^{-1}$)
	1 - 0.1	0.1 - 0.02	< 0.02		mmol(+) kg^{-1} soil		
ls	49	37	14	6.5	8.25	78.00	6.30

ls – loamy sand, Corg – organic carbon content, Hh – hydrolytic acidity,
S – sum of exchangeable base cations

RESULTS AND DISCUSSION

The rate of ammonifcation of L-aspartic acid, DL-alanine, L-arginine and urea in the control samples was varied (Tables 2-5). After 48 hrs of soil incubation, urea was nearly completely ammonified, compared to the successful ammonification of 87% L-arinine, 47% DL-alanine and 42% L-aspartic acid. The compounds which proved to be the most easily ammonified (L-arginine and urea) were ammonified at 26 and 25%, respectively, after 12 hours of incubation. Conversion of nitrogen organic compounds depended on the type of a herbicide and its dose, type of ammonified organic compound and duration of soil incubation (Tables 2-5).

Table 2

Effect of soil pollution with the herbicide Harpun 500 SC
on amount of ammonified nitrogen, in %

Rate of herbicide*	Time of incubation in hours			
	12	24	36	48
L-aspartic acid				
0	6.50 ± 0.17	20.37 ± 0.86	29.41 ± 2.38	41.71 ± 4.12
1	5.09 ± 1.45	22.42 ± 1.61	24.55 ± 0.77	47.33 ± 1.09
50	5.23 ± 1.67	34.47 ± 1.12	37.13 ± 0.99	51.30 ± 3.69
100	4.31 ± 1.16	32.99 ± 1.11	36.43 ± 0.69	37.30 ± 3.81
150	4.55 ± 1.01	23.17 ± 3.96	25.46 ± 1.66	30.31 ± 2.22
200	5.79 ± 0.99	18.24 ± 3.86	22.53 ± 1.34	24.69 ± 3.15
r	-0.27	-0.18	-0.32	-0.87
DL-alanine				
0	2.50 ± 0.36	8.10 ± 0.43	27.16 ± 2.18	47.18 ± 1.01
1	1.02 ± 0.74	10.37 ± 1.00	27.57 ± 0.59	56.46 ± 2.20
50	1.13 ± 0.43	10.73 ± 1.19	26.41 ± 2.05	63.37 ± 1.19
100	0.63 ± 0.41	13.46 ± 0.83	19.38 ± 2.72	65.92 ± 4.80
150	1.49 ± 0.67	12.07 ± 0.56	16.01 ± 1.44	70.78 ± 2.09
200	2.21 ± 0.53	11.29 ± 0.56	15.52 ± 1.15	65.41 ± 2.55
r	0.12	0.61	-0.96	0.79
L-arginine				
0	26.07 ± 1.69	36.16 ± 1.82	40.84 ± 2.48	87.18 ± 5.92
1	19.37 ± 1.16	42.85 ±3.42	43.31 ± 2.61	99.93 ± 1.85
50	17.57 ± 2.38	46.52 ± 4.46	46.60 ± 1.23	95.17 ± 0.78
100	17.97 ± 2.08	46.13 ± 0.86	48.90 ± 0.73	79.05 ± 5.56
150	18.23 ± 1.29	46.55 ± 1.48	51.94 ± 2.12	78.45 ± 4.58
200	18.77 ± 0.49	42.47 ± 0.61	51.85 ± 1.70	80.61 ± 3.14
r	-0.51	0.42	0.95	-0.76
Urea				
0	24.83 ± 1.14	56.29 ± 1.12	73.91 ± 1.14	98.31 ± 2.34
1	22.45 ± 0.90	54.58 ± 2.84	72.58 ± 0.99	61.38 ± 0.99
50	22.05 ± 2.47	55.12 ± 3.57	72.60 ± 2.85	61.50 ± 0.85
100	13.43 ± 2.92	53.93 ± 3.57	71.30 ± 0.87	58.46 ± 1.73
150	12.99 ± 1.51	54.02 ± 2.49	73.07 ± 2.78	58.52 ± 0.75
200	12.93 ± 0.96	53.80 ± 0.72	67.65 ± 1.75	56.20 ± 0.61
r	-0.92	-0.77	-0.74	-0.91
$LDS_{0.01**}$	a – 0.55, b – 0.50, c – 0.45, $a \cdot b$ – 1.23, $a \cdot c$ – 1.10, $b \cdot c$ – 1.01, $a \cdot b \cdot c$ – 2.46			

** LSD for: a – rate of herbicide, b – date of analysis, c – type of organic substance; r – correlation coefficient; *0 – control not polluted with herbicides, 1 – dose recommended by manufacturer; rates higher than recommended: 50-, 100-, 150- and 200-fold

Table 3

Effect of soil pollution with the herbicide Faworyt 300 SL
on amount of ammonified nitrogen, in %

Rate of herbicide*	Time of incubation in hours			
	12	24	36	48
L-aspartic acid				
0	6.50 ± 0.17	20.37 ± 0.86	29.41 ± 2.38	41.71 ± 4.12
1	6.11 ± 1.14	20.26 ± 0.08	30.58 ± 0.90	49.09 ± 1.00
50	4.45 ± 0.55	19.67 ± 0.29	41.12 ± 0.41	51.16 ± 1.89
100	4.84 ± 1.74	18.56 ± 0.37	44.80 ± 0.43	59.58 ± 0.85
150	4.04 ± 0.57	18.34 ± 0.43	51.66 ± 0.77	79.35 ± 2.18
200	4.02 ± 0.79	14.17 ± 0.17	63.57 ± 0.87	90.88 ± 1.06
r	-0.87	-0.91	0.99	0.97
DL-alanine				
0	2.50 ± 0.36	8.10 ± 0.43	27.16 ± 2.18	47.18 ± 1.01
1	5.53 ± 2.18	13.23 ± 1.43	23.52 ± 3.16	74.15 ± 1.49
50	6.72 ± 1.18	13.74 ± 1.26	22.25 ± 2.39	75.37 ± 2.30
100	9.15 ± 1.24	15.78 ± 2.59	21.04 ± 3.23	86.68 ± 4.03
150	9.42 ± 0.49	17.74 ± 2.86	16.37 ± 1.81	92.45 ± 4.63
200	10.66 ± 2.04	14.84 ± 1.71	16.02 ± 2.50	93.48 ± 2.92
r	0.92	0.71	- 0.94	0.84
L-arginine				
0	26.07 ± 1.69	36.16 ± 1.82	40.84 ± 2.48	87.18 ± 5.92
1	23.05 ± 0.85	37.42 ± 4.07	44.33 ± 4.14	88.84 ± 2.73
50	22.55 ± 1.01	46.49 ± 6.53	42.09 ± 1.10	87.12 ± 1.15
100	21.30 ± 1.86	52.89 ± 1.10	47.09 ± 1.87	94.90 ± 5.10
150	21.41 ± 0.88	53.52 ± 7.35	49.62 ± 2.45	89.77 ± 0.89
200	16.91 ± 0.35	54.34 ± 6.46	50.88 ± 3.93	85.49 ± 2.68
r	-0.89	0.92	0.92	-0.02
Urea				
0	24.83 ± 1.14	56.29 ± 1.12	73.91 ± 1.14	98.31 ± 2.34
1	29.05 ± 2.39	73.12 ± 3.52	75.32 ± 0.88	93.15 ± 1.43
50	30.91 ± 2.21	72.84 ± 3.42	71.46 ± 0.91	97.94 ± 2.37
100	20.90 ± 5.29	46.92 ± 2.23	68.02 ± 2.78	99.19 ± 2.17
150	16.29 ± 1.17	42.58 ± 1.56	67.89 ± 1.83	88.43 ± 3.23
200	15.66 ± 0.58	40.40 ± 1.22	62.36 ± 1.65	81.28 ± 2.71
r	- 0.86	-0.81	-0.97	-0.76
$LDS_{0.01**}$	*a* – 0.62, *b* – 0.57, *c* – 0.51, *a* · *b* – 1.40, a · *c* – 1.25, *b* · *c* – 1.14, *a* · *b* · *c* – 2.79			

* for designations cf. Table 2

Table 4

Effect of soil pollution with the herbicide Akord 180 OF on amount of ammonified nitrogen, in %

Rate of herbicide*	Time of incubation in hours			
	12	24	36	48
L-aspartic acid				
0	6.50 ± 0.17	20.37 ± 0.86	29.41 ± 2.38	41.71 ± 4.12
1	5.59 ± 0.44	21.60 ± 0.18	31.31 ± 3.55	41.14 ± 4.31
50	5.09 ± 0.43	21.14 ± 0.26	35.19 ± 4.28	43.66 ± 2.27
100	5.72 ± 0.42	21.04 ± 0.40	44.53 ± 2.14	44.77 ± 2.34
150	4.74 ± 0.72	22.35 ± 0.34	42.55 ± 2.18	44.81 ± 2.16
200	4.56 ± 0.25	22.24 ± 0.46	42.35 ± 3.35	44.87 ± 2.76
r	-0.80	0.75	0.87	0.89
DL-alanine				
0	2.50 ± 0.36	8.10 ± 0.43	27.16 ± 2.18	47.18 ± 1.01
1	2.37 ± 0.81	7.97 ± 0.84	26.43 ± 1.87	56.22 ± 3.36
50	2.48 ± 0.81	7.22 ± 0.27	26.12 ± 1.84	56.83 ± 1.74
100	1.04 ± 1.23	6.96 ± 0.39	24.50 ± 1.20	51.88 ± 3.19
150	1.23 ± 0.76	6.65 ± 0.24	23.91 ± 1.70	51.36 ± 3.71
200	1.35 ± 0.61	5.21 ± 0.38	22.09 ± 1.79	50.69 ± 1.63
r	-0.82	-0.96	-0.98	-0.21
L-arginine				
0	26.07 ± 1.69	36.16 ± 1.82	40.84 ± 2.48	87.18 ± 5.92
1	26.79 ± 3.21	41.53 ± 1.75	34.22 ± 2.81	95.32 ± 2.34
50	26.37 ± 1.67	43.36 ± 1.84	33.24 ± 6.00	97.17 ± 2.64
100	25.95 ± 1.51	46.79 ± 0.95	27.70 ± 2.50	97.60 ± 2.83
150	26.54 ± 0.51	43.89 ± 3.04	26.95 ± 4.03	98.27 ± 2.83
200	29.93 ± 0.81	39.66 ± 1.68	23.29 ± 2.68	99.79 ± 3.45
r	0.67	0.27	-0.93	0.75
Urea				
0	24.83 ± 1.14	56.29 ± 1.12	73.91 ± 1.14	98.31 ± 2.34
1	16.30 ± 1.06	60.38 ± 1.13	60.55 ± 3.72	95.70 ± 1.98
50	12.96 ± 0.67	59.95 ± 1.04	56.37 ± 2.23	93.46 ± 1.83
100	13.21 ± 0.70	61.52 ± 0.47	54.28 ± 3.86	91.30 ± 0.84
150	11.47 ± 0.92	80.04 ± 0.68	45.75 ± 2.65	97.09 ± 1.96
200	11.60 ± 0.57	79.96 ± 1.12	42.56 ± 2.87	97.48 ± 0.47
r	-0.73	0.91	-0.91	0.08
$LDS_{0.01**}$	*a* – n.s., *b* – 0.50, *c* – 0.45, *a* · *b* – 1.23, *a* · *c* – 1.10, *b* · *c* – 1.00, *a* · *b* · c – 2.46			

* for designations cf. Table 2

Table 5

Effect of soil pollution with the herbicide Mocarz 75 WG
on amount of ammonified nitrogen, in %

Rate of herbicide*	Time of incubation in hours			
	12	24	36	48
L-aspartic acid				
0	6.50 ± 0.17	20.37 ± 0.86	29.41 ± 2.38	41.71 ± 4.12
1	5.69 ± 0.32	15.82 ± 0.58	29.19 ± 0.79	28.53 ± 0.27
50	4.27 ± 0.56	12.98 ± 0.21	25.81 ± 0.98	22.82 ± 0.20
100	3.38 ± 0.41	12.25 ± 0.33	22.80 ± 1.44	17.59 ± 0.30
150	2.68 ± 0.58	11.80 ±0.26	21.83 ± 0.50	15.84 ± 0.63
200	3.08 ± 0.25	10.02 ± 0.27	21.32 ± 0.97	13.64 ± 0.19
r	-0.90	-0.85	-0.96	-0.86
DL-alanine				
0	2.50 ± 0.36	8.10 ± 0,43	27.16 ± 2.18	47.18 ± 1.01
1	2.99 ± 0.43	9.12 ± 0,38	23.71 ± 1.65	43.70 ± 1.83
50	2.99 ± 0.34	6.26 ± 0,57	21.23 ± 2.91	35.74 ± 2.33
100	2.84 ± 0.47	5.90 ± 0,47	21.23 ± 3.40	34.13 ± 0.87
150	2.63 ± 0.26	4.53 ± 0,42	17.09 ± 2.54	41.27 ± 1.38
200	2.76 ± 0.56	2.96 ± 0,25	15.74 ± 2.18	44.74 ± 0.43
r	-0.37	-0.97	-0.94	-0.10
L-arginine				
0	26.07 ± 1.69	36.16 ± 1.82	40.84 ± 2.48	87.18 ± 5.92
1	39.98 ± 1.12	35.48 ± 2.79	33.47 ± 1.34	86.00 ± 2.27
50	46.53 ± 1.22	19.95 ± 1.64	30.21 ± 2.67	86.09 ± 1.96
100	49.00 ± 1.61	16.71 ± 1.08	26.54 ± 1.99	85.62 ± 1.85
150	51.02 ± 1.09	12.48 ± 1.01	23.46 ± 1.60	82.52 ± 3.32
200	55.57 ± 0.29	9.44 ± 0.47	19.52 ± 0.84	80.59 ± 2.08
r	0.85	-0.94	-0.94	-0.93
Urea				
0	24.83 ± 1.14	56.29 ± 1.12	73.91 ± 1.14	98.31 ± 2.34
1	21.83 ± 1.41	48.39 ± 5.61	69.86 ± 1.61	98.42 ± 3.05
50	22.79 ± 1.72	45.58 ± 1.96	68.61 ± 1.12	98.73± 2.32
100	22.49 ± 1.77	42.26 ± 1.33	67.61 ± 3.10	96.57 ± 2.15
150	20.50 ± 1.36	42.77 ± 2.30	57.15 ± 2.15	95.57 ± 2.33
200	18.05 ± 1.44	37.41 ± 2.11	53.87 ± 2.18	97.43 ± 0.68
r	-0.85	-0.88	-0.95	-0.70
$LDS_{0.01^{**}}$	a – 0.45, b – 0.41, c – 0.37, $a \cdot b$ – 1.00, $a \cdot c$ – 0.90, $b \cdot c$ – 0.82, $a \cdot b \cdot c$ – 2.02			

* for designations cf. Table 2

The course of ammonification process was modified by the herbicide Harpun 500 SC (Table 2). It had a negative effect on the transformation of urea. For 36 hrs it intensified transformations of L-arginine and, when applied at the lowest doses (technological and 50-fold higher), it continued to do so for 48 hrs. However, it had a variable influence on the ammonification of L-alanine. After 24 hrs of incubation, the soil containing this herbicide and DL-alanine was determined to possess more ammonia nitrogen than after 36 hrs, although at the end of the incubation (48 hrs) the ammonification of this compound accelerated again. Excessive quantities of Harpun 500 SC (applied at the rates 100- and 200-fold higher than recommended) depressed the rate of the ammonification of L-aspartic acid.

The rate of the ammonification process as shaped by Faworyt 300 SL varied between the dates of analyses (Table 3). The transformations of L-asparic acid were initially inactivated by this herbicide, although after 36 and 48 hrs of incubation, Harpun was found to be stimulating the ammonification of this compound. The positive influence of this herbicide on decomposition of DL-alanine at 12 and 24 hrs disappeared at 36 h to reappear at 48 h. Faworyt 300 SL retarded the ammonification of L-arginine at 12 h, but accelerated it at 24 h and 36 h. When applied at the highest doses (over 100-fold higher than the recommended dose), this herbicide halted hydrolysis of urea for 36 hours, and when introduced to soil at the doses 150- and 200-fold higher than recommended, it had a negative effect on this process throughout the whole incubation period (48 h).

Another tested herbicide, Akrod 180 OF, had a weaker effect on ammonification (Table 4). It only had an inhibitory influence on the transformation of urea in soil and stimulated transformations of L-aspertic acid. When applied at the technological rate and 50-fold higher than recommended, it intensified decomposition of DL-alanine, while producing only a light effect on the ammonification of L-arginine. The rate of ammonification was changeable during the incubation. At 12 h it retarded the transformation of L-aspartic acid, DL-alanine and urea, but already at 24 h it stimulated the decomposition of all the organic compounds except DL-alanine. It continued to exert the negative influence on DL-alanine until 36 h of incubation. At that time it also negatively affected the transformations of L-arganine and urea, but had a positive effect on the ammonification of L-aspartic acid. After 48 hours, it reduced only the hydrolysis of urea, while stimulating the transformation of all the remaining organic compounds.

The herbicide Mocarz 75 WG also had a significant effect on ammonification (Table 5). In the soil containing the highest rate of this chemical, it was found out that ammonification was depressed by 51% for L-aspartic acid, 22% for DL-alanine, 18% for urea and 13% for L-arganine. This preparation inhibited transformations of all the tested organic compounds for 36 hours. After 48 hours, it ceased to have a negative influence on the ammonification of urea and had a much weaker adverse effect on decomposition of L-arginine. However, it continued to significantly inhibit the ammonification of L-aspartic acid and DL-alanine.

Owing to ammonification, nitrogen present in organic compounds is transformed to forms easily available to plants. Amino acids, which are a valuable source of organic nitrogen, can enhance the intensification of nitrogen ammonification (WYSZKOWSKA 2002). The literature (JONES et al. 2005, WYSZKOWSKA et al. 2006) suggests that mineralisation of amino acids in soil can occur very rapidly and be completed in the course of just a few hours. The authors' own studies have shown that introduction of herbicides to soil can retard the rate of ammonification. Nonetheless, urea and L-arginine were the two organic substances whose ammonification was the fastest.

Chemical pollutants entering soil destabilize ammonification, which has microbiological and biochemical consequences for soil environment (KUCHARSKI, WYSZKOWSKA 2008, WYSZKOWSKA et al. 2007).

The rate of ammonification is determined by several factors, but particularly by a type of organic compound, including amino acids. L-arganine, which belongs to alkaline amino acids, was most rapidly ammonified, whereas L-aspartic acid, which is an acidic amino acid, underwent ammonification most slowly. The chemical properties of L-aspartic acid may have led to acidification of soil and lower soil pH, which resulted in a slower rate of its mineralisation (MARTENS, BREMNER 1994, WYSZKOWSKA et al. 2006, WYSZKOWSKA ET al. 2007). Changing the conditions within soil environment, such as depressed soil reaction, is not indifferent to soil microorganisms, especially to ammonifying bacteria (BARABASZ et al. 2002).

CONCLUSIONS

1. All the tested herbicides had a significant effect on the course of ammonification.

2. The rate of ammonification depended on the type and dose of a herbicide, type of ammonified organic compound and time of incubation.

3. Among the tested organic compounds, i.e. L-aspartic acid, DL-alanine, L-arginine and urea, ammonification of urea and L-arginine was the fastest.

4. The strongest inhibitor of ammonification proved to be the herbicide Mocarz 75 WG, which for 36 hours retarded the transformation of all the tested organic nitrogen compounds, while the other preparations did not have such am unambiguous influence on ammonification.

REFERENCES

BARABASZ W, ALBIŃSKA D., JASKOWSKA M., LIPIEC J. 2002. *Biological effects of mineral nitrogen fertilization on soil microorganisms*. Pol. J. Environ. Stud., 11(3): 193- 198.

BONDE T. A., NIELSEN T. H., MILLER M., SORENSEN J. 2001. *Arginine ammonification assay as a rapid index of gross N mineralization in agricultural soils*. Biol. Fertil. Soils, 34: 179-184.

BOTTOMLEY P.J., TAYLOR A.E., BOYLE S.A., MCMAHON S.K., RICH J.J. CROMACK JR. K., MYROLD D.D. 2004. *Responses of nitrification and ammonia-oxidizing bacteria to reciprocal transfers of soil between adjacent coniferous forest and meadow vegetation in the Cascade Mountains of Oregon.* Microb. Ecol., 48: 500-508.

BRIERLEY E.D. WOOD M., SHAW P.J.A. 2001. *Influence of tree species and ground vegetation on nitrification in an acid forest soil.* Plant Soil, 229: 97-104.

CASTALDI S., CARFORA A., FIORENTINO A., NATALE A., MESSERE A., MIGLIETTA F., COTRUFO M. F. 2009. *Inhibition of net nitrification activity in a Mediterranean woodland: possible role chemicals produced by Arbutus unedo.* Plant Soil, 315: 273-283.

JONES D.L., KEMMITT S.J., WRIGHT D., CUTTLE S.P., BOL R., EDWARDS A.C. 2005. *Rapid intrinsic rates of amino acid biodegradation in soils are unaffected by agricultural management strategy.* Soil Biol. Biochem., 37: 1267-1275.

KARA E.E., ARIL M., UYGUR V. 2004. *Effects of the herbicide topogard on soil respiration, nitrification and denitrification in potato-cultivated soils differing in pH hort communication.* Biol. Fertil. Soils, 39: 474-478.

KOSTOV O., CLEEMPUT O. 2001. *Nitrogen transformation in copper-contaminated soils and effects of lime and compost application on soil resiliency*. Biol. Fertil. Soils., 33: 10-16.

KRAVE A. S., STRAALEN N. M., VERSEVELD H. W. 2002. *Potential nitrification and factors influencing nitrification in pine forest and agricultural soils in Centarl Java, Indonesia.* Pedobiologia, 46: 573-594.

KUCHARSKI J. 1997. *Relation between enzymes activity and soil fertility. Microbes in environment – appearance, activity and importance.* (Ed. W. BARABASZ). AR Kraków, 327-347.

KUCHARSKI J., JASTRZĘBSKA E., WYSZKOWSKA J. 2004. *Effects of some oil products on course of ammonification and nitrification*. Acta Agr. Silv. ser. Agr., 42: 249-255.

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

LINA Q., BROOKES P.C. 1999. *Arginine ammonification as method to estimate soil microbial biomass and microbial community structure.* Soil Biol. Biochem., 31: 1985-1997.

MARTENS D.A., BREMNER J.M. 1994. *Effects of preemergence and postemergence herbicides on urna hydrolysis and nitrification of urea nitrogen in soil*. Biol. Fertil. Soils, 17, 309-313.

PIETRIL J.C.A., BROOKES P.C. 2008. *Nitrogen mineralisation along a pH gradient of a silty loam UK soil.* Soil Biol. Biochem., 40: 797-802.

QIAN C., CAI Z. 2007. *Leaching of nitrogen from subtropical soils as affected by nitrification potential and base cations*. Plant Soil, 300: 197-205.

SIERRA J. 2006. *A hot-spot approach applied to nitrification in tropical acid soils.* Soil Biol. Biochem., 38: 644-652.

Statsoft. Inc. 2006. Statistica (data analysis software), version 6.0. www.statsoft.com.

WYSZKOWSKA J. 2002. *Biological properties of soil contaminated with hexavalent chromium.* Wyd. UWM., Rozpr. i Monogr., 65: 1-134.

WYSZKOWSKA J., BOROS E., KUCHARSKI J. 2007. *The effect of nickel on the ammonification process in soil.* Pol. J. Natur. Sc., 22(3): 383-394.

WYSZKOWSKA J., KUCHARSKI M., KUCHARSKI J., BOROWIK A. 2006. *Transformations of aspartic acid and l-glutamic acid in the presence of nitrification inhibitors.* Acta Agr. Silv. ser. Agr., 49: 507-515.

ZHU W., CARREIRO M. M. 1999. *Chemoautotrophic nitrification in forest soils along an urban-to-rural transect*. Soil Biol. Biochem., 31: 1091-1100.

J. Elementol. 2009, 14(3): 489–500

SILICA GRIT, CHARCOAL AND HARDWOOD ASH IN TURKEY NUTRITION

Teresa Majewska, Dariusz Mikulski, Tomasz Siwik

Chair of Poultry Science
University of Warmia and Mazury in Olsztyn

Abstract

The objective of this study was to determine whether silica grit is a necessary dietary additive for turkeys raised in intensive production farms, and whether it can be replaced by other supplements such as charcoal or hardwood ash. The conclusions were formulated based on performance results, blood hematological and biochemical indices, the slaughter quality of turkeys, as well as on the chemical and physicochemical properties of turkey meat. The experimental material comprised 360 male Big 6 heavy-type turkeys randomly divided into 4 treatments and raised on litter until 20 weeks of age. Birds of all treatments were fed identical complete pelleted basal diets. The experimental factor were various feed supplements in each group. The control treatment was fed a diet without supplements. The diets for experimental groups were supplemented with silica grit (SG), charcoal (CH) or hardwood ash (HA) in the amount of 0.3% of the administered feed mix. Dietary supplements were administered from the first day of life until the end of the rearing period. No significant changes in blood hematological and biochemical indices of turkeys were observed. Silica grit (SG) had an adverse effect on the performance results of turkeys, while the addition of both CH and HA had a highly beneficial impact. The best results were reported in respect of charcoal. The treatment fed a diet supplemented with CH was characterized by the lowest mortality rate of 4.4%, i.e. half that observed in the control group, body weight higher by 3.9% and rearing efficiency index higher by 9.7%.

Key words: silica grit, charcoal, hardwood ash, performance, slaughter quality, turkeys.

prof. dr hab. Teresa Majewska, Chair of Poultry Science, University of Warmia and Mazury in Olsztyn, ul. Oczapowskiego 5, 10-719 Olsztyn, Poland, e-mail: teresa.majewska@uwm.edu.pl

ŻWIREK KRZEMOWY, WĘGIEL DRZEWNY I POPIÓŁ Z DRZEW LIŚCIASTYCH W ŻYWIENIU INDYKÓW

Abstrakt

Celem badań było określenie, czy w żywieniu indyków utrzymywanych w warunkach intensywnej produkcji potrzebny jest żwirek krzemowy i czy można go zastąpić takimi dodatkami, jak węgiel drzewny i popiół z drzew liściastych. Wnioskowano na podstawie wyników odchowu, wskaźników hematologicznych i biochemicznych krwi oraz wartości rzeźnej, składu chemicznego i właściwości fizykochemicznych mięsa indyków. Materiał doświadczalny stanowiło 360 indorów typu ciężkiego Big 6, które rozdzielono losowo do 4 grup i odchowywano do 20. tygodnia życia na ściółce. Ptaki ze wszystkich grup żywiono jednakowymi granulowanymi pełnoporcjowymi mieszankami. Żywienie ptaków z poszczególnych grup różniło się tylko zastosowanymi dodatkami paszowymi. Grupa kontrolna nie otrzymywała żadnego dodatku. W grupach doświadczalnych zastosowano do paszy dodatek: żwirku krzemowego, węgla drzewnego lub popiołu z drzew liściastych w ilości 0,3% zadawanej mieszanki. Dodatki stosowano od 1. dnia życia do końca okresu odchowu. Nie odnotowano istotnych różnic we wskaźnikach hematologicznych i biochemicznych krwi indyków. Stwierdzono, że zastosowanie żwirku krzemowego pogorszyło wyniki odchowu indorów, natomiast dodatek zarówno węgla drzewnego, jak i popiołu z drzew liściastych wpłynął bardzo korzystnie na efekty produkcyjne odchowu tych ptaków. Najlepsze efekty otrzymano stosując dodatek węgla drzewnego. U indorów z tej grupy stwierdzono najmniejszą śmiertelność – 4,4%, o 3,9% większą masą ciała i o 9,7% lepszy wskaźnik efektywności odchowu, w porównaniu z grupą kontrolną.

Słowa kluczowe: żwirek krzemowy, węgiel drzewny, popiół z drzew liściastych, indyki.

INTRODUCTION

When raised in a natural environment, birds show a strong appetite for silica grit. In intensive poultry production farms, the use of silica grit was abandoned due to technical difficulties. Slaughter houses continue to oppose the use of silica grit in poultry feed due to possible overestimation of the weight of birds intended for slaughter and the complex procedure of gizzard cleaning. It was believed that silica grit had no nutritional value and that it did not affect the digestive process as silicon cannot be digested by birds. Yet feed mixing in the acidic environment of the gizzard releases silicon ions which form weak inorganic acids – metasilicic acid (H_2SiO_3) and orthosilicic acid (H_4SiO_4) of white coloring. Since they occur in sol or gel form, they have a very large absorptive surface and are strong adsorbents for gas, toxins and bacteria. Those acids have bactericidal and healing properties, they control metabolism, boost immunity and play a vital role in medical treatment. Poultry feed supplementation with silicon is not required under the applicable norms, but silicon ions determine the availability of other micronutrients (NICHOLAS et al. 2001). Silicon deficiency in animals inhibits growth, leads to atrophy of organs, disrupts the formation of bones and connective tissue, causes inflammations of the mucosa and of the skin

(BODAK et al. 1997). In birds, it causes flaccidity of the proventriculus, lack of appetite, anemia, diarrhea, paresis of the legs and wings (ELLIOT, EDWARDS 1991, ROLAND et al. 1993). In addition to feed grinding, the presence of silica grit in chicken gizzards also had a sterilizing, emulsifying and thermoregulating effect.

Published sources indicate that in intensive poultry production, silica grit may be replaced by pulverized charcoal, hardwood ash or straw. Those additives are easier to administer because they are not subject to sedimentation and they do not overload the gizzard. They are sterile and are a source of various elements occurring in natural proportions with an adequate nutritive value. Pulverized charcoal and silicic acid gel are believed to be the strongest natural adsorbents for gas, bacterial toxins and mycotoxins (EDRINGTON et al. 1997, KUTLU at al. 1999, WANG et al. 2006). They physically absorb gas and toxins. The absorptive surface of 1 g of charcoal can reach 1 ha.

The objective of this study was to determine whether silica grit is a necessary dietary additive for turkeys raised in intensive production farms, and whether it can be replaced by other supplements such as charcoal or hardwood ash. The conclusions were formulated based on performance results, blood hematological and biochemical indices, the slaughter value of turkeys, as well as on the chemical and physicochemical properties of turkey meat.

MATERIAL AND METHODS

The experiment was conducted at the State Turkey Evaluation Station of the University of Warmia and Mazury (Olsztyn, Poland), according to the guidelines of the local animal experimentation ethics committee. The experimental material comprised 360 male Big 6 heavy-type turkeys, sexed at the local commercial hatchery (Grelavi Co., Kętrzyn, Poland). One-day-old poults were randomly divided into 4 groups. The experiment was performed in three replications, each of 30 birds. Turkeys were raised on litter until 20 weeks of age in a building with a controlled environment. Birds of all groups were fed identical complete pelleted wheat-soybean diets in a five-stage system, supplied by the local commercial animal feed mill Agrocentrum Co., Kolno, Poland. The nutritional value of feed was consistent with BUT (British United Turkeys Ltd.) recommendations (Table 1). The experimental factor were various feed supplements in each group. The control treatment (Control) was fed a diet without supplements. The diets for experimental groups were supplemented with silica grit (SG) in the amount of 0.3% of the administered feed mix, charcoal (CH) at 0.3% and hardwood ash (HA) at 0.3%. Dietary supplements were administered from the first day of life until the end of the rearing period. Feed and fresh water were offered ad libitum.

Table 1

Composition (g kg^{-1}) and calculated nutrient content of the basal diets fed to turkeys from 1 to 20 weeks of age

Specification	Starter 1	Starter 2	Grower 1	Grower 2	Finisher
	1 – 4 weeks	5 – 8 weeks	9 – 12 weeks	13 – 16 weeks	17 – 20 weeks
Components					
Wheat	194.3	264.3	488.4	665.9	485.3
Maize	250.0	250.0	100.0	100.0	100.0
Triticale	-	-	-	-	150.0
Potato protein	50.0		-	-	-
Soybean meal, 46% CP	425.0	388.0	327.3	200.0	172.1
Soybean oil, 98%	22.0	21.0	43.2	-	20.0
Animal fat, 99%	-	-	-	-	40.9
Limestone	12.4	12.0	11.6	11.3	9.2
Monocalcium phosphate	32.4	27.0	14.8	12.2	11.7
Na_3PO_4	1.3	1.3	1.3	1.3	0.7
DL methionine 99	3.0	2.6	2.6	0.8	1.0
L lysine 99 HCL	3.6	3.2	4.1	3.0	3.2
L threonine	1.0	0.6	1.7	0.5	0.9
Vitamin-mineral premix*	5.0	5.0	5.0	5.0	5.0
Nutrients (calculated)					
ME, kcal kg^{-1}	2789	2822	3000	3146	3250
Crude protein, %	28.05	25.16	21.78	17.17	16.25
Lysine, %	1.82	1.58	1.39	1.00	0.96
Methionine + Cystine, %	1.18	1.05	0.94	0.65	0.64
Threonine, %	1.16	0.99	0.94	0.64	0.64
Tryptophan, %	0.32	0.29	0.25	0.19	0.18
Ca, %	1.34	1.20	0.90	0.80	0.70
Available P, %	0.74	0.65	0.45	0.40	0.37
Na, %	0.05	0.05	0.05	0.04	0.03
Cl, %	0.17	0.17	0.16	0.16	0.11

* supplied the following per kilogram of starter/grower-finisher diet: vitamin A (all-trans retinol acetate), 15,000/13,000 IU; cholecalciferol, 4,000/3,000 IU; vitamin E (all-rac-α-tocopherol acetate), 40/35 mg; vitamin K (menadione Na bisulfate), 2.5/2 mg; thiamin (thiamin mononitrate), 2.5/2 mg; riboflavin, 10/8 mg; niacin, 70/65 mg; vitamin B_6, 5/3.5 mg; pantothenic acid, 20/18 mg; folic acid, 2/1.5 mg; biotin, 0.3/0.2 mg; choline (choline chloride), 600/400 mg; Mn, 120/100 mg; Zn ($ZnSO_4 \cdot 7H_2O$), 90/80 mg; Fe ($FeSO_4 \cdot 7H_2O$), 60/50 mg; Cu ($CuSO_4 \cdot 5H_2O$), 10/8 mg; J, 1/0.8 mg; Se (Na_2SeO_3), 0.3/0.3 mg.

All husbandry practices and euthanasia were carried out with full consideration of animal welfare. Poults were vaccinated with Nobilis TRT on the first day of life. Stocking density was approximately 50 kg LBW m^{-2} of usable floor space in all pens. Brooder rings for poults (till 10 days of age) and additional heat sources (till 28 days of age) were installed in the pens. Heating was provided by a central heating system and electric heaters (red light). The brooder unit's temperature was set at 35°C and then altered as needed to suit bird comfort. Room temperature was set at 28°C on the day of placement, and was subsequently reduced by 2°C per week. The temperature and humidity were recorded on a daily basis at 8 AM and 3 PM. The lighting program in the room was as follows: 23 h light at about 100 lux till 3 days of age and 14 h light at 5-6 lx from day 4 until the end of the experiment. Relative humidity was approximately 65 to 70%. Air changes were 0.4--0.5 m^3 h^{-1} kg^{-1} of BW from 2 to 7 weeks of age and 7-8 m^3 h^{-1} kg^{-1} of BW from 8 weeks of age.

Visual health inspection of all birds was performed on a daily basis. Mortality rates, culling rates and the body weight of dead birds were also recorded every day. Feed intake per pen and the body weight of turkeys per pen were recorded on day 21, 70 and 140. The results provided a basis for calculating the Rearing Efficiency Index (REI), according to the formula:

$$REI = \frac{\text{average live body weight (kg)} \cdot \text{liveability (\%)} \cdot 100}{\text{feed conversion ratio (kg feed kg}^{-1}\text{ body weight)} \cdot \text{number of rearing days}}$$

Additionally, carcass characteristics (dressing percentage and breast, thigh and drumstick muscles) were determined after 20 weeks of the experiment.

At the end of the rearing period, 6 turkey-toms with the most average body weight were selected from each group and were sacrificed after 12 hours of fasting, according to the recommendations for euthanasia of experimental animals (Close et al. 1997). During slaughter, blood samples were collected from every bird for hematological and biochemical analyses. The hemoglobin content (HGB), hematocrit levels (HCT), red blood cell (RBC) and white blood cell (WBC) counts were determined by routine methods (Kokot 1989). The following biochemical parameters were determined: total protein content in the blood serum by the Biuret method, triglycerides content by the enzymatic method, activity of alkaline phosphatase (ALP), aspartate aminotransferase (AST) and creatine kinase (CK) with the use of the method proposed by Reitman and Fränkel (as cited in Krawczyński, Osiński 1967).

Charcoal, hardwood ash and silica grit samples were spectrally etched with pure acid (Merck) in a Milestone microwave labstation (manufactured in Italy) under the pressure of 100 atm until completely mineralized. Three analytical tests were performed for each investigated micronutrient. Quantitative analyses of total carbon content in SG, CH and HA were performed with the use of an Eurovector elemental analyzer (manufactured in Italy). Si, Ca, P, Mg and Zn levels were determined with the use of a plasma spectrometer interfaced with the Philips Scientific PU 7000 analytical system. The analytical device was applied to determine the content of the analyzed elements in Ar ICP (Inductively Coupled Plasma) flame.

Plucked, eviscerated and chilled (4°C, 12 h) carcasses were dissected to determine the dressing percentage of each primal. The proximate chemical composition, physical and chemical properties of breast muscle samples were determined. The dry matter, total protein, crude fat and ash content of meat were measured by traditional methods (AOAC, 1990). 5 g of raw meat was homogenized with 5 ml of distilled water and the ultimate pH (pHu) of meat was measured with a WTW inoLab level 2 pH-meter equipped with a Hamilton Polylite Lab electrode. The free water content of meat samples, prepared according to Honikel (1998), was determined by the Grau and Hamm method (Oeckel van 1999). Meat color brightness was measured as described by Różyczka et al. (1968) with the use of a SPEKOL spectrocolorimeter with an R-0-45 remission attachment at a wavelength of 560 nm.

The results were verified statistically by one-factorial analysis of variance (ANOVA). The significance of differences between groups was estimated by Duncan's test. A pen was considered as a replicate experimental unit. Treatment effects were considered to be significant at $P = 0.05$.

RESULTS AND DISCUSSION

During chemical analyses of feed additives (Table 2), three analytical measurements were performed for each investigated element. The highest Ca, P, Mg and Zn content was determined in hardwood ash where average levels of those micronutrients were also marked by high standard deviation.

All poults used in the experiment were in good condition as no deaths were observed in the first three weeks of rearing (Table 3). The first deaths in all groups, except for the group fed a diet supplemented with charcoal, were reported in the sixth week of rearing. Wattle formation begins around this period and it usually weakens the birds' immune system. The highest mortality rate of 6.7% observed at that time without a clearly identifiable cause occurred in the group of birds fed a diet supplemented with silica grit. Although no invasive diseases were reported in subsequent weeks of rearing, the highest mortality rate (11.1%) in the entire 20-week rearing period was also reported in the group of turkeys receiving silica grit. A possible cause could be the quality of the administered grit which was obtained directly from a gravel mine without prior rinsing. Gravel types other than

Table 2

Concentrations of selected nutrients in feed additives

Nutrient	Silica grit	Charcoal	Hardwood ash
C, %	0.08 ± 0.00	82.97 ± 4.26	13.23 ± 3.67
Si, %	38.01 ± 0.60	0.22 ± 0.03	4.12 ± 1.46
Ca, %	26.70 ± 0.09	1.27 ± 1.10	27.43 ± 4.42
Mg, %	0.42 ± 0.85	0.13 ± 0.11	1.45 ± 0.35
P, %	0.13 ± 0.01	0.49 ± 0.23	1.22 ± 0.23
Zn, mg kg^{-1}	15.57 ± 0.21	337.0 ± 44.90	1121.6 ± 305.6

Table 3

Effect of diets containing silica grit, charcoal or hardwood ash on the body weight and feed conversion ratio (FCR) of turkeys raised from 1 to 140 days of age

Specification	Control	Silica grit	Charcoal	Hardwood ash
Mortality (%)				
0-20 wk	8.8	11.1	4.4	6.6
Culling (%)				
0-20 wk	2.2	4.4	-	-
Body weight (kg)				
1 wk	0.16 ± 0.01	0.16 ± 0.01	0.16 ± 0.02	0.15 ± 0.02
3 wk	0.49 ± 0.04^{b}	0.52 ± 0.05^{ab}	0.54 ± 0.06^{a}	0.50 ± 0.03^{ab}
10 wk	4.91 ± 0.48^{b}	4.96 ± 0.59^{b}	5.09 ± 0.77^{a}	4.95 ± 0.59^{b}
20 wk	16.12 ± 1.26^{b}	16.23 ± 1.51^{b}	16.73 ± 1.14^{a}	16.73 ± 1.03^{a}
%	100.0	100.7	103.8	103.7
Feed conversion ratio, kg kg^{-1} body weight gain				
0 - 20 wk	2.81 ± 0.09	2.78 ± 0.10	2.79 ± 0.04	2.76 ± 0.06
%	100.0	98.9	99.3	98.2
Rearing Efficiency Index, points				
0 - 20 wk	373	371	409	404

Means within the same line with no common superscripts differ: $^{a-b}$=$P \le 0.05$.

river gravel contain compounds which are toxic for birds and can easily upset systemic homeostasis. Clinical symptoms of disease are not always manifested. Impaired immunity can lead to death for no apparent reason. The mortality rate in the control group reached 8.8% and in the group fed a diet supplemented with hardwood ash – 6.6%. The lowest mortality rate was reported among turkeys receiving charcoal (4.4%), i.e. half that observed in the control group. Culling was not necessary in the group of birds fed a diet with the addition of charcoal (Table 3).

The applied feed supplements had a statistically significant effect ($P < 0.05$) on the final body weights of turkeys. After 3 weeks of rearing, the body weight of birds fed a diet supplemented with charcoal was significantly higher ($P < 0.05$) than that of control group turkeys. After 10 weeks of rearing, their body weight was significantly higher ($P < 0.05$) in comparison with the remaining groups. After 20 weeks of rearing, the highest body weight was reported in groups receiving pulverized charcoal and hardwood ash, i.e. in groups marked by the lowest mortality rates. The final body weight of those birds was identical (16.73 kg) and significantly higher (more than 3%; $P < 0.05$) than that of control group turkeys and of birds fed diets with the addition of silica grit. Charcoal seems to have a particularly beneficial effect in the initial rearing period as 3-week-old turkeys of this group were 12% heavier ($P < 0.05$) than control birds. Average body weight results also indicate that silica grit may be added to feed only in the initial periods of life because as of the third week of rearing, the body weight of turkeys receiving grit was 7% higher in comparison with the control group. After this period, a higher mortality rate with a simultaneous drop in body weight were noted in birds fed diets supplemented with silica grit.

The supplements applied in this experiment improved the feed conversion ratio (by around 1.0-1.8%, Table 3), but the resulting differences were not statistically verified. The rearing efficiency index (REI) of turkeys fed charcoal and hardwood ash additives reached 409 and 404 points, respectively (Table 3), and it was approximately 9% higher than in the control group. The lowest feed efficiency was observed in control group birds which consumed 2.81 kg of feed per kg of body weight gain.

In a previous study (MAJEWSKA et al. 2002), after 18 weeks of rearing, the body weight of turkeys fed diets supplemented with 0.3% charcoal throughout the entire rearing period increased by 5.9% ($P < 0.01$), while FCR dropped by 6.5% in comparison with the control group. The mortality rate of turkeys receiving charcoal was only 1.0%, while it reached a high level of 12.7% in the control group. In another study (MAJEWSKA, ZABOROWSKI 2003), broiler chickens fed diets supplemented with 0.3% charcoal were 4% heavier after 6 weeks of rearing in comparison with control birds. The benefits of charcoal in the nutrition of broiler chickens were also recognized by other authors. EDRINGTON et al. (1997) fed broiler chickens a diet supplemented with 0.5% superactivated charcoal (SAC) and observed a 4.4% increase in body weight in comparison with the control group already after 21 days. The authors attributed this effect to the presence of available microelements and the detoxicating effect of charcoal. In the first 3 weeks of chicken rearing, KUTLU and UNSAL (1998) noted that dietary wood charcoal affected ($P < 0.01$) feed intake, body weight gain and feed conversion ratio. In a study carried out by KUTLU et al. (1999) who applied feed supplemented with 2.5% charcoal only in the first three weeks of rearing, the body weight of 6-week-old chicks increased by 165 g, i.e. 7.8%, and this increase was statistically higher ($P < 0.05$) than in the group of birds whose diet was not supplemented with charcoal. According to other authors (EDRINGTON et al. 1997, KUTLU et al. 1999, MAJEWSKA, SIWIK 2006), the addition of charcoal and superactivated charcoal did not affect the feed conversion ratio in broiler chickens.

The authors observed no significant changes in blood hematological and biochemical indices (Tables 4 and 5) which would be indicative of the effect of feed additives or a deterioration in the turkeys' health condition. In both the control treatment (not receiving additives) and the group of birds fed diets supplemented with SG, CH and HA, the above indicators were within physiological limits (KRASNODĘBSKA-DEPTA, KONCICKI 1999, 2000). Only a minor (statistically non-significant) increase in blood zinc levels was noted in birds receiving charcoal and hardwood ash. No changes in blood hematological and biochemical indices of chickens and turkeys were observed by ERINGTON et al. (1997) who supplemented feed with superactivated carbon or MAJEWSKA et al. (2002) who used charcoal feed additives.

Table 4

Effect of diets containing silica grit, charcoal or hardwood ash on blood hematological indices in 20-week-old turkeys

Specification	Control	Silica grit	Charcoal	Hardwood ash
RBC, 10^{12} l^{-1}	2.28 ± 0.20	2.32 ± 0.33	2.35 ± 0.22	2.38 ± 0.16
WBC, 10^{9} l^{-1}	15.50 ± 0.81	14.81 ± 1.26	17.05 ± 0.56	15.42 ± 1.42
HGB, g dl^{-1}	10.40 ± 0.78	11.36 ± 0.57	11.63 ± 0.97	11.15 ± 0.81
HCT, %	33.0 ± 1.72	32.32 ± 1.49	33.51 ± 3.38	33.63 ± 0.94

RBC – red blood cell, WBC – white blood cell, HGB – hemoglobin, HCT – hematocrit

In this experiment, the applied feed additives had no statistically significant effect on the carcass yield of 20-week-old turkeys (Table 6). The only significant difference ($P = 0.05$) was noted in respect of liver weight in turkeys receiving CH in comparison with turkeys fed a diet supplemented with SG whose livers were the lightest. This effect could be attributed to the fact that SG present in the proventriculus is capable of absorbing heat from the liver, thus simulating this organ. Yet the obtained results did not support this hypothesis. In a research conducted by MAJEWSKA and SIWIK (2006), the addition of SG, CH and HA did not affect the slaughter value of broiler chickens. Other authors (KUTLU, UNSAL 1998, KUTLU et al. 1999) noted that charcoal inclusion to the diets for chickens tended to reduce abdominal fat weight and abdominal fat percentages, while having no significant effects on carcass yield.

Diets supplemented with feed additives did not affect the chemical composition and physicochemical properties of meat (Table 7). The results of research carried out by KUTLU and UNSAL (1998) also showed that charcoal inclusion did not affect carcass dry matter, fat and protein content.

Table 5

Effect of diets containing silica grit, charcoal or hardwood ash on blood biochemical indices in 20-week-old turkeys

Specification	Control	Silica grit	Charcoal	Hardwood ash
Total protein, g dl^{-1}	5.25 ± 0.38	5.28 ± 0.59	5.24 ± 0.19	5.30 ± 0.19
Triglycerides, mg dl^{-1}	26.17 ± 2.08	26.59 ± 1.02	32.27 ± 5.20	30.19 ± 9.14
Total cholesterol, mg dl^{-1}	148.0 ± 30.8	152.3 ± 18.9	156.0 ± 13.1	160.7 ± 22.9
Ca, mg dl^{-1}	12.17 ± 0.71	11.57 ± 1.12	12.49 ± 1.65	12.21 ± 0.76
P, mg dl^{-1}	3.23±0.90	3.61±0.47	3.65±0.80	3.44±0.48
Zn, mg dl^{-1}	208.6 ± 24.0	214.6 ± 61.6	268.5 ± 68.0	273.5 ± 88.9
Mg, mg dl^{-1}	2.96 ± 0.11	3.14 ± 0.42	3.11 ± 0.54	3.72 ± 0.22
Uric acid, mg dl^{-1}	221 ± 80	322 ± 50	246 ± 99	191 ± 70
ALP, U l^{-1}	537 ± 96	547 ± 131	606 ± 122	558 ± 85
CK, U l^{-1}	4677 ± 747	5411 ± 950	5708 ± 561	4937 ± 192
AST, U l^{-1}	106 ±18	119 ± 38	136 ± 27	100 ± 12

ALP – alkaline phosphatase, CK – creatine kinase, AST – aspartate aminotransferase

All of the above cited authors noted that charcoal supplementation had a beneficial effect on bird health, production efficiency and product quality. Charcoal is a cheap and environment-friendly feed additive. The positive influence of charcoal results from the presence of mineral compounds and from the uptake and adsorption of gases. The ions produced when charcoal mixes with feed in the gizzard can activate enzymes, hormones, vitamins and antibodies. The minerals contained in charcoal mix with water to form lye, which can decrease the surface tension of intestinal digesta. Due to its extensive absorptive area, charcoal permits physical adsorption of gases and toxins produced during the digestive process, as well as toxic substances secreted by bacteria and fungi. Since activated charcoal contributes to enhancing the immune responses, the supplied nutrients may be effectively used to increase body weight and not to produce antibodies or develop defense mechanisms. There are no published data documenting the use of hardwood ash in poultry nutrition, but the results of this study indicate that this supplement could also enhance production efficiency. Charcoal and hardwood ash are environment-friendly and inexpensive additives, which contain mineral nutrients, do not undergo sedimentation (separation) in feed and, similarly to silica gel, have a very large absorptive area.

CONCLUSION

In conclusion, this experiment has shown that modern turkey rearing methods do not require the inclusion of silica grit in animal diets. Nevertheless, diet supplementation with charcoal or hardwood ash in the amount of 0.3% of the administered feed mix could have a beneficial effect on performance of turkeys. Turkeys fed a diet supplemented with 0.3% charcoal were characterized by the lowest mortality rate of 4.4%, i.e. half that noted in the control treatment, body weight higher by 3.9% and rearing efficiency index higher by 9.7%.

REFERENCES

AOAC 1990. Official Methods of Analysis of the Association of Official Analytical Chemists, In: HELRICH, K. (Ed.), 15th ed., Association of Official Analytical Chemists, Inc., Arlington, VA, USA.

B.U.T. Big 6 Commercial Performance Goals. British United Turkeys Ltd. Warren Hall, Broughton, Chester CH4 0EW, England.

BODAK E., DOBRZAŃSKI Z., TRZISZKA T. 1997. *Biofunction of Silicon and its role in animal production – a rewiew*. Med. Wet., 53 (6): 316-322.

CLOSE, B., K. BANISTER, V. BAUMANS, E. M. BERNOTH, N. BROMAGE, J. BUNYAN, W. ERHARDT, P. FLECKNELL, N. GREGORY, H. HACKBARTH, D. MORTON, C. WARWICK. 1997. *Recommendations for euthanasia of experimental animals*. Part 2. Lab. Anim., 31:1–32.

EDRINGTON T.D., KUBENA, HARLEY R.B.ROTTINGHAUS G.E. 1997. *Influence of a superactivated charcoal on the toxic effects of aflatoxin or T-2 toxin in growing broilers.* Poult. Sci., 76: 1205-2011.

ELLIOT M.A., EDWARDS H.M. 1991. *Comparison of the effects of cynthetic and natural zeolite on laying hen and broiler chicken performance*. Poult. Sci., 70 (10): 2115-2130.

HONIKEL K.O. 1998. *Reference methods for the assessment of physical characteristics of meat.* Meat Sci., 49:447-457.

KOKOT F. 1989. *Metodyka badań laboratoryjnych stosowanych w klinice [Methodology of laboratory tests performed in a clinic].* PWRiL, Warszawa. [in Polish]

KRASNODĘBSKA-DEPTA A., KONCICKI A. 1999. *Physiological values of selected serum biochemical indices in chickens.* Pol. J. Vet. Sci., 2: 49-57.

KRASNODĘBSKA-DEPTA A., KONCICKI A. 2000. *Fizjologiczne wartości wybranych wskaźników biochemicznych krwi kurcząt brojlerów [Physiological values of some biochemical blood parameters of broiler chikens].* Med. Wet., 56 (7): 456-460. [in Polish]

KRAWCZYŃSKI J., OSIŃSKI T. 1967. *Laboratoryjne metody diagnostyczne [Laboratory diagnostic methods].* PZWL, Warszawa. [in Polish]

KUTLU H.R., UNSAL I. 1998. *Effects of dietary wood charcoal on performance and fatness of broiler chicks.* Brit. Poult. Sci., 39: S31.

KUTLU H.R., UNSAL I., GORGULU M., BAYKAL L. 1999. *Effects of providing dietary wood charcoal to broiler chicks of different ages.* Brit. Poult. Sci., 40: 34-36.

MAJEWSKA T., PYREK D., FARUGA A. 2002. *A note on the effect of charcoal supplementation on the performance of Big 6 heavy tom turkeys.* J. Anim. Feed Sci., 11:135-141.

MAJEWSKA T., SIWIK T. 2006. *Silica grit, charcoal, hardwood ash and the preparation Humokarbowit as dietary additives for broiler chickens.* Pol. J. Nat. Sci., Suppl. 3: 445-449.

MAJEWSKA T., ZABOROWSKI M. 2003. *Charcoal in the nutrition of broiler chickens.* Med. Wet., 1: 81-83.

NICHOLAS A.S., VELLAS B., FERNSTROM J.D., UAUY R., ARROYO P. 2001. *Diet related prevention of Alzheimer's disease: different hypotheses. Nutrition and brain.* Proc. of the 5th Nestle Nutrition Workshop, pp. 219-230.

OECKEL M.J. VAN, WARNANTS N., BOUQUEE CH.V. 1999. *Comparison of different methods for measuring water holding capacity and juiciness of pork versus on-line screening methods.* Meat Sci., 5: 313-320.

ROLAND D.A., RABON H.W., RAO K.S., SMITH R.C., MILLER J.W., BARNES D.G., LAURENT S.M. 1993. *Evidence for adsorption of silicon and aluminum by hens fed sodium zeolite.* Poult. Sci., 72 (3): 447-455.

RÓŻYCZKA J., KORTZ J., GRAJEWSKA-KOŁACZYK S. 1968. *A simplified method of the objective measurement of colour in fresh pork meat.* Rocz. Nauk Rol., 90: 345-353.

WANG R.J., FUI S.X., MIAO C.H., FENG D.Y. 2006. *Effects of different mycotoxin adsorbents on performance, meat characteristics and blood profiles of avian broiler fed mold contaminated corn.* Asian-Australian J. Anim. Sci., 19 (1): 82-92.

J. Elementol. 2009, 14(3): 501–507

A STUDY ON BIOACCUMULATION OF SELECTED METALS IN MEAT AND INTERNAL ORGANS OF INTENSTIVELY FED KID GOATS

Roman Niedziółka, Krystyna Pieniak-Lendzion, Elżbieta Horoszewicz

Chair of Breeding Methods, Breeding of Poultry and Small Ruminants Podlasie Academy in Siedlce

Abstract

Owing to its wholesomeness and good taste, meat of goats and lambs is an increasingly more important in human nutrition. It is characterized by excellent nutritive value and high digestibility. It is also relatively rich in mineral compounds. The aim of this study has been to determine the content of some elements in muscles, liver and kidneys of male and felame kid goats, fattened to 150 day of life. The animals received full-ration mixture containing 169 g total protein and 6.0 MJ net energy. The mixture was given ad libitum and supplemented with meadow hay as bulk feed. During the whole period of fattening, the animals were kept under the conventional housing system. Samples for analysis were taken from *adductor femoris* muscles, liver and kidneys.

The muscle tissue of the analysed male goats contained less Cd (0.002 mg kg^{-1} ± 0.004) than that of the female goats (0.003 mg kg^{-1} ± 0.001). In turn, the level Pb was lower in the female goats (0.019 mg kg^{-1} versus 0.026 mg kg^{-1} in male goats), although the difference was statistically non-significant. In the internal organs, more Cd occurred in kidneys than in the liver, unlike Pb. The concentration Pb was higher in kidneys of the male than female goats (0.073 mg kg^{-1} ± 0.02), whereas the level of this element the liver was identical in both groups (0.084 mg kg^{-1}). In the muscule tissue, the sex differentiated the content of Fe ($p = 0.01$) as well as Mg and Zn ($p = 0.05$). The liver accumulated not only Pb and Cd, but also Cu and Zn. The concentration Cu in the liver of female goats was higher (146.79 mg kg^{-1} at $p = 0.01$) than in male goats by about 50 mg kg^{-1}. High disproportions between the sexes in the content of Fe in the liver were observed: 36.30 mg kg^{-1} for female goats and 21.99 mg kg^{-1} for male goats ($p = 0,01$). In kidneys, however, the concentration of Ca was very high, particularly for female goats particularly (81.01 ± 26.55 mg kg^{-1}).

Roman Niedziółka, Chair of Breeding Methods, Breeding of Poultry and Small Ruminants Podlasie Academy in Siedlce, ul. Prusa 14, 08-110 Siedlce, Poland, phone: (025) 643 1255, e-mail: owce@ap.siedlce.pl

Key words: goat kids, meat, liver, kidneys, bioaccumulation, elements.

BADANIA BIOAKUMULACJI WYBRANYCH METALI W MIĘSIE I NARZĄDACH WEWNĘTRZNYCH KOŹLĄT ŻYWIONYCH INTENSYWNIE

Abstrakt

Ze względu na walory zdrowotne i smakowe mięso kozie i jagnięce nabiera coraz większego znaczenia w żywieniu człowieka. Charakteryzuje się doskonałymi właściwościami pod względem wartości odżywczej i wysoką strawnością. Jest stosunkowo bogate w związki mineralne. Celem badań było określenie zawartości wybranych pierwiastków w mięśniach, wątrobie i nerkach koziołków i kózek żywionych do 150. dnia życia. Materiał doświadczalny stanowiły koziołki i kózki rasy białej uszlachetnionej oraz tuczone do 150. dnia życia. Zwierzęta żywiono pełnoporcjową mieszanką o wartości pokarmowej 168 g białka ogólnego i 6,0 MJ energii netto. Mieszankę podawano *ad libitum* i uzupełnioano dodatkiem strukturalnym siana łąkowego. Przez cały okres tuczu zwierzęta utrzymywano w chowie alkierzowym, w pomieszczeniach zamkniętych. Próby do analizy pobrano z mięśnia przywodziciela uda (*m. adductor*), wątroby i nerek.

W tkance mięśniowej analizowanych koziołków doświadczalnych stwierdzono niższą zawartość Cd (0,002 mg kg^{-1} ± 0, 0,004) niż u kózek (0,003 mg kg^{-1} ± 0,001). Natomiast poziom Pb był niższy w grupie kózek i wyniósł 0,019 mg kg^{-1} wobec 0,026 mg kg^{-1} – różnice okazały się nieistotne statystycznie. W narządach wewnętrznych wyższy poziom Cd stwierdzono w nerkach niż w wątrobie, a w przypadku Pb odwrotnie. Stężenie Pb było wyższe w nerkach koziołków i wyniosło 0,073 mg kg^{-1} ± 0,02. W wątrobach poziom tego pierwiastka był na tym samym poziomie (0,084 mg kg^{-1}). Płeć różnicowała istotnie zawartość Fe i Ca w tkance mięśniowej ($p \le 0,01$) oraz Mg i Zn ($p \le 0,05$). Wątroba była narządem koncentracji nie tylko Pb i Cd, ale Cu i Zn. Stwierdzono, że stężenie Cu w wątrobie kózek było wyższe ($p \le 0,01$, 146,79 mg kg^{-1}) w porównaniu z koziołkami o ok. 50 mg kg^{-1}. Zaobserwowano wysokie dysproporcje w zawartości Fe w wątrobie, ponieważ u kózek stwierdzono 36,30 mg kg^{-1}, a u koziołków 21,99 mg kg^{-1} ($p \le 0,01$). W nerkach wykazano zdecydowanie większe stężenie Ca, szczególnie u kózek (81,01 ± 26,55 mg kg^{-1}).

Słowa kluczowe: koźlęta, mięso, wątroba, nerki, bioakumulacja, pierwiastki.

INTRODUCTION

Human economic activity leads to increased levels of heavy metals in environment, which directly influence the ability of plants and animals to function properly. As a result, occurrence of increased contents of certain metals in edible parts of slaughter animals is an alarming phenomenon (KREŁOWSKA-KUŁAS 1989, WĘGLARZ 2007).

Heavy metals are found most often and are the most dangerous contaminants to human health. In particular, lead, cadmium and mercury are distinguished by high accumulation due to their faster absorption from the digestive tract and easy transfer through the organism's biological barriers (PARK 1990, LIDWIN-KAZIMIERKIEWICZ et al. 2006, NIEDZIÓŁKA et al. 2006, OLESZKIEWICZ 2007, WĘGLARZ 2007).

Numerous studies, for example by KRUPA and KOGUT (2000), Pieniak-LENDZION et al. (2006), NIEDZIÓŁKA et al. (2007), have shown that kidneys, livers, and muscle tissues of small ruminants from the Podlasie region as well as south-eastern Poland have low heavy metal content, which does not exceed the permissible levels. The present study was undertaken with the aim to determine contents of selected elements in the muscle tissue, livers and kidneys of kid goats offered concentrate rations of feed produced in the Podlasie region

MATERIALS AND METHODS

Experimental animals were 20 kid goats of the Polish White Improved breed. They remained with the mothers kept under standard conditions up to 55 days of age. Next, they were weaned and fattened up to 150 days of age in two feeding groups: group I – males, and group II – females. The grouping was to examine the impact of sex on the accumulation of elements when the same feeding regime was applied in both groups.

Throughout the whole period of the experiment, the animals were kept indoors. Following the fattening, the animals were slaughtered and their carcasses were chilled for 24 hours at 4^{o}C.

Samples of *adductor femoris* muscles, livers and kidneys were examined. Weighed samples were dried at 110^{o}C and then burned in an oven at 450^{o}C. Lead, cadmium, iron, copper and zinc contents were determined by atomic adsorption spectroscopy (AAS). Lead and cadmium contents were determined by means of the extraction method using 2% APDC (nickel pyrrolidine-dithiocarbamate) as an extraction agent. Methylisobutyl ketone saturated with deionised water was the organic phase. Iron, copper and zinc contents were determined directly from the mineralised material applying suitable dilutions. The determination was performed on an AAS-1 atomic adsorption spectrometer produced by Carl Zeiss Jena.

Statistical analysis included the ANOVA procedure, and was carried out using Stat. 6.0 PL (Statistica 2002).

RESULTS AND DISCUSSION

Excessively high doses of some elements, e.g. copper, may induce haemolysis and cause liver and kidney damages. Table 1 presents contents of the elements in the muscle tissues of male and female kid goats. Lead content was by 0.006 mg kg^{-1} higher in male muscles than in female meat, and amounted to 0.025 mg kg^{-1}. In contrast, female kid goat meat was charac-

Table 1

Concentration of elements in the muscle tissue of male and female kids

Elements	Male kids, N=10		Female kids, N=10	
	mg kg^{-1} of fresh tissue			
	$\bar{x}$	SD	$\bar{x}$	SD
Pb	0.025	0.01	0.019	0.01
Cd	0.002	0.001	0.003	0.001
Mn	0.24	0.04	0.35	0.16
Fe	16.70**	0.81	18.66**	3.69
Zn	28.41*	1.56	27.89*	0.72
Cu	0.94	0.39	1.23	0.44
Ca	20.76**	1.44	19.11**	2.65
Mg	156.55*	6.31	164.59*	10.47

The values are means ($\bar{x}$), standard deviation (SD);
*row mean value significant at $p \leq 0.05$;
**row mean value significant at $p \leq 0.01$.

terised by a higher cadmium concentration (by 0.003 mg kg^{-1}). The differences for both lead and cadmium were statistically insignificant. Higher cadmium and lead concentrations in the meat of kid goats and lambs were recorded for animals from central-eastern Poland slaughtered at 180 days of age (Niedziółka et al. 2007) as well as adult goats and sheep reared in the Rzeszów region (Krupa and Kogut 2000).

The Minister of Health regulation (Journal of Law 2001) states that cadmium and lead contents in muscle tissue can reach up to 0.05 and 0.20 mg kg^{-1}, respectively. It is worth noting that in 2003 the Minister of Health introduced a detailed norm (Journal of Law 2003), which set a lower permissible lead content in muscle tissue, i.e. 0.10 mg kg^{-1} fresh tissue.

It was found that male kid goat meat had a significantly (p = 0.01) lower iron and magnesium content (respectively, 16.70 and 156.55 mg kg^{-1}). By contrast, female kid goat meat was characterised by less calcium and zinc (respectively, 19.11 (p = 0.01) and 27.89 mg kg^{-1} (p = 0.05)). The Polish norm of 2000 specified that the maximum Zn content could not exceed 80 mg kg^{-1} fresh muscle tissue. From 2000 to 2003 there were no limits for copper and zinc content in food like eggs and meat (Journal of Law 2001, 2003). In other studies (Johnson et al. 1995) the muscle tissue of young kid goats contained an average of 28.1 mg 100 g^{-1} calcium, 92.3 mg 100 g^{-1} zinc and 4.4 mg 100 g^{-1} copper. Excess of lead and magnesium deficiency is typical of and common among different age groups of Poles. Thus, magensium supplementation or a diet including magnesium have a positive effect on the population and on children in particular (Dunicz-Sokołowska et al. 2006, Oleszkiewicz 2007).

No significant differences in the lead content in the liver were found between the sex groups (0.084 mg kg^{-1} fresh tissue, Table 2). Only a slightly lower cadmium level was found in female livers, that is 0.020 mg kg^{-1} versus 0.021 mg kg^{-1} for males. The values were about 20-fold lower than the level accepted by the Minister of Health regulation of 2003 (Journal of Law 2003). Lead and cadmium contents in mammalian livers should not exceed

Table 2

Concentration of elements in the liver of male and female kids

Elements	Male kids, N=10		Female kids, N=10	
	mg kg^{-1} of fresh tissue			
	$\overline{x}$	SD	$\overline{x}$	SD
Pb	0.084	0.01	0.084	0.06
Cd	0.021	0.01	0.020	0.01
Mn	3.54*	0.33	3.86*	1.31
Fe	21.99**	7.79	36.30**	14.74
Zn	30.34	1.41	31.99	2.01
Cu	96.23**	11.97	149.79**	23.11
Ca	35.59*	7.26	33.01*	8.61
Mg	127.57**	9.40	134.98**	14.16

The values are means ($\overline{x}$), standard deviation (SD);
*row mean value significant at $p \leq 0.05$;
**row mean value significant at $p \leq 0.01$.

0.50 mg kg^{-1}. The concentration of both lead and cadmium was also much lower compared with other studies (Krupa and Kogut 2000, Niedziółka et al. 2007). Copper was mainly accumulated in the liver irrespective of the sex. Significant ($p = 0.01$) differences were found in copper content. The copper level, higher by 52.56 mg kg^{-1}, was recorded for females (149.79 mg kg^{-1}) as compared to males. The results were lower (21.99 mg kg^{-1}), and the variation in the accumulation according to the analysed tissues confirmed the results of earlier studies (Niedziółka et al. 2006). The maximum permissible zinc content in liver, according to the Minister of Health norm of 2000 (Journal of Law 2001), was set at 80 mg kg^{-1} fresh tissue but since 2003 all such limits have been abandoned (Journal of Law 2003). It is estimated that in most countries, including the wealthiest ones, the daily zinc intake is less than 10 mg whereas the adult person's demand is 12-20 mg (Węglarz 2007). In the present work, the zinc level in the liver was similar in males and females, reaching 31.99 and 30.34 mg kg^{-1}, respectively.

Lead and cadmium levels in the kidneys of examined animals (Table 3) were 0.073 mg kg^{-1} and 0.038 mg kg^{-1}, respectively. Significant differences ($p \leq 0.05$) were found in iron content in the kidneys of investigated animals,

Table 3

Concentration of elements in the kidneys of male and female kids

Elements	Male kids, N=10		Female kids, N=10	
	mg kg^{-1} of fresh tissue			
	$\overline{x}$	SD	$\overline{x}$	SD
Pb	0.073	0.02	0.057	0.01
Cd	0.031	0.01	0.038	0.01
Mn	1.53	0.46	1.32	0.15
Fe	49.88*	10.28	45.36*	8.80
Zn	20.80	4.23	20,68	1.71
Cu	3.49	0.36	3.99	0.35
Ca	75.86**	18.44	81.07**	26.55
Mg	135.58**	7.74	127.30**	7.59

The values are means ($\overline{x}$), standard deviation (SD);
*row mean value significant at $p \leq 0.05$;
**row mean value significant at $p \leq 0.01$.

similarly to calcium and magnesium ($p \leq 0.01$), all the results being similar to the findings of previous studies (Niedziółka et al. 2006). Krupa and Kogut (2000) concluded that kidneys of kid goats reared in the Rzeszów region were not suitable for consumption because lead and cadmium contents were higher than the norms. The results of a study by Zięba (2003) showed an evidently higher cadmium content in flesh organs, several-fold higher level in livers and more than ten-fold higher concentration in kidneys than in muscles. In turn, lead content varied, reaching up to 0.06 in muscles, and to 0.092 and 0.061 mg kg^{-1} in livers and kidneys, respectively. Analysis of individual tissues showed that the highest was the level of calcium in kidneys, and in females it was significantly ($p = 0.01$) and by 5.21 mg kg^{-1} higher, reaching 81.07 mg kg^{-1}.

SUMMARY

The results of the present study indicate a higher accumulation of toxic metals in flesh organs. It was found that lead and cadmium concentrations were similar in both sex groups. The bulk of copper and zinc accumulated in livers of both males and females. Irrespective of the sex, the highest and the lowest magnesium levels were found in muscle tissue and livers, respectively, and the differences reached between ten and twenty per cent. In turn, in kidneys the highest was the accumulation of iron: from 45.36 mg kg^{-1} (in females) to 49.88 mg kg^{-1}(in males), whereas in muscle tissue the greatest was the concentration of magnesium.

The values of all the elements, including toxic ones, did not exceed the upper levels of the Polish norms for meat tissue and internal organs, which indicates that the meat was fully suitable for human consumption.

REFERENCES

Dunicz-Sokołowska A., Radomska K., Długaszek M., Graczyk A 2006. *Contents of bioelements and toxic metals in the Polish population determined by hair analysis.* Part. 1. *Children aged 1 to 10 years.* Magnes. Res., 34-35.

Johnson D.D., Eastridge J.S., Neubaure D.R., MC Gowan C.H. 1995. *Effect of sex class on nutrient content of meat from young goat.* J. Anim. Sc., 73: 296-301.

Krełowska-Kułas M. 1998. *Badanie zawartości metali w mięśniach i narządach wewnętrznych [Examination of the content of heavy metals in muscles and internal organs].* Zesz. Nauk. AR Kraków, 179-183. [in Polish]

Krupa J., Kogut B. 2000. *Zawartość kadmu i ołowiu w mięśniach, wątrobie i nerkach kóz i owiec z okolic Rzeszowa [Content of cadmium and lead in muscles, liver and kidneys of goats and sheep in the Rzeszów region].* Żywność, Nauka.-Technologia-Jakość, 1(22): 109-115. [in Polish]

Lidwin-Kaźmierkiewicz M., Rajkowska M., Protasowicki M. 2006. *Wpływ gotowania mięsa wołowego na zawartość Cd, Cu, Hg, Pb, Zn [The influence of beef cooking on the content of Cd, Cu, Hg, Pb and Zn].* Rocz. Nauk. PTZ, 2(1): 127-132. [in Polish]

Madruga M.S., Arruda S.G.B., Nascimento J.A. 1999. *Castration and slaughter age effects on nutritive of the mestico goat meat.* Meat. Sci., 52: 119-125.

Niedziółka R., Pieniak-Lendzion K., Horoszewicz E. 2007. *Concentration of Cd and Pb in the muscle, liver and kidney lambs and goat kids fattened in the Podlasie mountains.* Brit. Soc. Anim. Sci., 1, Proceedings, 98-99.

Oleszkiewicz J. 2007. *„Ołowiany diabeł" ['The lead devil']* J. Elementol., 12(2): 149-153. [in Polish]

Park Y.W. 1990. *Effect of breed, sex and tissues on concentrations of mocro-minerals in goat meat.* J. Food. Sci., 55, 308-311.

Pieniak-Lendzion K., Niedziółka R., Horoszewicz E. 2006. *Bioaccumulation of some metals in muscle tissue, liver and kidneys of young male and female goats.* Part II. Polish. J. Environ. Stud., 15 (2A): 456-458.

Rozporządzenie Ministra Zdrowia z dnia 27.12.2000 roku. *w sprawie wykazu dopuszczalnych ilości substancji dodatkowych i innych substancji obcych dodawanych do środków spożywczych lub używek, a także zanieczyszczeń, które mogą znajdować się w środkach spożywczych lub używkach*. Dz.U. z 2001 r., nr 7, poz. 72. [Oridnance of the Minister for Health of 27th December 2000, Journal of Law, 2001, no 7, item 72]. [in Polish]

Rozporządzenie Ministra Zdrowia z dnia 31.01.2003 r. *w sprawie maksymalnych poziomów zanieczyszczeń chemicznych i biologicznych które mogą znajdować się w żywności, składnikach żywności, dozwolonych substancjach dodatkowych, substancjach pomagających w przetwarzaniu albo na powierzchni żywności.* Dz.U., z 2003 r., nr 37, poz. 326. [Oridnance of the Minister for Health of 31st January 2003, Journal of Law, 2003, no 37, item 326] [in Polish]

Statistica – Przewodnik (Guide), 2002 – StatSoft z.o.o. Kraków.

Węglarz K. 2007. *Metale ciężkie – źródła zanieczyszczeń i wpływ na środowisko [Heavy metals – sources of pollution and effect on environment].* Wiad. Zoot., 3(254): 31-38. [in Polish]

Zięba M. 2003. *Zawartość metali ciężkich w tkankach owiec z regionu południowo-wschodniej Polski [Concentrations of heavy metals in tissues of sheep from south-eastern Poland].* Prz. Hod., 6: 21-24. [in Polish]

J. Elementol. 2009, 14(3): 509–516

MINERAL COMPOSITION OF HIGH BLUEBERRY LEAVES AND FRUITS DEPENDING ON SUBSTRATE TYPE USED FOR CULTIVATION*

Ireneusz Ochmian, Józef Grajkowski, Grzegorz Mikiciuk, Krystyna Ostrowska, Piotr Chełpiński

Pomology Department
West Pomeranian University of Technology in Szczecin

Abstract

In 2004-2006, an experiment was established at the Experimental Station Rajkowo belonging to the Agricultural University (West Pomeranian University of Technology) in Szczecin. High blueberry bushes were planted in an alkaline reaction (pH 7.1) heavy soil using three different substrates. The bushes were planted in trenches filled with peat, cocoa husk and sawdust. The fertilization of the plants was limited to a sole nitrogen supply (30 kg N ha^{-1}), because chemical analyses of both the soil and the substrates showed high and/or moderate content of other nutrients. In order to decrease cocoa husk reaction and to maintain the reaction of peat and sawdust, the bushes were irrigated with H_2SO_4 acidified water up to pH 2.5-3.5.

The bed prepared of cocoa husk was characterized by the highest N (23.8 mg 100 g^{-1}), Zn content (51.6 mg kg^{-1}), and pH (5.0). On the other hand, sawdust substrate had the lowest salinity (0.35 g NaCl kg^{-1}). Sawdust substrate showed the highest level of Mn (56.5 mg kg^{-1}) and Cu (7.4 mg kg^{-1}) and the lowest of Ca (83 mg 100 g^{-1}), whereas, peat substrate showed the highest salinity (0.87 g NaCl kg^{-1}) and the lowest pH (3.8). The usage of cocoa husk substrate resulted in the highest content of N (23.6), K (6.9 g kg^{-1}) and Mn (104.5 mg kg^{-1}) in cv. Sierra blueberry leaves as well as N (16.0) and K (6.5 g kg^{-1}) in berries. The plants grown in sawdust substrate showed the highest level of Ca (1.75 g kg^{-1}), Cu (3.2) and Zn (12.4 mg kg^{-1}), in the leaves as well as S (1.7) and Cu (3.5 mg kg^{-1}) in berries.

Key words: highbush blueberry, macro-, microelements, substrates, leaves, fruit.

dr Ireneusz Ochmian, Pomology Departament, West University of Technology, ul. Janosika 8, 71-424 Szczecin, Poland, phone: 091 449 61 63, e-mail: ochmian@go2.pl, ireneusz.ochmian@zut.edu.pl

*The study was supported by the grant of the Scientific Research Committee No.0395/P06/2004/26.

RÓŻNICE W SKŁADZIE CHEMICZNYM LIŚCI I OWOCÓW BORÓWKI WYSOKIEJ W ZALEŻNOŚCI OD RODZAJU PODŁOŻA ZASTOSOWANEGO DO UPRAWY

Abstrakt

W latach 2004-2006, w Sadowniczej Stacji Badawczej Katedry Sadownictwa Akademii Rolniczej w Szczecinie (obecnie Zachodniopomorski Uniwersytet Technologiczny), posadzono krzewy borówki wysokiej na glebie zwięzłej o odczynie zasadowym (pH 7,1) z zastosowaniem trzech różnych rodzajów podłoża. Krzewy posadzono w rowy wypełnione torfem, przekompostowaną łuską kakaową oraz trocinami z drzew iglasto-liściastych. Nawożenie roślin ograniczono do azotu (saletra amonowa 30 kg N ha^{-1}), z powodu wysokiej lub/i średniej zawartości pozostałych składników pokarmowych w glebie oraz podłożach. W celu obniżenia (łuska kakaowa) lub utrzymania niskiego odczynu podłoża (torf, trociny) w czasie okresu wegetacji krzewy nawadniano wodą zakwaszaną H_2SO_4 do odczynu 2,3-3,9.

Podłoże przygotowane z łuski kakaowej miało najwyższą zawartość azotu (23,8 mg 100 g^{-1}), cynku (51,6 mg kg^{-1}) oraz najwyższe pH (5.0), ponadto zawierało najmniej soli (0,35 g NaCl kg^{-1}). Podłoże przygotowane z trocin zawierało najwięcej manganu (56,5 mg kg^{-1}) i miedzi (7,4 mg kg^{-1}) oraz najmniej wapnia (83 mg 100 g^{-1}), natomiast było najbardziej zasolone (0,87 g NaCl kg^{-1}) i miało najniższy odczyn (3,8). Krzewy odmiany Sierra posadzone w łusce kakaowej zawierały najwięcej azotu (23,6), potasu (6,9 g kg^{-1}) i manganu (104,5 mg kg^{-1}) w liściach oraz azotu (16,0) i potasu (6.5 g kg^{-1}) w owocach. Natomiast rośliny posadzone w trocinach zawierały najwięcej wapnia (1,75 g kg^{-1}), miedzi (3,2) i cynku (12,4 mg kg^{-1}) w liściach oraz siarki (1,7) i miedzi (3,5 mg kg^{-1}) w owocach.

Słowa kluczowe: borówka wysoka, makro- i mikroelementy, podłoże, liście, owoce.

INTRODUCTION

Fruits of high blueberry are appreciated for their good taste and processing value as well as their dietary and healthy virtue. Because of its value, the species has been cultivated for years in North America. The first breeding project was launched in 1908 in Florida (LYRENE 1997) and from the USA highbush blueberries were imported to Europe (STRIK 2005). In Poland, first experiments on highbush blueberry cultivation were undertaken in 1946, although the development of large-scale production methods started in 1976 (SMOLARZ 2006). In recent years, considerable increase in blueberry production has been observed in Poland. In 2005, blueberry production in Poland was 3,500 t and in 2006 the growing area covered 2,100 ha (ENTROP 2006).

All plants of the *Ericaceae* family species have specific soil requirements, different from those of other orchard plants. The bushes are widespread in pine forests on acid and moisture soils (BLASING et al. 1987). Because such soils are less and less available, establishing new plantations has become more and more difficult. It is thought that cropping this plant is possible on mineral soil which is irrigated and bedded (MOORE 1993). In the United States, cotton by-products, pecan hulls (KREWER et al. 2002), pit-coal ash, sludge from sewage-treatment plants as well as peat are used for mulching (PLISZKA 1977).

Fertilization needs of the berries are not high in comparison with other species, but they are specific. The basic fertilizer component in berry cropping is nitrogen (KOZIŃSKI 2006, KREWER and NESMITH). When a plantation is covered by a sawdust bed, all fertilizer doses need to be increased by 50-100%. The berry does not require much phosphorus and displays no response to phosphorus fertilization (ECK 1985). It is highly recommendable to double fertilizer doses on peat soil (OSTROWSKI and MAZURCZAK 1991). Abundance of beds and soil in chemical components depends much on their origin, cultivation technology and, first of all, fertilization and irrigation (BRYLA 2008, KREWER and NESMITH).

From the practical and economical point of view, the components used for mulching should be relatively cheap, easily accessible, and should meet habitat requirements of the species. Therefore, the usage of agricultural and forest by-products for this purpose seems to be especially justified. The objective of the present study was to evaluate different substrates (cocoa husk, sawdust and peat) on chemical composition of fruits and leaves of highbush blueberry cv Sierra.

MATERIAL AND METHODS

The experiment was carried out in 2004-2006 at the Experimental Station Rajkowo belonging to the Agricultural University (West Pomeranian University of Technology) in Szczecin. The purpose of the field trial was to evaluate highbush blueberry cultivation in a neutral reaction (pH 7.1) heavy soil using three different substrates. Bushes of cv. Sierra were planted in ditches 35 cm deep and 100 cm wide filled by substrates. In this culture system, three types of substrates were used: acid muck soil (peat), conifer sawdust obtained from a local sawmill, and cocoa husk, a by-product obtained from Gryf Chocolate Confectionary Plant in Szczecin.

Table 1

Physicochemical properties of substrates used in the experiment

Specification	Peat	Cocoa husk	Sawdust
Field water capacity (% vv^{-1})	44.8	36.9	31.3
Full water capacity (% vv^{-1})	80.6	85.3	82.6
pH*	3.8	5.0	4.7
Soil salinity (g NaCl kg^{-1})	0.87	0.35	0.56

*The substrates reaction was measured in KCl at the end of highbush blueberry vegetative season.

Among the substrates tested, the greatest field water capacity was found for peat (44.8%) and the least for sawdust (31.3%). Regarding full water capacity, cocoa husk substrate was predominant (85.3%). These differences necessitated diversifying the watering of the substrates (Table 1).

Water used for peat substrate irrigation had higher pH (3.7-3.9) because peat reaction was suitable for blueberry cultivation. For cocoa husk and sawdust substrates, water of pH ~2.4 was used to depress reaction of these substrates (Table 2). Among the substrates tested, the peat maintained a constant pH 3.8 during field trial, while the highest reaction throughout all the experiment was observed for cocoa husk substrate (pH 5).

Table 2

Physicochemical properties of water used in the experiment

Raw water				Acidified water for sawdust and cocoa husk		Acidified water to irrigate peat	
Fe^{+3} (mg l^{-1})	Ca^{+2} (mg 11)	EC (mS cm^{-1})	pH	EC (mS cm^{-1})	pH	EC (mS cm^{-1})	pH
0.17	94.0	0.80	7.01	2.46	2.36	2.01	3.72

The supplemental irrigation was applied through the drip line type T-Tape with acidified water (with H_2SO_4 up to pH 2.5-3.9 measured in H_2O). The intensity of water supply was adjusted to soil moisture by means of the tensiometer monitoring twice a week. Measuring tubes (30 cm) were installed 15 cm below the soil surface and 2.2 PF was used as a threshold value for irrigation. Having reached the threshold, the soil was irrigated to approximately 1.0 PF.

The fertilization of the plants was limited to nitrogen supply only because chemical analyses of both the soil and the substrates showed high and/or moderate content of other nutrients. Each type of media used in the raised bed system was fertilized with the ammonium nitrate on three occasions at a total dose of 30 kg N ha^{-1}. The fertilizer was spread evenly on the bed tops at the width of 1 m.

The chemical analyses were carried out on the leaves and fruits collected from 4-6 year old bushes. One hundred leaves out of plants growing on a particular substrate were sampled each year at the beginning of August for chemical analyses. The fruits of each harvest in the season were packed in polyethylene bags (250 g) and kept frozen (-25°C) until analyzed. Having completed fruit collecting, all fruit samples were combined, thawed at room temperature and then dried. The dried fruit were pulverized. The content of macro- and microelements was determined according to the Polish Standards. Total nitrogen content was determined with Kjeldahl's method. The content of K and Ca was measured with the atomic emission spectrometry, whereas Mg, Cu, Zn, Fe and Mn content was determined with the flame atomic absorption spectroscopy. Phosphorus content was determined with Barton's method.

The results were subjected to statistical analysis using Statistica 7.1. Each year, the study trial consisted of a a randomized block design in three replications. The analysis of variance in the form of 3-year synthesis for a fixed model was used. The values were evaluated by Duncan's test and the differences at $P < 0.05$ were considered significant.

RESULTS AND DISCUSSION

The cocoa husk bed showed higher content of available P (17.16 mg 100 g^{-1}) compared to sawdust (7.54 mg 100 g^{-1}), although both substrates were not significantly different from peat (Table 3). During the experiment, this form of phosphorus significantly decreased in the peat bed because of irrigating with acidified water. In contrast, it increased in the cocoa husk bed (data not presented). The higher level of P in cocoa husk could be an after-effect of mineralization of organic matter. MERCIK and SAS (1998) also observed that P content decreased in low pH beds, although visual evaluation of plants and analyses of leaves did not prove any deficiency of this element.

Table 3

Content of soil reaction, absorbing macro- and microelements in used beds – synthesis of years 2004-2006

Macro- and microelements	Peat	Sawdust	Cocoa husk
mg 100 g^{-1}			
Total N	11.18 *b**	6.20 *a*	23.84 *c*
P	13.04 *ab*	7.54 *a*	17.16 *b*
K	29.65 *a*	52.49 *b*	51.26 *b*
Ca	170.1 *b*	83.4 *a*	208.5 *b*
Mg	28.62 *a*	21.94 *a*	26.71 *a*
mg kg^{-1}			
Cu	1.81 *a*	7.36 *b*	1.58 *a*
Zn	12.65 *a*	25.93 *a*	51.66 *b*
Fe	202 *a*	231 *ab*	279 *b*
Mn	32.03 *a*	56.47 *b*	24.13 *a*

*Means values marked with the same letter do not differ significantly at $P < 0.05$ according to Duncan range test.

The level of K found in the peat bed was over two-fold lower than in the cocoa husk and sawdust beds (~52 mg 100 g^{-1}). During the three years of the experiment, K level also decreased in the substrates due to the uptake by plants and migration into deeper layers of the soil. However, its content in the beds met fertilizer requirements of the plants.

The peat and cocoa husk substrates were characterized by higher Ca content. Moreover, cocoa husk showed highest N, Zn, and Fe content, whereas sawdust had the highest level of Mn and Cu.

In general, the leaves exhibited higher accumulation of macro- and microelements compared to berries (Table 4). Both leaves and fruits originating from bushes grown in cocoa husk substrate were characterized by the highest total N (23.56 and 16.03 g kg^{-1}) and K (6.87 and 6.54 g kg^{-1}) content. According to HANSON (2006), the sufficient range of N for leaves collected in mid-summer is between 1.7-2.1%. In this assay, all the plants showed similar or higher values. On the other hand, nitrogen content in fruit varied from 1.4% (14.22 g kg^{-1}) to 1.6% (16.03 g kg^{-1}); for comparison, SKUPIEŃ (2004) obtained 1.7-2.8% N divergence in four cultivars of blueberries. The highest P content was found in the leaves of plants grown in the cocoa husk bed and the lowest in that of sawdust. The fruit-P was not substrate dependent.

Table 4

Macro- and microelements content in leaf and fruit of blueberry 'Sierra' cultivar – synthesis of years 2004-2006

Macro- and microelements*	Leaves			Fruit		
	peat	sawdust	cocoa husk	peat	sawdust	cocoa husk
	g kg^{-1} d.w.					
Total N	21.79 *a*	21.03 *a*	23.56 *b*	14.53 *a*	14.22 *a*	16.03 *b*
P	1.08 *ab*	0.91 *a*	1.22 *b*	1.06 *a*	1.07 *a*	1.15 *a*
K	5.66 *a*	5.53 *a*	6.87 *b*	5.99 *a*	5.90 *a*	6.54 *b*
Ca	0.75 *a*	1.75 *b*	0.90 *a*	0.10 *a*	0.10 *a*	0.06 *a*
Mg	1.03 *a*	1.18 *a*	1.02 *a*	0.40 *a*	0.39 *a*	0.34 *a*
S	2.20 *b*	1.66 *a*	1.48 *a*	1.16 *a*	1.68 *b*	1.16 *a*
mg kg^{-1} d.w.						
Cu	0.92*a*	3.24 *b*	1.28 *a*	0.79 *a*	3.52 *b*	1.35 *a*
Zn	9.09 *a*	12.38 *b*	10.10 *a*	6.31 *a*	11.31 *b*	11.37 *b*
Fe	58.74 *a*	62.90 *b*	60.50 *ab*	22.37 *a*	29.92 *b*	26.40 *ab*
Mn	73.86 *a*	89.56 *b*	104.47 *c*	33.62 *c*	27.21 *b*	21.55 *a*

*The values are presented on dry weight basis.

Further, the largest amount of Ca was found in the leaves of plants cultivated in sawdust (1.75 g kg^{-1}) whereas for fruits it was at an approximate level regardless the bed used (0.06-0.1 g kg^{-1}). Both for leaves and fruits no effect of substrate was observed regarding Mg level. For 'the cultivars Spartan, Bluecrop, Jersey and Blueray SKUPIEŃ (2004) determined lower Mg content in fruit (0.16-0.18 g kg^{-1} f.w. after recalculation).

The usage of peat in the assay favored S accumulation in the leaves (2.20 g kg^{-1}) whereas the usage of sawdust enhanced S level in berries (1.68 g kg^{-1}) compared to other substrates. GLONEK and KOMOSA (2006) found 1.1--1.2 g S kg^{-1} leaf d.w. (after recalculation) irrespective of the fertilization rate applied.

The highest level of Cu was measured in leaves and fruit when the sawdust medium was used (3.24 and 3.52 mg kg^{-1}, respectively). Regarding Zn content, it was the highest in leaves of plants grown in sawdust (12.38 mg kg^{-1}) and berries of plants on the cocoa husk and sawdust substrates (~11.3 mg kg^{-1}). SKUPIEŃ (2004) found much lower fruit Cu (0.17-0.30 mg kg^{-1} f.w.) and Zn range (1.08-1.30 mg kg^{-1} f.w.).

The leaves and fruits of bushes grown in sawdust showed the highest Fe content (62.9 and 29.9 mg kg^{-1}, respectively) whereas the lowest iron concentration was found in plants grown in peat (58.7 and 22.4 mg kg^{-1}, respectively). Over 36-37 μg g^{-1} of iron was found by MERHAUT and DARNELL (1996).

The use of cocoa husk enhanced Mn (104.47 mg kg^{-1}) level in leaves, while plants originating from peat showed an increased Mn level in fruits. GLONEK and KOMOSA (2006) found similar Fe (53.9-57.7 mg kg^{-1}) and Mn (107.6-128.0 mg kg^{-1}) leaf status, which slightly increased in fertilized plants compared to control ones.

CONCLUSIONS

1. The bed prepared of cocoa husk was characterized by the highest N and Zn content and pH despite substrate irrigation with acidified water (pH 2.3-2.5) for many years. On the other hand, sawdust substrate had the lowest salinity. Sawdust substrate showed the highest level of Mn and Cu and the lowest of Ca, whereas peat substrate showed the highest salinity and the lowest pH.

2. The use of cocoa husk substrate resulted in the highest content of N, K and Mn in cv. Sierra blueberry leaves as well as N and K in berries.

3. The plants grown in sawdust substrate showed the highest level of Ca, Cu and Zn in leaves as well as S and Cu in berries.

4. The three-year study showed that it is possible to cultivate highbush blueberries in clay soil of alkaline reaction provided that the plants be grown in trenches filled with peat or sawdust substrates and drip irrigation with acidified water be applied.

REFERENCES

Blasing D., Naumann W.D., Hartge K.H. 1987. *Einfluss des Standortes auf die Entwicklung von Kulturheidelbeeren (Vaccinium corymbosum L.).* I. *Erhebung in erwerbsmassig genutzten Kulturheidelbeeranlagen auf humosen Sandboden.* Gartenbauwissenschaft, 52 (4): 185-191.

Bryla, D.R. 2008. *Water requirements of young blueberry plants irrigated by sprinklers, microsprays and drip*. Acta Hort., 792: 135-139.

Eck, P. 1985. *Response of highbush blueberry on a berryland sand to potassium fertilization*. Acta Hort., 165: 227-228.

Glonek J., Komosa A. 2006. *The effect of fertigation on the nutrient status and yield of highbush blueberry cv. 'Bluecrop'*. Acta Hort., 715: 371-374.

Entrop A-P. 2006. *Heidelbeeerlehrfahrt zur "Blueberry World" nach Venlo/ Nlederlande am 23/24.6.2005*. Mitt.OVR 61 (1): 19-24.

Hanson E.J. 2006. *Nitrogen fertilization of highbush blueberry*. Acta Hort., 715: 347-351.

Kozinski B. 2006. *Influence of mulching and nitrogen fertilization rate on growth and yield of highbush blueberry*. Acta Hort., 715: 231-236.

Krewer G., Ruter J., NeSmith D. S., Clark J., Otts T., Scarborough S., Mullinix B., Hepp R. F., 2002. *Performance of low cost organic materials as blueberry substrates and soil amendments*. Acta Hort., 574: 273-279.

Krewer G., NeSmith D. S. *Blueberry fertilization in soil***.** University of Georgia Ext. Fruit Publication 01: 1. http://www.smallfruits.org/Blueberries/production/blueberryfert.pdf .

Lyrene PM. 1997. *Value of various taxa in breeding tetraploid blueberries in Florida*. Euphytica, 94: 15–22.

Mercik S., Sas L. 1998. *Ujemny wpływ nadmiernego zakwaszenia gleby na rośliny [Negative influence on excssive acidification of soil on plants]*. Zesz. Probl. Post. Nauk Rol., 456: 29-39. [in Polish]

Merhaut D.J. Darnell R.L. 1996. *Vegetative growth and nitrogen/karbon partitioning In blueberry as influence by nitrogen fertilization*. J. Amer. Hort. Sci., 121(5): 875-879.

Moore JN**.** 1993. *Adapting low organic upland mineral soils for culture of highbush blueberries*. Acta Hort., 346: 221-229.

Ostrowski W., Mazurczak G. 1991. *Zakładanie i prowadzenie towarowej plantacji borówki wysokiej [Establishing and running a commercial plantation of high berry]*. AR, Szczecin. [in Polish]

Pliszka, K. 1977. *A primary study on growth and development of highbush blueberries in central Poland*. Acta Hort., 6: 153-162.

Skupień K. 2004. *Zawartość wybranych składników mineralnych w owocach czterech odmian borówki wysokiej (Vaccinium corymbosum L.) [Content of some mineral components in fruits of four cultivars of high berry (Vaccinium corymbosum L.)]*. J. Elementol., 9(1): 43-49. [in Polish]

Smolarz, K. 2006. *History of highbush blueberry (V. corymbosum L.) growing in Poland*. Acta Hort., 715: 313-316.

Strik B. 2005. *Blueberry: an expanding world berry crop*. Chron. Hort., 45 (1): 7-12.

J. Elementol. 2009, 14(3): 517–525

DISTRIBIUTION OF NICKEL FRACTIONS IN FOREST LUVISOLS IN THE SOUTH PODLASIE LOWLAND

Krzysztof Pakuła, Dorota Kalembasa

Chair of Soil Science and Plant Nutrition
University of Podlasie

Abstract

Sequential extraction methods enable identification of chemical fractions of heavy metals in soil environment as well as evaluation of their availability and potential toxicity to biotic elements of a trophic chain. The study aimed at separating nickel fractions from particular genetic horizons of forest Luvisols by means of three sequential extraction methods (modified Tessier's, Zeien and Brümmer's as well as Hedley's with Tiessen and Moir's modifications methods), and to compare the metal content in four fractions: easily soluble, exchangeable, organic, and residual, along with their distribution within studied soils' profiles. Nickel concentrations in the examined fractions varied: the largest amounts of the heavy metal (regardless of the analytical procedure applied) were found in residual fraction F_{resid} (mineral horizons) and organic fraction F_{org} (forest litter horizons – Ol), while the smallest ones occurred in easily soluble fraction F1 (all genetic horizons). Statistical processing revealed significant dependences between the four nickel fractions as well as between the fractions and selected properties of analyzed soils (except soil pH and total nickel content Ni_t).

Keywords: sequential extraction, nickel fractions, Luvisols, forest.

ROZMIESZCZENIE FRAKCJI NIKLU W LEŚNYCH GLEBACH PŁOWYCH NA NIZINIE POŁUDNIOWOPODLASKIEJ

Abstrakt

Metody ekstrakcji sekwencyjnej umożliwiają identyfikację frakcji chemicznych metali ciężkich w środowisku glebowym, a także ocenę ich dostępności i potencjalnej toksyczności dla biotycznych elementów łańcucha troficznego. Celem pracy było wydzielenie frakcji ni-

dr inż. Krzysztof Pakuła, Chair of Soil Science and Plant Nutrition, University of Podlasie, ul. Prusa 14, 08-110 Siedlce, Poland, pakulak@ap.siedlce.pl; kalembasa@ap.siedlce.pl

klu w poszczególnych poziomach genetycznych leśnych gleb płowych trzema metodami ekstrakcji sekwencyjnej (zmodyfikowanej metody Tessiera, metody Zeiena i Brümmera oraz metody Hedleya w modyfikacji Tiessena i Moira), a także porównanie zawartości tego metalu w czterech frakcjach: łatwo rozpuszczalnej, wymiennej, organicznej i rezydualnej oraz ocena ich rozmieszczenia w profilu badanych gleb. Zawartość niklu w badanych frakcjach była zróżnicowana. Najwięcej tego metalu, niezależnie od zastosowanej procedury analitycznej, stwierdzono we frakcji rezydualnej – F_{resid} (poziomy mineralne) oraz organicznej – F_{org} (poziomy ściółki leśnej – Ol), a najmniej we frakcji łatwo rozpuszczalnej – F1 (wszystkie poziomy genetyczne). Obliczenia statystyczne wykazały, że badane cztery frakcje niklu były wysoko istotnie zależne między sobą oraz wybranymi właściwościami analizowanych gleb (z wyjątkiem pH gleby i zawartości ogólnej Ni_t).

Słowa kluczowe: ekstrakcja sekwencyjna, frakcje niklu, gleby płowe, las.

INTRODUCTION

Environmental pollution may contribute to the accumulation of heavy metals, which is an important link in bio-geochemical cycles of elements. Nickel is a heavy metal which is essential for the growth and development of living organisms, but in excess can be toxic (Kabata-Pendias, Pendias 1999). Different (regarding their solubility) chemical fractions (forms), the percentage of which depends on the soil properties (pH value, content of organic and mineral colloids, organic matter, redox potential, and parent rock's abundance), make up the total content of this metal in soil. Sequential extraction methods enable identification of chemical fractions of metals, their mobility within soil environment as well as their availability and potential toxicity to biotic elements of a trophic chain (Kalembkiewicz, Sočo 2005).

The research was aimed at examining the nickel content in four fractions extracted by means of three sequential extraction methods as well as at comparing their distribution within the profile of forest Luvisols.

MATERIAL AND METHODS

Luvisols developed from loams and localized within three forest complexes in the South Podlasie Lowland were studied. The following were determined in air-dried soil samples collected from particular genetic horizons of the three profiles: granulometric composition of mineral horizons by aerometric method (according to PN-R-04033); pH in 1 mol $KCl \cdot dm^{-3}$ (pH_{KCl}) by potentiometry; soil's sorption capacity (T = CEC) on the basis of hydrolytic acidity value (Hh) and sum of basic cations (S) determined by means of Kappen's method; organic carbon content (Corg) by the oxidation-titrimetric method (Kalembasa, Kalembasa 1992); total nickel content (Ni_t) by the ICP-EAS technique after wet digestion in concentrated nitric acid in a microwave system. Nickel sequential fractionation was made applying three analytical procedures (Table 1): modified Tessier's method (own modifications consisted in adjusting the extraction solutions to determinations by means of ICP-EAS) (TESSIER et al. 1979, KALEMBASA, PAKUŁA 2006), Zeien and Brümmer's method (ZEIEN, BRÜMMER 1989), as well as Hedley's method with Ties-

Table 1

Nickel sequential extraction methods used in the examined forest Luvisols

Fraction	Extraction reagent and condition
Tessier et al. method (in own modification)	
F1 easily soluble	20 cm^3 deionized H_2O, pH=7, shake for 1 h
F2 exchangeable	20 cm^3 1 mol $NH_4Cl \cdot dm^{-3}$, pH=5.5, shake for 1 h
F3 bound to carbonates	20 cm^3 1 mol $CH_3COOH \cdot dm^{-3}$, pH=3, shake for 5 h
F4 bound to Fe-Mn oxides	40 cm^3 0.2 mol $(NH_4)_2C_2O_4 \cdot dm^{-3}$ + 0.2 mol $H_2C_2O^4 \cdot dm^{-3}$, pH=3, shake for 4 h
F_{org} bound to organic matter	40 cm^3 0.1 mol $NaOH \cdot dm^{-3}$, pH=12.5, shake for 3 h
F_{resid} residual*	
Zeien and Brümmer's method	
F1 easily soluble	20 cm^3 unbuffered 1 mol $NH_4NO_3 \cdot dm^{-3}$, shake for 24 h
F2 exchangeable	20 cm^3 1 mol $CH_3COONH_4 \cdot dm^{-3}$, pH=6, shake for 24 h
F3 bound to MnOx	20 cm^3 1 mol $NH_2OH \cdot HCl \cdot dm^{-3}$ + 1 mol $CH_3COONH_4 \cdot \cdot dm^{-3}$, pH=6, shake for 0.5 h
F_{org} bound to organic matter	20 cm^3 0.025 mol $C_{10}H_{22}N_4O_8 \cdot dm^{-3}$, pH=4.6, shake for 1.5 h
F5 bound to amorphic FeOx	40 cm^3 0.2 mol $(NH_4)_2C_2O_4 \cdot dm^{-3}$+0.2 mol $H_2C_2O_4 \cdot dm^{-3}$, pH=3, shake for 4 h
F6 bound to crystalline FeOx	40 cm^3 0.2 mol $(NH_4)_2C_2O_4 \cdot dm^{-3}$+0.2 mol $H_2C_2O_4 \cdot dm^{-3}$ + 0.1 mol $C_6H_8O_6 \cdot dm^{-3}$, pH=3, 0.5 h in boiling water
F_{resid} residual*	
Hedley's method modified by Tiessen and Moir	
F1 easily soluble	20 cm^3 deionized H_2O, pH=7, shake for 1 h
F2 exchangeable	20 cm3 0.5 mol $NaHCO_3 \cdot dm^{-3}$, pH=8.5, shake for 16 h
F_{org} bound to organic matter	20 cm^3 0.1 mol $NaOH \cdot dm^{-3}$, pH=12.5, shake for 16 h
F4 bound to carbonates	20 cm^3 1 mol $HCl \cdot dm^{-3}$, shake for 16 h
F5 bound to stable organic-min. and mineral compounds	10 cm^3 conc. HCl in a water bath at 80°C for 10 min
F_{resid} residual*	

* Calculation as difference between total content of nickel and sum of the above determined fractions.

sen and Moir's modifications (referred to as Hedley's method in further parts of present paper) (TIESSEN, MOIR 1993).

Soil samples (1 g) were subjected to extraction with sequentially replaced extraction solutions. After centrifuging, particular nickel fractions were determined in the extracts by means of the ICP-EAS technique. The accuracy of the analytical procedures was confirmed by applying the standard addition method to every analyzed sample. Control samples consisting of used solutions were also included in the analyses.

In order to compare the applied sequential extraction schemes, four fractions defined as easily soluble (water-soluble F1), exchangeable (F2), bonded to organic matter (organic fraction F_{org}), and residual (F_{resid}) fractions were selected from six fractions extracted with Tessier's and Hedley's methods, and from seven fractions obtained with Zeien and Brümmer's method. The four fractions were selected because they could be directly separated in particular analytical procedures and because the percentage of their total amount in the soil best represents a diverse character of the analyzed bindings with solid phase components in the soil, from the most labile (fractions F1 and F2), through potentially labile (F_{org}), to very stable mineral and organic-mineral complexes (F_{resid}).

The following values were calculated for particular genetic horizons of soils: nickel enrichment coefficient (WW) in relation to its content in the mother rock, and mobility index (WM) as the percentage of the sum of F1 and F2 fractions, which enables evaluation of the current bio-availability of nickel.

Dependencies between total nickel content and its quantities in particular fractions as well as selected soil properties were evaluated by means of linear correlation.

RESULTS AND DISCUSSION

The physical, physicochemical and chemical properties of soils chosen for the study are characteristic for forest Luvisols developed from material deposited during the Middle-Polish glaciation (Table 2). The total nickel content ranged from 4.75 to 20.2 $mg \cdot kg^{-1}$ in the soils, which did not exceed the geochemical background level for boulder loam deposits (CZARNOWSKA 1996). The differences in the metal concentration were probably caused by the dual structure of the soil profile (surface horizons consisted of sandy deposits, while enrichment horizons and parent rock were made of loamy deposits). Particular genetic horizons were were ordered according to the total nickel content as floows: IIBt > IIC > Ol > Ah > Eet. The distribution of nickel in the soil profile resulted from a great abundance of this metal in the parent rock (low values of enrichment coefficient WW in sandy horizons Ah

Table 2

Some properties (mean* and ranges** for three soil profiles) of the forest Luvisols

Genetic horizon	Sand 2-0.05	Silt 0.05-0.002	Clay <0.002	pH_{KCl}	CEC***	Corg	Ni_t****	WW*****
	% fraction of diameter in mm				$mmol(+)\cdot kg^{-1}$	$g\cdot kg^{-1}$	$mg\cdot kg^{-1}$	
Ol	-	-	-	-	631.0	458.0	17.8	0.99
	-	-	-	4.81-5.16	545.0-700.0	443.0- 472.0	14.5-19.2	0.74-1.18
Ah	72*	23	5	-	89.4	16.1	5.70	0.32
	69-76**	19-25	4-6	3.08-3.36	75.2-107.0	12.0-20.3	4.98-6.35	0.25-0.39
Eet	77	19	4	-	48.9	3.67	4.75	0.26
	73-80	17-22	3-5	3.55-3.65	43.6-54.0	2.30-5.00	3.76-5.87	0.19-0.36
	44	26	30	-	170.0	1.83	20.2	1.12
IIBt	37-53	23-31	24-32	3.32-3.48	146.0-187.0	1.60-2.00	18.3-21.8	1.11-1.13
	43	31	26	-	289.0	1.42	18.0	-
IIC	35-53	25-37	22-28	6.81-7.43	238.0-388.0	1.30-1.60	16.2-19.6	-

***CEC – Cation Exchenge Capacity
****total content of nickel
*****enrichment coefficient values

and Eet) and soil-forming lessivage processes (in mineral horizons), as well as biological accumulation (in organic horizons), which was confirmed by the highest values of WW index in Bt and Ol horizons. KWASOWSKI et al. (2000), and UZIAK et al. (2001) reported similar nickel distribution in forest Luvisols. KABATA-PENDIAS AND PENDIAS (1999) as well as KONECKA-BETLEY et al. (1999) underline that nickel occurrence in a soil profile corresponds to its content in parent rocks (C), whereas its elevated concentrations in surface horizons (Ol) result from biological accumulation and anthropopression.

Different shares of the nickel fractions determined by means of the three sequential extraction methods were recorded in the soils (Table 3). The mean percentage of the nickel fractions in its total content for particular genetic horizons can be presented in a form of the following sequences:

Ol: organic (30.2) > residual (25.5) > exchangeable (13.8) > easily soluble (12.1);

Ah: residual (42.5) > organic (6.93) > exchangeable (5.88) > easily soluble (2.34);

Eet: residual (48.6) > exchangeable (5.60) > organic (5.44) > easily soluble (1.70);

IIBt: residual (63.4) > exchangeable (1.19) > organic (0.75) > easily soluble (0.61);

IIC: residual (71.5) > organic (0.51) > exchangeable (0.39) > easily soluble (0.29).

In the mineral horizons of the analyzed soils, nickel dominated in the residual fraction (F_{resid}) – Table 3. Most of the nickel in this fraction was separated by means of Tessier's method (73.59 %), while the least applying

Table 3

The mean percentage contribution of selected nickel fractions in the soils

Genetic horizon	Fraction				WM*
	F1	F2	F_{org}	F_{resid}	(%)
Tessier et al. method					
Ol	9.38	13.90	32.21	25.36	23.28
Ah	1.56	6.14	7.02	53.10	7.70
Eet	1.13	5.99	5.44	65.80	7.12
IIBt	0.43	1.19	0.72	85.24	1.62
IIC	0.25	0.38	0.52	90.20	0.63
Zeien and Brümmer method					
Ol	17.70	15.10	23.10	35.63	32.80
Ah	3.92	6.53	5.22	43.47	10.45
Eet	2.85	6.21	4.38	46.10	9.06
IIBt	0.97	1.54	0.58	65.43	2.51
IIC	0.39	0.48	0.41	77.70	0.87
Hedley method in modification by Tiessen and Moir					
Ol	9.28	12.50	35.20	15.57	21.78
Ah	1.55	4.98	8.55	30.90	6.53
Eet	1.13	4.59	6.51	33.80	5.72
IIBt	0.43	0.84	0.94	39.47	1.27
IIC	0.24	0.31	0.61	46.60	0.55

Fraction: F1– easy soluble, F2 – exchangeable, F_{org} — ound to organic matter, F_{resid} — esidual;
*mobility coefficient values

Hedley's method (37.69%, on average). Lower contribution of nickel in the residual fraction (Hedley's method) resulted from the extraction of the previous fraction (F5) using concentrated HCl, which made the metal release from more eroded minerals and less durable organic-mineral bindings.

The lowest amount of nickel from the residual fraction was recorded in organic horizons (Ol) (from 15.57 to 35.65 %), and its contribution increased with the profile's depth, reaching the maximum values in the parent rock. A similar profile distribution of this nickel fraction in soils was also observed by ABOLLINO et al. (2002) as well as KAASALAINEN, YLI-HALLA (2003). KABATA--PENDIAS, PENDIAS (1999) reported that nickel in mineral horizons is characterized by great affinity to clay minerals, while in organic horizons, it is chelated by organic matter. PALUMBO et al. (2000), ANDERSEN et al. (2002) and KRÓLAK (2004) determined from 60.0 to 84.0 % of the residual fraction of nickel in Luvisols (agricultural and forest).

In the organic fraction (F_{org}), the largest percentage of nickel was determined in forest litter horizons (Ol); the largest amounts were separated with Hedley's method (35.20 %), while the smallest ones were obtained with Zeien and Brümmer's method (23.10 %) – Table 3. IThis confirmed a more selective action of NH_4-EDTA (Zeien and Brümmer's method) compared to

NaOH solution (applied in the other two methods), which may destabilize relatively strong organic-mineral bindings, including bonds with iron and manganese oxides (Hlavay et al. 2004). Distribution of nickel in the organic fraction of the soil profile, regardless of the applied analytical procedure, decreased with the depth, which was also observed by Palumbo et al. (2000) and Abollino et al. (2002), who determined the largest quantities of organic nickel bindings in surface horizons: 14.0% and 1.56%, respectively. In organic horizons of acidic soils, organic nickel forms prevail. They may be transported inside the soil profile, where they are mineralized (Andersen et al. 2002).

The highest nickel contribution (9.28-17.70%) in the bio-available fractions, easily soluble (F1) and exchangeable (F2), were found in the organic horizons (Ol), while the lowest ones (0.24-0.48%) were in the parent rock (C) – Table 3. The lowest amounts of nickel were extracted by means of Tessier's and Hedley's method, while the highest ones – with Zeien and Brümmer's method, which was confirmed by the calculated values of mobility index (WM). Hlavay et al. (2004) as well as Kalembkiewicz, Sočo (2005) also reported higher efficiencies of NH_4NO_3 and CH_3COONH_4 solutions for separating easily soluble and exchangeable fractions as compared to chlorides (NH_4Cl) and de-ionized water. Królak (2004) found about 12.0% available nickel fractions in humus horizons of arable soils, while Palumbo et al. (2000) from 1.2 to 2.9% its total content. Kaasalainen and Yli-Halla (2003) recorded 11.0-16.0% of nickel in F1 and F2 fractions of surface horizons of contaminated soils. Decrease of easily soluble and exchangeable fractions with the depth was observed by Abollino et al. (2002) and Andersen et al. (2002).

On the basis of the linear correlation coefficients (Table 4), highly significant dependence was verified between nickel content in particular fractions (extracted using the three analytical procedures): easily soluble, exchangeable, organic (positive) as well as residual (negative) in the examined forest Luvisols. Statistical analysis revealed highly significant negative influence of organic carbon content (C_{org}) and sorption capacity – CEC (except Zeien and Brümmer's methods) as well as a positive effect of clay fraction (ř < 0.002 mm) on nickel concentration in the residual fraction. Stable and non-exchangeable nickel bindings with the mineral part of soil (colloidal particle size) determine the contribution of F_{resid} in the total amount of the metal in soil. Nickel levels in F1, F2, and F_{org} fractions were correlated with C_{org} and CEC values (positively) as well as clay amount (negatively). Susceptibility of nickel towards chelate bindings with organic matter determines great mobility of the heavy metal. Bio-available fractions can be exchangeably adsorbed on soil colloids. Palumbo et al. (2000) as well as Kaasalainen and Yli-Halla (2003) reported significant influence of the soil properties (organic carbon content, sorption capacity, and clay fraction < 0.002 mm content) on nickel speciation in the soil environment. No effects of the total nickel content (Ni_t) and soil pH on the metal speciation in the examined soils were found.

Table 4

The coefficient values of the correlation between the fractions of nickel and some properties of the soils

Specification	F1	F2	F_{org}	F_{resid}	Ni_t	pH_{KCl}	C_{org}	CEC	Ø < 0.002
Tessier et al. method									
F1	x	0.927*	0.996*	-0.892*	0.215	0.133	0.993*	0.837*	-0.564*
F2		x	0.954*	-0.985*	-0.150	-0.113	0.879*	0.602*	-0.801*
F_{org}			x	-0.925*	0.134	0.094	0.981*	0.799*	-0.628*
F_{resid}				x	0.201	0.173	-0.836*	-0.561*	0.808*
Zeien and Brümmer method									
F1	x	0.956*	0.999*	-0.726*	0.160	0.089	0.984*	0.806*	-0.605*
F2		x	0.967*	-0.889*	-0.120	-0.106	0.892*	0.622*	-0.783*
F_{org}			x	-0.746*	0.118	0.085	0.977*	0.790*	-0.640*
F_{resid}				x	0.380	0.383	-0.598*	-0.221	0.861*
Hedley method in modification by Tiessen and Moir									
F1	x	0.951*	0.994*	-0.915*	0.213	0.131	0.993*	0.836*	-0.565*
F2		x	0.978*	-0.981*	-0.087	-0.065	0.909*	0.653*	-0.769*
F_{org}			x	-0.945*	0.112	0.077	0.975*	0.786*	-0.643*
F_{resid}				x	0.109	0.234	-0.866*	-0.575*	0.736*

* significant at $\alpha = 0.01$

CONCLUSIONS

1. In the examined forest Luvisols, the total nickel content did not exceed the geochemical background level for boulder loam deposits in Poland. Particular genetic horizons of these soils can be lined up in the following sequence for mean nickel concentrations: IIBt > IIC > Ol > Ah > Eet.

2. Sequential fractionation of nickel in particular genetic horizons by means of Tessier's, Zeien and Brümmer's, and Hedley's methods revealed that its content in the separated fractions varied. Organic horizons of the forest litter (Ol) contained the largest amounts of easily soluble, exchangeable, and organic nickel fractions, while parent rock horizons (C) were the richest in the residual fraction.

3. Chemical analyses revealed that the largest amounts of nickel were contained in easily soluble and exchangeable fractions separated applying Zeien and Brümmer's method; in organic fraction – Hedley's method; and in stable bindings of residual fraction – Tessier's method.

4. Statistical processing revealed that nickel concentration in the four analyzed fractions was highly significantly correlated (positively or negatively) between those fractions as well as with organic carbon content (C_{org}), sorption capacity and clay fraction level in forest Luvisols.

REFERENCES

ABOLLINO O., ACETO M., MALANDRINO M., MENTASTI E., SARZANINI C., PETRELLA F. 2002. *Heavy metals in agricultural soils from Piedmont, Italy. Distribution, speciation and chemometric data treatment*. Chemosphere, 49: 545–557.

ANDERSEN M.K., RAULUND-RASMUSSEN K., HANSEN C.B., STROBEL B.W. 2002. *Distribution and fractionation of heavy metals in pairs of arable and afforested soil in Denmark*. Eur. J. Soil Sci., 53: 491-502.

CZARNOWSKA K. 1996. *Ogólna zawartość metali ciężkich w skałach macierzystych jako tło geochemiczne gleb [Total content of heavy metals in parent rock as a geochemical background of soils]*. Rocz. Glebozn., 47, supl.: 43-50. [in Polish]

HLAVAY J., PROHASKA T., WEISZ M., WENZEL W.W., STINGEDER G. J. (2004). *Determination of trace elements bound to soils and sediment fractions (IUPAC technical report).* Pure Appl. Chem., 76 (2): 415-442.

KABATA-PENDIAS A., PENDIAS H. 1999. *Biogeochemia pierwiastków śladowych [Biogeochemistry of trace elements].* Wyd. PWN, Warszawa, 398 pp. [in Polish]

KALEMBASA S., KALEMBASA D. 1992. *The quick method for the determination of C:N ratio in mineral soils.* Pol. J. Soil Sci., 25 (1): 41-46.

KALEMBASA D., PAKUŁA K. 2006. *Fractions of zinc and copper in the forest Luvisols of the South Podlasie Lowland*. Pol. J. Environ. Stud., 15 (2a): 98-113.

KALEMBKIEWICZ J., SOČO E. 2005. *Ekstrakcja sekwencyjna metali z próbek środowiskowych [Sequential extraction of metals from environmental samples].* Wiad. Chem., 59 (7-8): 697-721. [in Polish]

KAASALAINEN M., YLI-HALLA M. 2003. *Use of sequential extraction to assess metal partitioning in soils.* Environ. Pollut., 126: 225–233.

KONECKA-BETLEY K., CZĘPIŃSKA-KAMIŃSKA D., JANOWSKA E. 1999. *Przemiany pokrywy glebowej w Kampinowskim Parku Narodowym (1991-1994) [Soil cover modifications in Kampinoski National Park (1991-19940.* Rocz. Glebozn., 50 (4): 5-29. [in Polish]

KRÓLAK E. 2004. *Zależność pomiędzy zawartością metali ciężkich w opadzie całkowitym, glebie i roślinie wskaźnikowej Taraxacum sp. na Nizinie Południowopodlaskiej. [Dependence between content of heavy metals in total rainfall, soil and indicator plant Taraxacum sp. in the South Podlasie Lowland].* Rozprawa Naukowa, 75, Wyd. AP w Siedlcach. [in Polish]

KWASOWSKI W., CHOJNICKI J., OKOŁOWICZ M., KOZANECKA T. 2000. *Metale ciężkie w glebach powierzchni wzorcowych (GPW) w Puszczy Białej [Heavy metals in model surface soils in Biała Forest].* . Rocz. Gleb., 51 (3/4): 85-95. [in Polish]

PALUMBO B., ANGELONE M., BELLANCA A., DAZZI C., HAUSER S., NERI R., WILSON J. 2000. *Influence of inheritance and pedogenesis on heavy metal distribution in soils of Sicily, Italy*. Geoderma, 95: 247-266.

TESSIER A., CAMPBELL P.G.C., BISSON M. 1979. *Sequential extraction procedure for the speciation of particulate trace metals*. Anal. Chem., 51: 844–851.

TIESSEN H., MOIR J.O. 1993. *Characterization of available P by sequential extraction.* [In:] Carter M.R. (eds) Soil Sampling and Methods of Analysis. Canadian Society of Soil Science, Lewis Publishers, Canada: 75–86.

UZIAK S., MELKE J., KLIMOWICZ Z. 2001. *Wpływ użytkowania na zawartość metali ciężkich w glebach „Ściany Wschodniej" [Effect of land use on the content of heavy metals in soil of the 'Eastern Wall'].* Acta Agrophysica, 48: 127-132. [in Polish]

ZEIEN H., BRÜMMER G.W. 1989. *Chemische Extraktion zur Bestimmung von Schwermetallbindungsformen in Böden.* Mitteilg. Dtsch. Bodenkundl. Gesellsch., 59: 505–510.

J. Elementol. 2009, 14(3): 527–537

THE CONTENT OF MINERAL ELEMENTS IN TWO LAMB GENOTYPES DEPENDENT ON THE SYSTEM OF MAINTENANCE*

Mirosław Pięta, Krzysztof Patkowski

Chair of Sheep and Goat Breeding
University of Life Science in Lublin

Abstract

The aim of the study was to determine the content of macro- and microelements in the blood serum and in the longissimus dorsi muscle *(Musculus longissimus dorsi – Mld)* of lambs, dependent on the system of maintenance of the lambs with their mothers, the genotype and the year of research. Lambs were reared together with their mothers in two maintenance systems: indoor system in a sheep fold and outdoor system in the open air with unlimited access to pasture. The lambs were of two genotypes: PLS (Polish Lowland sheep Uhruska variety) and BCP (the synthetic prolific meat line sheep). The concentration of elements in the blood serum was estimated in the second and third month of life, as well as in the *Mld*, after slaughter of the lambs at a weight of 25-28 kg.

The results of the study show that the mineral compositions of blood change with the lamb's age and related method of feeding. The system of maintenance had a modifying effect on the calcium, copper and zinc content in the blood serum, especially in the third month of the lambs' life. It was noted that the copper content in the longissimus dorsi muscle in the lambs kept with their mothers in the outdoor system was higher compared to the lambs kept indoors. A similar tendency was observed in the content of mineral elements in the blood serum and in the longissimus dorsi muscle, dependent on the genotype and maintenance system. The concentration of elements (except sodium) in the blood serum in the lambs comprised within the reference values set for adult sheep.

Key words: sheep, maintenance system, lambs, genotype, macroelements, microelements.

prof. nadzw. dr hab. Mirosław Pięta, Chair of Sheep and Goat Breeding, University of Life Sciences in Lublin, Akademicka 13, 20-950 Lublin, e-mail: miroslaw.pieta@up.lublin.pl

* Scientific study financed by science funds in 2005-2008 under the title of Project No. 2P06Z010029.

ZAWARTOŚĆ SKŁADNIKÓW MINERALNYCH U JAGNIĄT DWÓCH GENOTYPÓW W ZALEŻNOŚCI OD SYSTEMU UTRZYMANIA

Abstrakt

W pracy przedstawiono wyniki badań dotyczących określenia zawartości makroelementów i mikroelementów w surowicy krwi oraz mięśniu najdłuższym grzbietu (*Musculus longissimus dorsi – Mld*) jagniąt w zależności od systemu utrzymania ich przy matkach, genotypu i roku badań. Jagnięta odchowywano z matkami w dwu systemach utrzymania: alkierzowym w owczarni oraz pastwiskowym, na wolnym powietrzu, z nieograniczonym dostępem do pastwiska. Były to jagnięta dwóch genotypów – PLS – polska owca nizinna odmiany uhruskiej i BCP – jagnięta syntetycznej linii plenno-mięsnej. Zawartość pierwiastków w surowicy krwi oceniono w drugim i trzecim miesiącu życia jagniąt, zaś w *Mld* – po uboju tryczków o masie ciała 25-28 kg.

Stwierdzono, że skład mineralny krwi zmienia się wraz z wiekiem jagniąt i związanym z tym sposobem żywienia. System utrzymania wpływał modyfikująco na zawartość wapnia, miedzi i cynku w surowicy krwi jagniąt, zwłaszcza w trzecim miesiącu życia. Stwierdzono wyższą zawartość miedzi w mięśniu najdłuższym grzbietu u jagniąt utrzymywanych z matkami na pastwisku, w porównaniu z jagniętami utrzymywanymi w alkierzu. Zaobserwowano podobną tendencję w zawartości składników mineralnych w surowicy krwi jagniąt a ich zawartością w mięśniu najdłuższym grzbietu (*Mld*) w przypadku genotypu i systemu utrzymania. Stężenie pierwiastków (oprócz sodu) w surowicy krwi jagniąt mieściło się w granicach norm referencyjnych dla owiec dorosłych.

Słowa kluczowe: owce, system utrzymania, jagnięta, genotyp, makroelementy, mikroelementy.

INTRODUCTION

The content of mineral substances in fodder for animals is influenced by many factors. The chemical composition of the soil in which plants are grown has a decisive influence on the mineral content of the plants. The chemical composition of soil largely depends on its geological origin as well as on the level of fertilisation. Obtaining higher and higher crop yields is usually achieved at the expense of decreasing the mineral abundance of soil. One of the possible negative effects of excessive fertilisation is the phenomenon of antagonism between mineral nutrients of soil. Ion antagonism is of great importance in plants taking in mineral nutrients (Przybysz et al. 2003, He et al. 2004, Górski et al. 2005). The content of the mineral elements in plants does not always show the abundance of the soil. Changes in the chemical content can also cause modifications in the botanical content of grasslands. This is particularly common when pasture is intensively fertilised with nitrogen (Kozłowski et al. 2006), which causes the growth of grass with a concurrent decrease in the share of papilionaceous plants and herbs. Continuous use of pasture by animals changes the composition of the plants as well (Baryła, Kulik 2006). The chance that animals in the wild will eat plants in various stages of the development is higher. Hence, deficiencies in the elements are not observed in these animals or they are insignificant (HUMMAN-ZIEHANK et al. 2008).

The level of mineral elements in fodder as well as in sheeps is insignificant, although mineral elements perform an important role in physiological and biochemical processes (CROOKSHANK et al. 1967, ZERVAS et al. 2001). They are also essential for proper metabolic processes in animal organisms, growth and development, as well as the level of productivity (BIS-WENCEL 2001, KHAN et al. 2007, KINCAID 1999, LANE et al. 1968). Mineral elements also affect the level of animal immunity (BOLAND et al. 2005). The interactions that occur between particular elements are of considerable significance for the mineral transformations in animals (MILLER 1985).

Many authors (BARANOWSKI 1992, 2000, FICK et al. 1976, KLATA et al. 2000, KOŁACZ et al. 1994, PEARL et al. 1983) have proved that the concentration of mineral elements in the blood serum does not show their actual demand. The content of mineral elements in tissues (muscle tissue, bone tissue), organs (heart, kidneys, liver) and in milk can be of great significance.

The aim of the present study was to analyse the mineral content in the blood serum and in the *Musculus longissimus dorsi* (*Mld*) in lambs of Polish Lowland Sheep and in the synthetic BCP line kept with their mothers in indoor and outdoor systems.

MATERIAL AND METHODS

The experiment was carried out in the Small Ruminants Research and Experimental Station in Bezek. The station belongs to the Department of Sheep and Goat Breeding at the University of Life Sciences in Lublin. The experiment was carried out twice in 2006 and 2007. Young rams of two genotypes were examined: the Polish Lowland sheep (PLS) and the BCP synthetic line. The sheep of Uhruska variety are kept as a preservative herd of a native breed that was created in this region after World War II. The herd is included in the programme of preservative breeding. The BCP synthetic line was created in Bezek with the participation of the following breeds of sheep: PLS Uhruska variety, prolific breeds (Romanowska, Finnish, Olkuska, Booroola), Berrichone du Cher and Charolaise. The breeding is of a prolific meat specialization.

The lambs with their mothers were bred in two systems:

Indoors – kept year-round in a sheep house. They could make use of a fenced sheep yard. The lambs from this group during the period of rearing were fed with hay and concentrates (crushed oats, and ground grain or bran).

Outdoors – kept year-round in the open air, with permanent access to a roofed shelter. In the summer, pasture was the form of basic nutrition.

For this purpose, approx. 5 ha of pasture was enclosed with a net, and the sheep, at any time of the day, could make use of the pasture's green fodder. The lambs bred by the mothers, in the same way, could also graze on the pasture. The lambs were provided with concentrates in feeders (at first crushed oat, then ground grain or bran). Care was also taken to provide the lambs with hay.

The lambs of both groups were born at the end of April and in May, due to the delayed date of the mothers being mated.

Blood from the lambs was taken from their outer jugular vein twice: when they were two and three months old. Blood sampling was always done in the morning. Each year, the blood was taken from 10 lambs within the same genotype and the maintenance system and from young rams and ewes. Each year, five young rams were slaughtered from each maintenance system and genotype. When slaughtered, the body mass was about 25-28 kg. Samples from the *musculus longissimus dorsi* were taken from the carcass for determination of the mineral content. In the blood serum and in the fresh muscle mass *Mld*, the content of such elements as Na, K, Ca, Mg, Zn, Cu and Fe was established. The ASA method was applied using a Solar 939 UNICAM device. The content of phosphorus in the blood serum was determined using the colorimetric method with Cormay monotests.

The results were elaborated statistically using a multi-factor analysis of variance (SAS 2003) for orthogonal data. Means and standard deviations were put in tables.

RESULTS AND DISCUSSION

The level of calcium in the blood serum in the second month of life was significantly lower in the lambs kept outdoors, compared to the group kept indoors. A similar tendency was observed for potassium (Table 1). Calcium is found mainly in the osseous tissue, as well as in the systemic tissues and fluids. Calcium is crucial for maintaining regular activity of the neural tissue and for ensuring the proper coagulation and work of the heart. An important role in the assimilation of calcium by the body is played by phosphorus and vitamin D. The level of calcium in the blood serum depends on the season of the year and on the physiological condition (Khan et al. 2007). Its concentration influences the accumulation of heavy metals (Pearl et al. 1983). At the same time, no differences were recorded between the systems of maintenance with regard to the content of microelements.

In the third month of life, the calcium concentration in the blood serum increased. It was especially distinct in the outdoor group, where the level of phosphorus and copper was also higher. The level of sodium, irrespective of age, was below the reference values set for adult sheep. The role of sodium

Table 1

The content of macro- and microelements in the lamb blood serum dependent on the maintenance system

Element		Maintenance system			
		indoor		outdoor	
		mean	standard deviation	mean	standard deviation
Second month of life					
Macroelements (mmol · dm^{-3})	Mg	0.908	0.242	0.979	0.320
	Ca	3.14^{xx}	1.19	2.17^{xx}	0.61
	K	4.89^{x}	0.77	4.37^{x}	0.75
	Na	134.76	24.12	123.73	24.67
	P	2.58	0.36	2.75	0.54
Microelements (μmol · dm^{-3})	Cu	10.13	2.21	10.59	3.12
	Zn	14.74	4.84	14.52	3.53
	Fe	48.37	14.69	55.99	22.19
Third month of life					
Macroelements (mmol · dm^{-3})	Mg	1.033	0.378	1.076	0.261
	Ca	3.30^{xx}	0.71	3.87^{xx}	0.68
	K	4.57	0.66	4.41	0.75
	Na	125.92	21.23	127.28	29.51
	P	2.36^{xx}	0.44	2.65^{xx}	0.67
Microelements (μmol · dm^{-3})	Cu	10.09^{x}	3.12	11.46^{x}	2.92
	Zn	15.34^{x}	3.56	13.42^{x}	2.79
	Fe	34.65	12.30	31.12	10.93

Statistically significant differences at $^{xx}p < 0.01$, $^{x}p < 0.05$

and potassium is mainly connected with regulating the osmotic pressure of cells and the water metabolism in the body. Potassium is the main mineral element of cell cytoplasm as well as of wool ash. Sodium deficiency in sheep is supplemented by fodder salt. Sheep generally show good tolerance to high doses of salt (Kincaid 1999, Skrzypczak 1983).

The iron content in lambs decreased with age (Tables 1, 2, 3), more clearly in the outdoor system than in the indoor system.

No statistically significant differences were revealed in the mineral composition between the various genotypes of lambs. Only the level of copper proved to be higher in the PLS lambs, compared to the BCP lambs (Table 2).

Table 2

The content of macro- and microelements in the lamb blood serum dependent on the genotype

Element		Genotype			
		Polish Lowland sheep		Synthetic line BCP	
		mean	standard deviation	mean	standard deviation
Second month of life					
Macroelements ($mmol \cdot dm^{-3}$)	Mg	0.975	0.278	0.913	0.290
	Ca	2.57	0.92	2.74	1.19
	K	4.64	0.88	4.63	0.72
	Na	130.57	25.68	127.92	24.30
	P	2.65	0.62	2.68	0.23
Microelements ($\mu mol \cdot dm^{-3}$)	Cu	11.24^{x}	3.03	9.48^{x}	1.99
	Zn	15.23	4.95	14.04	3.25
	Fe	55.29	19.25	49.08	18.64
Third month of life					
Macroelements ($mmol \cdot dm^{-3}$)	Mg	1.014	0.241	1.095	0.389
	Ca	3.70	0.60	3.47	0.86
	K	4.35	0.56	4.63	0.81
	Na	125.24	26.02	127.95	25.33
	P	2.46	0.73	2.54	0.39
Microelements ($\mu mol \cdot dm^{-3}$)	Cu	10.70	2.99	10.85	3.21
	Zn	14.75	3.06	14.01	3.56
	Fe	35.48	12.93	30.29	9.80

Statistically significant differences at $^{x}p < 0.05$

A higher concentration of magnesium in the lamb blood serum was recorded in 2006, irrespective of the lambs' age. In the second year, a higher content of calcium, potassium, sodium, phosphorus and copper was recorded in the third month of life (Table 3). Nearly 30% of all the magnesium forms part of the osseous tissue. Hence, magnesium deficiency is manifested in the form of grass tetany, excessive irritability of the nervous system or convulsions and may even cause death (KANIA 1998, KHAN et al. 2007, REID et al. 1979). The content of magnesium in the blood serum, in this case, closely correlates to its content in the fodder provided. The absorption of magnesium remains strongly influenced by an adequate concentration of zinc in the feed (SALLES et al. 2008).

Table 3

The content of macro- and microelements in the lamb blood serum dependent on the investigations year

Element		Year of investigations			
		2006		2007	
		mean	standard deviation	mean	standard deviation
Second month of life					
Macroelements (mmol · dm^{-3})	Mg	1.133^{xx}	0.220	0.755^{xx}	0.201
	Ca	3.26^{xx}	1.10	2.06^{xx}	0.54
	K	4.59	0.49	4.67	1.03
	Na	135.19	10.32	123.30	32.67
	P	2.60	0.22	2.73	0.61
Microelements (μmol · dm^{-3})	Cu	11.15	2.22	9.57	2.92
	Zn	12.69^{xx}	2.48	16.58^{xx}	4.67
	Fe	46.98^{x}	12.46	57.38^{x}	22.95
Third month of life					
Macroelements (mmol · dm^{-3})	Mg	1.300^{xx}	0.268	0.808^{xx}	0.119
	Ca	3.32^{xx}	0.64	3.85^{xx}	0.76
	K	4.30^{x}	0.75	4.68^{x}	0.61
	Na	109.40^{xx}	19.34	143.79^{xx}	18.20
	P	2.19^{xx}	0.38	2.82^{xx}	0.58
Microelements (μmol · dm^{-3})	Cu	8.37^{xx}	1.95	13.18^{xx}	1.83
	Zn	13.66	3.23	15.10	3.29
	Fe	30.29	11.73	35.49	11.21

Statistically significant differences at $^{xx}p < 0.01$, $^{x}p < 0.05$

Regarding the content of macroelements in the longissimus dorsi muscle, no significant differences were revealed between the lambs from two systems of maintenance. A higher copper content was recorded in the lambs kept outdoors compared to those kept indoors (Table 4). It may be observed that the macro- and microelement content in the longissimus dorsi muscle in the PLS lambs was higher than in the BCP lambs (Table 5). In 2007, as compared to 2006, the content of magnesium, calcium, copper, zinc and iron recorded was higher (Table 6). The mineral element content in the blood serum corresponded to the content in the longissimus dorsi muscle. Similar results were obtained by Baranowski (2000), Fick et al. (1976), and Klata et al. (2000).

Table 4

The content of macro- and microelements in the longissimus dorsi muscle of lambs dependent on the maintenance system

Element		Maintenance system			
		indoor		outdoor	
		mean	standard deviation	mean	standard deviation
Macroelements (mmol·kg^{-1} f. m.)	Mg	12.043	1.251	12.620	2.317
	Ca	1.192	1.029	1.585	1.466
	K	81.11	20.22	77.64	28.67
	Na	27.620	3.896	27.971	5.846
	Cu	10.476^{x}	8.429	16.297^{x}	10.892
Microelements (µmol·kg^{-1} f. m.)	Zn	435.8	60.8	431.1	93.5
	Fe	447.3	178.3	486.6	238.6

Statistically significant differences at $^{x}p < 0.05$

Table 5

The content of macro- and microelements in the longissimus dorsi muscle of lambs dependent on the genotype

Element		Genotype			
		Polish Lowland sheep		Synthetic line BCP	
		mean	standard deviation	mean	standard deviation
Macroelements (mmol·kg^{-1} f. m.)	Mg	12.891^{x}	2.381	11.772^{x}	0.884
	Ca	1.698	1.565	1.080	0.798
	K	82.77	24.93	75.98	24.35
	Na	28.959	4.700	26.632	4.948
	Cu	13.459	10.259	13.314	10.112
Microelements (µmol·kg^{-1} f. m.)	Zn	441.0	97.7	425.9	52.88
	Fe	468.8	179.8	465.2	239.3

Statistically significant differences at $^{x}p < 0.05$

Table 6

The content of macro- and microelements in the longissimus dorsi muscle of lambs dependent on the investigations year

Element		Year of investigations			
		2006		2007	
		mean	standard deviation	mean	standard deviation
Macroelements (mmol·kg^{-1} f. m.)	Mg	11.363^{xx}	0.459	13.300^{xx}	2.219
	Ca	0.828^{xx}	0.588	1.949^{xx}	1.510
	K	91.56^{xx}	4.46	67.20^{xx}	30.11
	Na	29.350^{x}	3.153	26.241^{x}	5.865
	Cu	10.689	8.807	16.083	10.707
Microelements (μmol·kg^{-1} f. m.)	Zn	384.3^{xx}	40.4	482.6^{xx}	75.713
	Fe	399.9^{x}	121.4	534.1^{x}	255.6

Statistically significant differences at ^{xx} $p< 0.01$,^{x} $p< 0.05$

CONCLUSIONS

1. Changes were observed in the content of some elements in the blood serum, dependent on the age of the lambs. These changes are especially noticeable in the case of iron.

2. The system of lamb maintenance significantly influenced the concentration of calcium, phosphorus and zinc in the blood serum.

3. The content of macro- and microelements in the longissimus dorsi muscle in the PLS lambs was slightly higher than in the BCP lambs.

4. The content of mineral elements (except sodium) fell within the reference values set for adult ewes, which proves that the lambs' rearing and nutrition were conducted properly, regardless of the maintenance system.

5. The results presented concern young lambs, for which no reference standards have been formulated as regards the content of the macro- and micro- maintenance elements in the blood serum and in the muscles. These results can be used in the future in order to establish such standards.

REFERENCES

Baranowski P. 1992. *Niektóre wskaźniki hematologiczne oraz stężenie składników mineralnych w surowicy krwi i krwinkach czerwonych owiec mieszańców merynos polski x suffolk [Some hematologic indices and concentration of mineral components in blood serum and red cells in Polish Merino x Sufolk cross sheep].* Med. Wet., 48 (3): 138-140. [in Polish]

BARANOWSKI P. 2000. *Wybrane wskaźniki krwi u owiec dorosłych oraz makroelementy w surowicy i tkance kostnej jagniąt w zależności od różnych czynników środowiska [Some blood indices in adult sheep and macroelements in serum and bone tissue of lambs depending on various environmental factors].* Wyd. AR w Szczecinie, ser. Rozprawy, 194. [in Polish]

BARYŁA R., KULIK M. 2006. *Zawartość azotu i podstawowych składników mineralnych w runi pastwiskowej w róznych latach jej użytkowania [Content of nitrogen and basic mineral components in pasture cover in different years of pasture use].* Ann. UMCS, E, 61: 157--164. [in Polish]

BIS-WENCEL H. 2001. *Rozpoznawanie i zapobieganie niedoborom mineralnym u przeżuwaczy w regionie południowo-wschodniej Polski [identification and prevention of mineral deficits in ruminants in south-eastern Poland].* Wyd. AR Lublin, ser. Rozprawy, 243. [in Polish]

BOLAND T.M., KEANE N., NOWAKOWSKI P., BROPHY P.O., CROSBY T.F. 2005. *High mineral and vitamin E intake by pregnant ewes lowers colostral immunoglobulin G absorption by the lamb.* J. Anim. Sci., 83: 871-878.

CROOKSHANK H. R., ROBBINS J. D. , KUNKEL H. O. 1967. *Relationship of dietary mineral intake to serum mineral level and the incidence of urinary calculi in lambs.* J. Anim. Sci., 26: 1179-1185.

FICK K. R., AMMERMAN C. B., MILLER S. M., SIMPSON C. F., LOGGINS P.E. 1976. *Effect of dietary lead on performance, tissue mineral composition and lead absorption in sheep.* J. Anim. Sci., 42 (2): 515-523.

GÓRSKI K., SABA L., BOMBIK T., BOMBIK E. 2005. *Poziom wybranych mikroelementów (Fe, Cu, Zn) w glebie, paszach i surowicy krwi krów w rejonie południowego Podlasia [Level of some micrpnutrients (Fe, Cu, Zn) in soil, feeds and blood serum of cows in South Podlasie].* Rocz. Nauk. PTZ, 1 (2): 309-318. [in Polish]

HE P.P., LV X.Z., WANG G.Y. 2004. *Effects of Se and Zn supplementation on the antagonism against Pb and Cd in vegetables*. Environm. Int., 30: 167-172.

HUMANN-ZIEHANK E. ,GANTER M., HENNIG-PAUKA I , BINDER A. 2008. *Trace mineral status and liver and blood parameters in sheep without mineral supply compared to local roe deer (Capreolus capreolus) populations.* Small Rum. Res., 75: 185-191.

KANIA B.F. 1998: *Znaczenie magnezu dla zwierząt.* Med. Wet., 54 (6:) 378-382.

KHAN Z.I.,. HUSSAIN A, ASHRAF M., ASHRAF M.Y., MCDOWELL L.R. 2007. *Macromineral status of grazing sheep in a semi-arid region of Pakistan.* Small Rum. Res., 68: 279–284.

KINCAID R.L. 1999. *Assessment of trace mineral status of ruminants: A review.* Proc. Am. Soc. Anim. Sci., 1-10.

KLATA W., BARANOWSKI P., BARANOW-BARANOWSKI S. 2000. *Składniki mineralne surowicy krwi i kości kręgów ogonowych jagniąt rasy merynos polski [Mineral components in blood serum and caudal vertebra bones in the Polish Merino lambs].* Med. Wet., 56 (9): 586--588. [in Polish]

KOŁACZ R., DOBRZAŃSKI Z., PATKOWSKA-SOKOŁA B., BODKOWSKI R.1994. *Wpływ przemysłu miedziowego na akumulację metali ciężkich w tkankach i narządach [Influence of the copper industry on accumulation of heavy metals in tissues and organs].* Zesz. Nauk. Prz. Hod. PTZ, 13: 316-317. [in Polish]

KOZŁOWSKI S., GOLIŃSKI P., ZIELEWICZ W., BINIAŚ J. 2006. *Badania nad nawożeniem pastwiska nawozami płynnymi [Studies on fertilization of pastures with liquid fertilizers].* Ann. UMCS, E, 61: 341-352. [in Polish]

LANE A. G., CAMPBELL J. R. , KRAUSE G. F. 1968. *Blood mineral composition in ruminants.* J. Anim Sci., 27: 766-770.

MILLER E.R. 1985. *Mineral disease interactions.* J. Anim. Sci., 60 (6): 1500-1507.

PEARL D. S., AMMERMAN C. B., HENRY P. R., Littell R. C. 1983. *Influence of dietary lead and calcium on tissue lead mineral composition in sheep accumulation and depletion, lead metabolism and tissue* J. Anim. Sci., 56: 1416-1426.

PRZYBYSZ T., BOROWIEC J., KAMIŃSKA A. 2003. *Próba statystycznej interpretacji wyników badań chemizmu siedlisk łąkowych Lubelszczyzny [An attempt at statistical interpretation of the results of studies on the chemism of meadow habitats in the Lublin region].* Ann. UMCS, E, 58: 79-92. [in Polish]

REID R. L., JUNG G. A., WOLF C H. , KOCHER R. E. 1979. *Effects of magnesium fertilization on mineral utilization and nutritional quality of alfalfa for lambs.* J. Anim. Sci., 48: 1191--1201.

SALLES M.S.V, ZANETTI M.A., SALLES F.A. 2008. *Effect of monensin on mineral balance in growing ruminants reared under different environmental temperatures*. Anim. Feed Sci. Tech., 141: 233–245.

SAS Institute Inc. 2003. *User's Guide*, v. 9.1.3, Cary.

SKRZYPCZAK W. 1983. *O znaczeniu elektrolitów Na, K, Cl dla organizmu zwierząt [On the role of electrolytes Na, K, Cl in animal organisms].* Prz. Hod., 10: 12-14. [in Polish]

ZERVAS G., RISSAKI M., DELIGEORGIS S. 2001. *Free-choice consumption of mineral lick blocks by fattening lambs fed ad libitum alfalfa hay and concentrates with different trace mineral content.* Liv. Prod. Sci., 68: 251–258.

J. Elementol. 2009, 14(3): 539–544

EFFECT OF VARIOUS DOSES OF OAT ADDED TO A FEED MIXTURE ON THE CONTENTS OF SELECTED MINERALS IN TURKEY MEAT

Janusz F. Pomianowski[1], Teresa Majewska[2], Jerzy Borowski[3], Wacław Mozolewski[1]

[1]Chair of Commodity Science and Food Analysis
[2]Chair of Poultry Science
[3]Chair of Human Nutrition
University of Warmia and Mazury in Olsztyn

Abstract

Owing to its high protein and low fat content, turkey meat is regarded as dietetic. It also has a beneficial composition in terms of amino acid and mineral content.

The study involved an analysis of selected elements: zinc, magnesium, calcium, sodium, potassium and phosphorus in breast muscles of male turkeys BIG 6 fed on fodder with various content of oatmeal (0%; 5% and 10%).

The content of selected elements was determined in previously mineralised samples: zinc, magnesium and calcium – by AAS, using a UNICAM 939AAA Solar flame atomic absorption spectrometer; sodium and potassium – by flame photometry with a Carl Zeiss Jena FLAPHO 4 flame photometer, and phosphorus – by colorimetric measurement with the hydroquinone reagent at a wavelength of 610 nm. The results were processed with a single-factor analysis of variance with the Statistica 8pl computer program, and the significance of differences was determined with Duncan's test.

Oatmeal application in the analysed doses in turkey feeding has been shown to reduce the content of the elements under study in turkey meat. The differences for the analysed elements have not been shown to be statistically significant (P = 0.05), except for sodium, whose content – unlike that of other elements – grew with the oatmeal content in the fodder

Key words: turkey meat, mineral components, feed mixtures.

dr inż. Janusz F. Pomianowski, Chair of Commodity Science and Food Analysis, University of Warmia and Mazury in Olsztyn, pl. Cieszyński 1, 10-726 Olsztyn, e-mail: janusz.pomianowski@uwm.edu.pl

WPŁYW ZRÓŻNICOWANEGO UDZIAŁU OWSA W PASZY NA ZAWARTOŚĆ WYBRANYCH PIERWIASTKÓW W MIĘSIE INDYCZYM

Abstrakt

Mięso indycze ze względu na dużą zawartość białka i małą ilość tłuszczu uznaje się za dietetyczne. Ponadto ma ono korzystny skład aminokwasowy i mineralny.

W pracy analizowano zawartość wybranych pierwiastków: cynku, magnezu, wapnia, sodu, potasu i fosforu w mięśniach piersiowych indorów BIG 6 żywionych paszą o różnym udziale śruty owsianej (0%; 5% i 10%).

Zawartość wybranych pierwiastków oznaczano w uprzednio zmineralizowanych próbkach: cynk, magnez i wapń – metodą ASA, stosując spektrofotometr płomieniowej absorpcji atomowej UNICAM 939AAA Solar; sód i potas – metodą fotometrii płomieniowej z użyciem fotometru płomieniowego FLAPHO 4 firmy Carl Zeiss Jena, fosfor – metodą kolorymetryczną z zastosowaniem odczynnika hydrochinonowego przy długości fali 610 nm. Wyniki poddano jednoczynnikowej analizie wariancji stosując program komputerowy Statistica 8pl, a istotność różnic oceniono testem Duncana.

Wykazano, że stosowanie śruty owsianej w skarmianiu indyków w ocenianych dawkach wpływa na obniżenie ilości badanych pierwiastków w mięsie indyczym. Nie wykazano, by różnice te były istotne statystycznie ($P \leq 0.05$) dla analizowanych pierwiastków, z wyjątkiem sodu, którego ilość w odróżnieniu od innych pierwiastków wzrastała wraz ze wzrostem ilości stosowanej śruty owsianej w paszy.

Słowa kluczowe: mięso indycze, składniki mineralne, pasza.

INTRODUCTION

Due to a high content of protein and a low content of fat, turkey meat is recognized as dietetic. It is additionally characterized by a beneficial amino acid and mineral composition. It contains considerable amounts of potassium, magnesium, zinc and selenium with a relatively low content of sodium. In the breeding practice of these birds, a number of producers have used feed mixtures similar to those for hens. This practice, however, seems to be inappropriate owing to the different behavior of these birds, the different pH value of their alimentary tract and susceptibility to a number of diseases.

Likewise, millet oat is a cereal known for its high content of minerals (ranging from 2% to 3.4%). It is also characterized by high contents of zinc and soluble silica. Owing to the above, as well as to high contents of dietary fibre, oat may be applied as feed for turkeys, which show a considerable demand for dietary fibre (BARTNIKOWSKA et al. 2000ab, MAJEWSKA et al. 2004).

The study was aimed at evaluating the effect of varied feeding of turkeys with feed mixtures containing different doses of oat on contents of selected minerals in their meat.

MATERIAL AND METHODS

The experimental material were breast muscles of heavy BIG 7 turkey toms originating from a feeding experiment conducted at a poultry farm of the Chair of Poultry Science, Faculty of Animal Bioengineering, University of Warmia and Mazury in Olsztyn. The birds were fed *ad libitum* pelleted feed mixtures in a 3-variant system. Three feeding groups were established: (A) control group without addition of oat, group (B) with 5% addition of oat meal, and group (C) with 10% addition of oat meal to a feed mixture. The protein value and energy value of the feed mixtures were consistent with dietary requirements for slaughter turkeys at respective stages of growth (SMULIKOWSKA, RUTKOWSKI 2005). After termination of the feeding experiment, 8 birds with body weights similar to the average were selected from each group, fasted for 12 h and slaughtered. After 24-h chilling at a temperature of 4°C, their carcasses were weighed and subjected to dissection. For analyses, *ca* 200 g of samples were collected from the breast muscle. Portions of meat were packed into PE foil bags and transferred to the Chair of Commodity Science and Food Analysis, University of Warmia and Mazury in Olsztyn, for further analyses.

Comminuted samples of meat were weighed and wet-mineralized in a mixture of HNO_3 and $HClO_4$ (at a 3:1 ratio). The mineralization was conducted in a block by the Tecator company with a programmed temperature.

Contents of selected minerals in the resultant mineralizate were determined as follows:

- zinc, magnesium and calcium – with the AAS method using a UNICAM 939AAA Solar flame atomic absorption spectrophotometer,
- sodium and potassium – with the method of flame photometry by means of a FLACHO 4 flame photometer by Carl Zeiss Jena,
- phosphorus – with the colorimetric method using a hydroquinone reagent at a wavelength of 610 nm.

The results were subjected to a one-way analysis of variance using Statistica 8pl software, and the significance of differences was evaluated with Duncan's test.

RESULTS AND DISCUSSION

The results of analyses are presented in Table 1. Out of all minerals analyzed, the breast muscles of turkeys had the highest content of potassium. Mean concentrations of this element in the samples examined varied between 360.78 mg 100 g^{-1} in meat of the turkeys fed a feed mixture with the highest 10% dose of oat (group C) and 385.90 mg 100 g^{-1} in meat of the

Table 1

The content of elements in turkey breats (mg 100 g^{-1})

Element	Statistical measures	Feeding group		
		A	B	C
Zinc	mean	0.93	0.83	0.80
	range	0.85 – 1.01	0.75 – 0.91	0.72 – 0.91
	SD	0.08	0.13	0.10
Magnesium	mean	23.77	23.25	22.94
	range	22.12 – 24.72	21.37 – 24.27	21.67 – 24.79
	SD	1.43	3.45	2.07
Calcium	mean	1.85	1.78	1.65
	range	1.69 – 2.06	1.60 – 1.92	1.40 – 1.90
	SD	0.19	0.22	0.27
Sodium	mean	59.47a	63.46b	66.89a
	range	57.46 – 61.64	61.69 – 65.72	62.43 – 69.41
	SD	2.10	2.06	3.87
Phosphorus	mean	247.93	244.08	240.82
	range	236.90 – 252.75	240.75 – 248.90	234.80 – 245.30
	SD	7.93	9.99	24.31
Potassium	mean	385.90	374.72	360.78
	range	384.20 – 387.30	356.75 – 385.40	351.95 – 365.60
	SD	1.21	9.46	7.66

Statistically significant differences at $P \leq 0.05$.

birds fed a feed mixture without oat addition (group A). Generally, analyses demonstrated a decrease in potassium content of meat along with an increasing dose of oat meal in feed mixtures. Similar observations were made for phosphorus, the content of which ranged from 240.82 mg 100 g^{-1} in meat of the birds from group C to 247.93 mg 100 g^{-1} in meat of the control toms (group A). However, the statistical analysis of the above results demonstrated a lack of significant differences in contents of both potassium and phosphorus between the feeding groups of birds.

In assaying sodium, oat meal addition to feed mixtures was observed to produce a significant effect on its concentration in meat, which ranged from 59.47 mg 100 g^{-1} in meat of the birds fed a feed mixture without oat addition (group A) to 66.89 mg 100 g^{-1} in meat of the birds receiving 10% oat meal in their feed mixture (group C). These differences turned out to be statistically significant (P=0.05). Adverse relations were observed for the magnesium content of meat. The highest mean content of this element (23.77

mg 100 g^{-1}) was determined in meat of the toms fed a feed mixture without an oat meal addition (group A), a slightly lower one (23.25 mg 100 g^{-1}) in meat of the turkeys fed a feed mixture with a 5% dose of oat meal (group B), and the lowest (22.63 mg 100 g^{-1}) in meat of the birds receiving a 10% dose of oat meal in the feed mixture (group C).

The second most abundant element in the meat examined was calcium. In this case, the results of assays also appeared to be differentiated depending on the feed mixture administered to birds. The lowest content of this element (1.65 mg 100 g^{-1}) was determined in meat of the birds fed a mixture with the highest dose of oat meal (group C), a slightly higher one (1.78 mg 100 g^{-1}) in meat of the turkeys from group B fed a feed mixture with a 5% dose of oat meal, and the highest one (1.85 mg 100 g^{-1}) in meat of the control birds (group A). However, the differences observed were not proven to be statistically significant.

Out of all elements assayed, the lowest concentrations were reported for zinc. The highest content of this element (0.93 mg 100 g^{-1}) was determined in meat of the control birds (group A), whereas its lower contents were observed in meat of the toms fed feed mixtures containing oat meal, i.e. 0.83 mg 100 g^{-1} and 0.80 mg 100 g^{-1} in the birds fed feed mixtures with 5% (group B) and 10% (group C) of oat meal, respectively. Nevertheless, the differences between the feeding groups were not statistically significant.

Similar concentrations of the elements examined were reported by other authors [Bojarska et. al 2000], who evaluated the contents of minerals in carcasses and muscles of turkeys. Those authors point to the possibility of decreasing contents of the elements along with the age of the birds. In addition, they emphasize differentiated concentrations of those elements as affected by the type of muscle. The results obtained in that study are also consistent with "Food Composition Tables" (Kunachowicz et al. 2005). The few discrepancies occurring between the results are likely to stem from the fact that the authors had expressed the contents of particular elements in respect of the whole carcass. Those discrepancies refer to potassium, sodium and magnesium (whose contents determined in the reported study were slightly higher) and to zinc (whose content was slightly lower). Such low concentrations of zinc in the breast muscles of turkeys were also reported in a study by Nadolna et al. (1996) on the nutritive value of chickens and turkeys.

A comparative analysis of contents of the elements examined in meat of turkeys with those found in beef and meat of ostrich demonstrates considerable differences (Sales, Oliver-Lyons, 1996). Other authors (Al-Najdawi, Abdullah 2002), who examined chickens from the Jordanian market, indicate remarkably higher contents of the assayed elements in the meat of chickens as compared to that of turkeys. Worthy of notice are also differences in the concentrations of individual mineral substances as affected by the type of muscle examined or origin of birds (Gardzielewska et al. 1997).

SUMMARY

The applied feed mixtures with various doses of oat meal were found to affect the contents of elements in the meat samples examined. However, the statistical analysis proved their significant effect only on the increased content of sodium ($P = 0.05$). In the other samples, no significant effect of the oat meal dose applied was shown on either an increase or decrease in the concentrations of the assayed micro- and macroelements.

REFERENCES

AL.-NAJDAWI R., ABDULLAH B. 2002. *Proximate composition, selected minerals, cholesterol content and lipid oxidation of mechanically and hand-deboned chickens from the Jordanian market*. Meat Sci., 62:243-247.

BARTNIKOWSKA E., LANGE E.; RAKOWSKA M. 2000a. *Ziarno owsa - niedoceniane źródło składników odżywczych i biologicznie czynnych.* Cz. I. *Ogólna charakterystyka owsa. Białka, tłuszcze [Oat grain – an undervalued source of nutrients and biologically active components.* Part I. *General description of oat. Proteins, fats].* Biul. IHAR, 215:209-222. [in Polish]

BARTNIKOWSKA E., LANGE E., RAKOWSKA M. 2000b. *Ziarno owsa – niedoceniane źródło składników odżywczych i biologicznie czynnych.* Cz. II. *Polisacharydy i włókno pokarmowe, składniki mineralne, witaminy [Oat grain – an undervalued source of nutrients and biologically active components.* Part II. *Polysaccharides and fibre, mineral components, vitamins].* Biul. IHAR, 215: 223-237. [in Polish]

BOJARSKA U., BATURA J., MARKIEWICZ K. 2000. *Zawartość składników mineralnych w tuszkach i mięśniach indyków [Content of minerals in carcasses and muscles of turkeys].* Zesz. Nauk. PTZ Prz. Hod., 49:267. [in Polish]

GARDZIELEWSKA J., SOCHACKA A., GOLACH Z., CYRAN A., NATALCZYK-SZYMKOWSKA W. 1997. *Mięso kurcząt brojlerów jako źródło magnezu, sodu, potasu, wapnia, fosforu i litu [Broiler chicken meat as a source of magnesium, sodium, potassium, calcium, phosphorus and lithium].* Zesz. Nauk. AR Szczecin, Zootechnika, 34:33-38. [in Polish]

KUNACHOWICZ H., NADOLNA I., PRZYGODA B., IWANOW K. 2005. *Tabele składu i wartości odżywczej żywności [Tables of food composition and nutritive value].* PZWL, Warszawa. [in Polish]

MAJEWSKA T., MIKULSKI D., ŚWIĘCICKA-GRABOWSKA G., WÓJCIK R. 2004. *Owies w żywieniu młodych indyków rzeźnych [Oat in nutrition of young slaughterturkeys].* Med. Wet., 60(6): 657-661. [in Polish]

NADOLNA I., KUNACHOWICZ H., IWANOW K, KŁYS W. 1996. *Wartość odżywcza mięsa kurzego i indyczego [Nutritive value of chicekn and turkey meat].* Bezpieczna Żywność, 1-2: 17-20. [in Polish]

SALES J., B. OLIVER-LYONS. 1996. *Ostrich meat: a review*. Food Australia, 48 (11): 504-511.

Zalecenia żywieniowe i wartość pokarmowa pasz. Normy żywienia drobiu. Red. SMULIKOWSKA S., RUTKOWSKI A. 2005. *[Nutritional recommendations and nutritive value of feeds. Nutritional norms for poultry].* PAN, Warszawa. [in Polish]

J. Elementol. 2009, 14(3): 545–551

AN ASSESSMENT OF THE INFLUENCE OF FLUORIDE, MODIFIED TRANSDERMAL REPLACEMENT HORMONE THERAPY AND SUPPLEMENT HORMONE THERAPY ON UNMANAGEABLE OSTEOPOROSIS IN POSTMENOPAUSAL WOMEN

Małgorzata Stanosz[2], Stanisław Stanosz[1], Andrzej Puchalski[1]

[1]Department Menopause and Andropause
Pomeranian Academy of Medicine, Szczecin, Poland
[2]West Pomeranian Osteoporosis Center “OSTEOMED” Szczecin, Poland

Abstract

The study was conducted on 40 women in the early postmenopausal period, aged 52.3±3.1 years with primary osteoporosis unmanageable in treatment, divided into 2 groups based on a randomized list. Group I (n-20) was administered orally fluoride 0.25 mg kg^{-1} 24 h^{-1} with modified transdermal hormone therapy/HRT, and group II (n-20) was administered orally fluoride and supplement hormonal therapy(HST) in 21 therapeutic cycle. The serum concentrations of osteocalcin (OC), procollagen(PICP), insulin-like growth factor I (IGF-1), prolactin basic (PRL) and prolactin after metoclopramide (PRL/ MCP) 4 times by using radioimmunoassy methods, before treatment and after 1, 3, 12 months of treatment. Bone mineral density (BMD) L2 – L4 was determined before treatment and at 12 month with a dualenergy x-ray absorptiometry scanner (Lunar DPX-1Q). In group I women receiving fluoride and transdermal HRT IGF-1 increased significantly while the concentrations of OC and PICP significantly decreased after 3 and 12 months of treatment but no statistically significant changes in the PRL concentration occurred. In group II women receiving orally fluoride and HST, a significant decrease in the concentration of IGF-1, OC after 3 and 12 months and a significant increase in the concentration of PRL and PRL/ MCP after 1, 3 and 12 months of treatment compared with the baseline values appeared.

prof. dr hab. med. Stanisław Stanosz, Department Menopause and Andropause, Pomeranian Academy of Medicine, ul. Unia Lubelska 1, 71-256 Szczecin, Poland, phone: +48 914873 225, e-mail: magnum @med.pam.szczecin pl

The concentration of type I procolagen (PICP) showed no statistically significant changes. Increase in bone mineral density was statistically significant L1, L2 ($p < 0.05$), L3, L4 ($p < 0.01$) compared with the baseline in the group receiving transdermal HRT. In women receiving fluoride and orally HST increase in the bone mineral density for L1 and L2 was non-insignificant, whereas for L3 and L4 it was significantly higher compared with the baseline ($p < 0.05$).

Key words: osteoporosis, fluoride, osteocalcin, modified hormone replacement therapy, hormone supplement therapy.

OCENA WPŁYWU FLUORU, ZMODYFIKOWANEJ PRZEZSKÓRNEJ HORMONOTERAPII ZASTĘPCZEJ I DOUSTNEJ HORMONOTERAPII WSPOMAGANEJ W LECZENIU OSTEOPOROZY OPORNEJ U KOBIET W OKRESIE POMENOPAUZALNYM

Abstrakt

Badaniem objęto 40 kobiet we wczesnym okresie pomenopauzalnym, w wieku 52,3±3,1 lat, podzielonych wg listy randomizowanej na dwie grupy: grupę I. (n-20) otrzymującą doustnie fluor w dawce 0,25 mg kg^{-1} 24 h^{-1} oraz zmodyfikowaną przezskórną hormonoterapię zastępczą (HTZ), grupę II (n-20) otrzymującą doustnie fluor w dawce 0,25 mg kg^{-1} 24 h^{-1} i hormonoterapię wspomaganą (HTW) w postaci tabletek. Cykle terapeutyczne w obu grupach trwały 21 dni w miesiącu z następową przerwą 7 dni w celu wystąpienia krwawienia z odstawienia przez okres jednego roku. W surowicy oceniano stężenia osteokalcyny (OC), prokolagenu (PICP), insulinopodobnego czynnika wzrostu (IGF-1), podstawową prolaktynę (PRL) i po teście z metoklopramidem (PRL/MCP) radioimmunologicznie czterokrotnie: przed leczeniem oraz po 1. 3. i 12. miesiącu leczenia. Gęstość mineralną trzonów kręgów lędźwiowych L2 –L4 badano przed leczeniem i po 12 miesiącach leczenia densytometrem, firmy Luna (DPX-1Q), metodą DEXA. U kobiet z grupy I otrzymującej doustnie fluor i przezskórnie HTZ wystąpił znamienny wzrost stężenia IGF-1, znamienne obniżenie OC, PLCP po 3 i 12 miesiącach leczenia oraz brak statystycznych zmian w stężeniu prolaktyny. Natomiast u kobiet z grupy II otrzymującej w postaci tabletek doustnie fluor i hormonoterapię wspomaganą HTW wystąpiło znamienne obniżenie stężeń IGF-1, OC po 3 i 12 miesiącach leczenia oraz znamienny wzrost stężenia prolaktyny podstawowej i po teście z metoklopramidem po 1. 3. i 12. miesiącu leczenia w porównaniu z wartościami wstępnymi. Stężenia prokolagenu w czasie stosowania doustnie fluoru i HTW nie wykazywały znamiennych różnic. Gęstość mineralna L2-L4 wykazywała znamienne przyrosty u kobiet z grupy I. Natomiast u kobiet z grupy II gęstość mineralna L1, L2 nie wykazywała przyrostu znamiennego, a w kręgu L3, L4 występował znamienny przyrost w porównaniu z wartościami wyjściowymi ($p < 0,01$).

Słowa kluczowe: osteoporoza, fluor, osteokalcyna, zmodyfikowana hormonalna terapia zastępcza, hormonalna terapia wspomagana.

INTRODUCTION

Osteoporosis is one of the most important problems in developed societies. The deficiency of bone mass or skeletal osteopenia results in fractures of the spine, the distal of the radius and ulna bones and the neck of the femoral bone (OKOPIEŃ et al. 2005). Fluoride is an important element in the mineralization of bone and teeth (SOWERS et al. 2005) The proper use of topical and systemic fluoride has resulted in major reductions in dental caries and associated disability (PALMER et al. 2005), although the therapeutic effect in osteoporosis depends on their dose (YAMAGUCHI 2007). High doses of fluorides applied in the treatment of osteoporosis reduce mineral part of the compact bone (BUSSE et al. 2006).During the long-term therapy with fluorides, it was established that an increase in the bone mineral density is accompanied by an increase in the bone fracture frequency (GIACHINI et al. 2004, REID et al. 2007, VESTERGAARD et al. 2008).

It is the consequence of disturbances in bone architecture, expressed by the loss of connections between the thinned bone trabeculares which leads to a decrease in the bone mechanical endurance (PEPENE et al. 2004, RINGE et al. 2005, OTSUKA et al. 2008).

OBJECTIVE

The aim of the study was to assess the influence of two kinds of hormonal therapy and the minimal effective fluoride dose on concentrations of osteocalcin, procolagen, insulin-like growth factor I, prolactin basal and prolactin after metoclopramide in serum as well as the degree of mineralization of the lumbar spine of unmanageable osteoporosis in early postmenopausal women.

MATERIAL AND METHODS

The study was conducted on 40 women in the early postmenopausal period, aged 52.3 + 3.1 years with osteoporosis unmanageable in treatment and no history of general diseases; there were no significant differences between groups in terms of age, body mass index and parity. The women were divided into 2 groups based on a randomized list. Group I ($n = 20$) was treated fluoride (Fluossen, Polfa) 0.25 mg kg^{-1} 24 h^{-1} orally with modified transdermal hormone therapy (HTR) in the form of patches (Systen Janssen–Cilag), according to STANOSZ et al. (1995). Group II ($n = 20$) was treated floride 0.25 mg kg^{-1} 24 h^{-1} orally and hormone supplement therapy orally, by taking Cyclomenorette (Wyeth, Munster). The therapeutic cycle in each group lasted 21 days, with a treatment free interval of 7 days. Estimation in serum concentrations of insulin-like growth factor I (IGF-1) was performed by using immunoenzymatic assay (Boldon IDS), propeptide of type I procollagen (PICP), kit from Orion Diagnostica, osteocalcin (OC), by radioimmunoassay (a kit from DRG), basal prolactin (PRL) and prolactin after metoclopramide (PRL/MCP) radioimmunoassay kit from bioMerieux. In all women, concentrations in serum were measured 4 times, before treatment and after 1, 3, 12 months of treatment. Bone mineral density (BMD) L2 – L4 was determined before treatment and at 12 months with a dualenergy x-ray absorptiometry scanner (Lunar DPX – 1 Q).

Statistical calculations were performed using the Statistica 6.188 PL. package made by StaSoft (STANISZ 1998).

RESULTS

The results are shown in Tables 1 to 2. As shown in Table 1, after 3 and 12 month of treatment in group I. (modified transdermal HRT) and group II (orally administered HST) significant OC levels were found. The concentration of PICP in group I after 3 and 12 was significantly decreased ($p < 0.05$). In group II, receiving orally given HST, the concentration of PICP did not change significantly. In group I of women receiving modified transdermal HRT, increased IGF-1 concentrations were found during the whole therapy with a significant increase after 1 month ($p < 0.05$) and 3, 12 month ($p < 0.01$). In the women receiving oral HST, IGF-1 concentrations were significantly decreased after 3 and 12 months of therapy ($p < 0.05$). The concentration of prolactin in women receiving transdermal modified HRT (group I) during the period of our study, under basic condition (PRL) and after MCP stimulation test (PRL/MCP) no statistically significant changes were observed. In the group of women receiving orally HST (group II), the concentration of prolactin basal after 3 month ($p < 0.05$) and 12 months ($p < 0.001$) was significantly increased. Prolactin level after MCP stimulation test (PRL/MCP) was also increased significantly ($p < 0.001$) in group II during the entire course of treatment.

Table 1

Concentrations of osteocacin (OC), procolagen (PICP), insulin-like growth factor I (IGF-1), prolactin basal (PRL), prolactin after metoclopramide (PRL/MCP)

Group *n*	Time	OC µg L^{-1}	PICP ng L^{-1}	IGI-1 µg L^{-1}	PRL µg L^{-1}	PRL/MCP µg L^{-1}
I 20 Baseline 1	1 mo	8.4+3.5	155.+41.1	88.1+20.2	19.1+6.4	210.+79.2***
	3 mo	7.6+4.1*	161.2+55.3	81.3+19.2*	20.4+7. 1*	282.4+99.1***
	12 mo	7.2+3.1*	166.4+57.2	79.2+16.1*	26.3+8.3***	290.3+87.1***

Data are shown as mean + SD, *p* indicates level of significance, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$

Table 2

Bone mineral density of the lumbar spine (BMD) L2 – L4 g cm^{-2} in women in early postmenopausal period receiving fluoride, modified transdermal hormone therapy (HRT) and oral supplement hormone therapy (HST)

Group *n*	Time	L1	L2	L3	L4	BMD L2-L4	BMD L2-L4 (%)
I 20 Baseline		0.851	0.921	0.984	0.961	0.948	
		+0.075	+0.086	+0.087	+0.075	+0.075	
	12 mo	0.876*	0.955 *	1.019**	1.006**	0.993**	
		+0.038	+0.069	+0.099	+0.112	+0.098	4.7
II 20 Baseline		0.876	0.924	0.970	0.905	0.933	
		+0.071	+0.068	+0.087	+0.085	+0.080	
	12 mo	0.884	0.939	0.992*	0.933*	0.951*	1.02
		+0.080	+0.084	+0.077	+0.079	+0.083	

L1 indicates BMD of the first lumbar spine; L2 BMD of the second lumbar spine; L3 BMD of third lumbar spine, L4 BMD of the fourth lumbar spine; L1 – L4 mean values of BMD in grams per square centimeter; BMD L2 – L4, BMD of the lumbar spine L2 – L4

*$p < 0.05$; **$p < 0.01$ significance of differences in comparison with baseline results (Student t test for paired variables); $p < 0.01$ – significance of differences in comparison with control group (ANOVA + Tukey's post hoc test; Student t test)

The results of BMD L2-L4 of the lumbar spine of baseline and after 12 months of treatment are presented in Table 2. The increase in BMD L2-L4 was statistically significant ($p < 0.01$) in women receiving modified transdermal HRT (group I) and also in women (group II) undergoing orally given HST ($p < 0.05$) in comparison with the values before treatment. The mean increase in BMD after one year of treatment was 4.7% in group I women who received modified transdrmal HRT and 1.02% in group II women, administered orally given HST.

RESULTS AND DSCUSSION

Significantly elevated BMD L2-L4 ($p < 0.01$) after 12 months of modified transdrmal HRT may be caused by an elevated level of IGF-1 and decreased PRL level (Stanosz et al. 2009). In contrast, women receiving orally given HST for 12 months were observed not to experience any significant increase in BMD L2-L4, which may be associated not only with elevated PICP and PRL levels ($p < 0.001$) in serum. Significantly lower IGF-1 concentration after 3 and 12 months in group II ($p < 0.05$) may be an indication of diminishing bone mass and of the risk factor of osteoporotic fractures in postmenopausal women. Despite the great progress in the field of recognizing the pathomechanisms responsible for the development of osteoporosis, the currently applied prophylaxis and treatments are still widely considered to be unsatisfactory. New combined therapy schemes are constantly being searched for and new medications are being developed. Currently employed treatment patterns use the bone resorption inhibitors or the bone formation stimulators. The former group of drugs consists of biphosphonians, estrogens, progestagens, calcitonin, vitamin D3, calcium derivates preparations, tiazides (GALUS 2005, MACLAUGHLIN et al. 2006, PALMER et al. 2005). The latter group is composed of fluorides, anabolic steroids, (OKOPIEŃ et al. 2005) parathormone and some of the growth stimulation factor (OHTA 2005).

Fluorides are considered to be the most powerful stimulators of bone formation, giving the possibility to achieve significant trabercular bone mass increase. Studies have showed that higher bone fracture frequency (arms and legs) was observed among patients given high doses of fluoride (VESTERGAARD et al. 2008). However, administration of small doses of fluoride combined with calcium resulted in significant increase of bone mass from 5 to 10% and decrease of bone fracture frequency. The authors' own studies on women treated for osteoporosis for one year with no fluorides applied revealed that the serum and urine concentrations of fluorides decreased significantly down to the trace values. The reason for such an evident drop in fluoride content seems to be their incorporation into the hydroxyapatite structure of bones (WHELAN et al. 2006) Fluorides are considered to diminish the dissolution of apatite crystals through the direct replacement of hydroxyl ions in the crystalline net. The results of the study presented prove that 0.25 mg kg^{-1} 24 h^{-1} is an optimum daily dose of fluoride and transdermal HRT in the osteoporosis treatment as it ensures the fluoride blood concentration to stabilize on the top level of the physiological range.Conclusions

1. Modified transdermal HRT and fluoride modify concentrations of prolactin, osteocalcin, insulin-like growth factor, procolagen and bone metabolism.

2. Lower albeit significant increase in BMD of lumbar spine in women receiving orally given HST may be a result of significantly lower concentration of IGF-1 and significantly increased prolactin.

REFERENCES

BUSSE B., JOBKE B., WERNER M., FURST M., RUTHER W., DELLING G. 2006. *Fluorosis a forgotten entity. Case of a woman with coxarthrosis and newly diagnosed fluorosis.* Pathologe, 27(1): 73-9

GALUS K. 2005. *Prevention and treatment of osteoporosis.* Pol. Arch. Med. Wewn., 114 (6/): 1236-43.

GIACHINI M., PIERLEONI F. 2004. *Fluoride toxicity.* Minerva Stomatol., 53 (4): 171-7

RINGE JD., DORST A., FABER H., KIPSHOVEN C., ROVATI LC., SETNIKA I. 2005. *Rheumatol.* Int., 25 (4): 296-300.

MACLAUGHLIN EJ., SLEEPER RB., MCNATTY D., RAEHL CL. 2006. *Management of age-related osteoporosis and prevention of associated fractures.* Ther. Clin. RiskManag., 2 (3): 281-95.

OHTA H. 2005. *Raloxifene and bone quality issues.* Clin. Calcium, 15 (6): 1012-9.

OTSUKA M., OSHINBE A., LEGEROS RZ., TOKUDOME Y., ITO A., OTSUKA K., HIGUCHI WI. 2008. *Efficacy of the injectable calcium phosphate ceramics suspensions containing magnesium, zinc and fluoride on the bone mineral deficiency in ovariectomized rats.* J. Pharmacol. Sci., 97 (1): 421-32.

OKOPIEŃ B., KRYSIAK R., LABUZEK K., HERMAN ZS. 2005. *Anabolic therapy in osteoporosis.* Part 1. Pol. Merkur. Lek., 18 (108): 712-4.

PALMER C., WPLFE SH. 2005. *Position of the American Dietetic Association: the impact of fluoride on health.* J. Am. Dit. Asspc., 105 (10): 1620-8.

PEPENE CE., SECK T., DIEL I., MINNE HW., ZIEGLER R., PFEILSCHIFTER J. 2004. *Influence of fluoride salts,hormone replacement therapy and calcitonin on the concentration of insulin-like growth factor /IGF/I. IGF-II and transforming growth factor beta 1 in human iliac crest bone matrix from patients with primary osteoporosis.* Eur. J. Endocrinol., 150 (1): 81-91.

REID IR., CUNDY T., GREY AB., HORNE A., CLEARWATER J., AMES R., ORR-WALKER BJ., WU F., EVANS MC., GAMBLE GD., KING A. 2007. *Addition of monofluorophosphate to estrogen therapy in postmenopausal osteoporosis: a randomized controlled trial*. J. Clin. Endocrinol. Metab., 92 (7): 2446-52.

GIACHINI M., PIERLEONI F. 2004. *Fluoride toxicity.* Minerva Stomatol., 53 (4): 171-177.

RINGE JD., DORST A., FABER H., KIPSHOVEN C., ROVATI LC., SETNIKAR I. 2005. *Efficacy of etidronate and sequential monofluorophosphate in severe postmenopausal osteoporosis: a pilot study.* Rheumatol. Int., 25 (4): 296-300.

SOWERS M., WHITFORD GM., CLARK MK., JANNAUSCH ML. 2005. *Elevated serum fluoride concentration in women are not related to fractures and bone mineral density*. J. Nutr., 135(9): 2247-52.

STANISZ A. 1998. *Course of statistics based on a program Statistica StaSoft.* Ist ed. Cracow.

STANOSZ S., LISIECKA B., WESOŁOWKA B., GOERTZ K., KULIGOWSKI D., KOŚCIUSZKIEWICZ B. 1995. *The influence of modified sequential therapy on lipids metabolism in postmenopausal women*. Ginecol. Pol., 66 (5): 284-8.

STANOSZ S., ŻOCHOWSKA E., SAFRANOW K., SIEJA K., STANOSZ M. 2009. *Influence of modified transdermal hormone replacement therapy on the concentrations of hormones,growth factors, and bone mineral density in women with osteopenia.* Metabolism, 58 (1): 1-7.

VESTERGAARD P., JORGENSEN NR., SCHWARZ P., MOSEKIDE L. 2008. *Effects of treatment with fluoride on bone mineral density and fracture risk – a meta-analysis.* Osteoporosis Int., 19 (3): 257-68.

WHELAN AM., JURGENS TM., BOWLES SK. 2006. *Natural health products in the prevention and treatment of osteoporosis: systematic review of randomized controlled trials.* Ann. Pharmacother., 40 (5): 836-49.

YAMAGUCHI M. 2007. *Fluoride and bone metabolism.* Clin. Calcium, 17 (2): 217-23

J. Elementol. 2009, 14(3): 553–562

EFFECT OF DIFFERENT POTASSIUM SOIL LEVELS AND FORMS OF POTASSIUM FERTILIZERS ON MICRO-ELEMETAL NUTRION STATUS OF APPLE TREES IN EARLY FRUITION PERIOD

Adam Szewczuk[1], Andrzej Komosa[2], Ewelina Gudarowska[1]

[1]Chair of Horticulture
Wrocław University of Environmental and Life Sciences
[2]Chair of Horticultural Plant Nutrition
Poznań University of Life Sciences

Abstract

The experiment was established in the spring of 1999 on grey brown podsolic soil formed from boulder clay. Apple trees of cv. Golden Delicious were planted on rootstock at 3.5×1.2 m (2381 trees·ha^{-1}). The first factor in the experiment consisted of the levels of potassium in the arable soil layer: 120, 160 and 200 mg K·kg^{-1} of soil d.m., on the basis of annual chemical analyses and determined by universal method. The second factor comprised three forms of potassium fertilizers: potassium chloride (KCl-60%), potassium sulphate (K_2SO_4) and potassium nitrate (KNO_3). Each year the analyses of macro- and microelements in the soil and leaves were made.

The increase in available potassium levels from 120 to 200 mg K·kg^{-1} of soil d.m did not have influence on the content of available forms of iron, manganese, zinc, copper and boron in the herbicide fallow strips in the tree rows or in the arable (0-20 cm) and subarable (20-40 cm)soil layers. Application of potassium sulphate fertilizer caused a significant increase in the content of zinc in herbicide strips in both soil layers compared with potassium chloride and potassium nitrate. Different potassium fertilizer forms did not cause any significant changes in amounts of available forms of iron, manganese, copper and boron.

dr hab. Adam Szewczuk, Chair of Horticulture, Wroclaw University of Environmental and Life Sciences, pl. Grunwaldzki 24A, 50-363 Wrocław, phone (071) 320 17 39, e-mail: adam.szewczuk@up.wroc.pl

The increase in available potassium levels from 120 to 200 mg K·kg^{-1} of soil d.m significantly reduced the content of manganese in leaves of trees. However, no significant influence on the content of iron, zinc, copper and boron in leaves of apple trees was found.

Key words: apple trees, potassium fertilizers, chlorides, sulphates, nitrates, microelements.

WPŁYW RÓŻNYCH POZIOMÓW POTASU I RODZAJU NAWOZÓW POTASOWYCH NA STAN ODŻYWIENIA JABŁONI MIKROELEMENTAMI PO WEJŚCIU DRZEW W OKRES OWOCOWANIA

Abstrakt

Doświadczenie założono wiosną 1999 r. na glebie płowej wytworzonej z glin lekkich zwałowych. Drzewa odmiany Golden Delicious na podkładce M.26 posadzono w rozstawie 3,5 x 1,2 m (2381 drzew·ha^{-1}). Pierwszym czynnikiem badań był zróżnicowany poziom zawartości potasu: 120, 160 i 200 mg K·kg^{-1} w ornej warstwie gleby, oznaczonego metodą uniwersalną, drugim rodzaj zastosowanego nawozu potasowego: chlorek potasu (sól potasowa 60%), siarczan potasu i saletra potasowa. Corocznie wykonywano analizy gleb i liści na zawartość mikroelementów.

Wzrost zawartości przyswajalnych form potasu w zakresie 120-200 mg K·kg^{-1} gleby nie miał istotnego wpływu na zawartość przyswajalnych form żelaza, manganu, cynku, miedzi i boru w pasach herbicydowych gleby, zarówno w warstwie 0-20, jak i 20-40 cm. Stosowanie siarczanu potasu istotnie zwiększało zawartość cynku w pasach herbicydowych zarówno w warstwie 0-20, jak i 20-40 cm gleby w stosunku do chlorku potasu (sól potasowa 60%) i azotanu potasu (saletra potasowa). Nie stwierdzono wpływu form nawozów potasowych na zawartość przyswajalnych form żelaza, manganu, miedzi i boru w glebie.

Wzrost zawartości przyswajalnych form potasu w glebie w zakresie 120-200 mg K·kg^{-1} gleby istotnie zmniejszał zawartość manganu w liściach jabłoni. Nie wykazano natomiast wpływu wzrastających poziomów potasu w glebie na zawartość w liściach jabłoni żelaza, manganu, miedzi i boru.

Słowa kluczowe: jabłoń, potas, nawozy potasowe, chlorki, siarczany, azotany, mikroelementy.

INTRODUCTION

Fertilization is the most important condition for proper growth and yielding of trees. Optimal nutrient supply for trees should take into account the vigour of the tree growth, the level of yielding as well the nutrient content in the soil and in the plant. Potassium fertilization and favourable N/K ratio can increase the frost tolerance of generative organs of fruit trees to some extent. The excess of lime can be compensated by potassium fertilization (Szűcs 2005). Potassium is taken up by apple trees in high levels, even greater than is actually needed to grow and yield properly. Excessive amounts of potassium in soil can cause worse absorption of magnesium (Lipecki and Jadczuk 1998, Pietranek and Jadczuk 2005) and calcium. Research on potassium fertilization of apple trees has brought variable results. Leszczyński and Sadowski (1990) pointed out to the positive effects of K fertilization on the tree nutrient status, while Pacholak (1984) presented an opposite opinion.

The content of potassium and phosphorus for orchard soil is usually determined by Egner-Riehm's method and the content of magnesium by Schachtschabel's method. In horticulture, a universal method after Nowosielski is used for determining all macro- and microelements. Komosa and Stafecka (2002) found out that the universal method is good for orchard soils, too. Most often, potassium is applied as potassium chloride. Being more expensive, potassium sulfate is used rarely. However, some studies conducted in Poland indicated a low sulfur level in soils of farmlands as well as in orchards (Jakubus 2001). That is why, using potassium sulfate for fertilization in apple orchard could by an important source of sulfur (Komosa, Szewczuk 2002, Szewczuk et al. 2008).

The aim of the present research has been to determine the influence of different level of potassium fertilization, as well anion accompanying potassium: chlorides, nitrites, sulfates, on the content of microelements in leaves of cv. Golden Delicious apple trees and on their nutrition status.

MATERIAL AND METHODS

The experiment was carried out in 2002-2004 years in the Experimental Station belonging to Wrocław University of Environmental and Life Sciences. In the spring of 1999, two-year-old apple trees of cv. Golden Delicious on rootstock M 26 were planted at the spacing 3.5×1.2 m (2381 trees ha^{-1}) on grey brown podsolic soil formed from boulder clay. The experiment was established in a randomized split-plot design in four replications with 4 trees per plot. The experimental plot covered 67.2 m^2 and had 16 trees, of which 4 in the middle were studied and the remaining 12 made the isolation.

Herbicide fallow strips were in the tree rows grass alleys were maintained and between them. Before planting, the macro- and microelements content of the soil was determined (Table 1). According to the content index worked out by Komosa and Stafecka (2002), the following were established: $N\text{-}NH_4$+$N\text{-}NO_3$ 6-20, P 30-60, K 50-80, Ca 250-400, Mg 30.0-60 and $S\text{-}SO_4$ 10-30 $mg \cdot kg^{-1}$ g soil d.w. and Fe 75.0-120.0, Mn 25.0-40.0, Zn 3.0-6.0, Cu 1.0-4.0 and B 0.3-1.5 $mg \cdot kg^{-1}$ soil d.m. and <50 mg Cl, <50 mg Na·kg g^{-1} soil, EC <0.5 $mS \cdot cm^{-1}$, i.e. low contents of nitrogen, sulfur and iron, standard amounts of phosphorus, manganese, copper and boron, high contents of potassium, magnesium, calcium, zinc, chloride and an admissible level of sodium in the arable soil layer (0-20 cm). In the subarable soil layer (20--40 cm), low contents of nitrogen, phosphorus, potassium, sulfur, zinc and copper, medium levels of calcium, magnesium, iron, manganese and boron,

Table 1

Content of macro and microelements in the soil before planting of apple trees (1998)

The soil layer (cm)	mg·kg^{-1} soil d.w.						
	$N-NH_4$	$N-NO_3$	P	K	Ca	Mg	$S-SO_4$
0-20 20-40	3.1 t.a.*	23.4 5.5	38.0 17	96.0 22	1278 279	86.0 42	t.a. t.a.
mg·kg^{-1} soil d.w.							
	Fe	Zn	Mn	Cu	B	Na	Cl
0-20 20-40	66.4 72.9	9.3 2.5	25.3 39.2	2.9 0.9	0.77 0.50	6 4	55 63
	pH (H_2O)	EC mS·cm^{-1}					
0-20 20-40	6.99 6.98	0.22 0.28					

t.a.* trace amounts

and high content of chlorides, pH (in H_2O) 6.99 and 6.98, EC 0.22 (0-20 cm) and 0.18 mS·cm^{-1} (20-40 cm) were observed.

The field trail was established as a two-factor experiment. The first factor consisted of increasing levels of potassium in the arable soil layer: 120, 160 and 200 mg K·kg^{-1} soil d.m., based on annual chemical analyses. The second factor comprised three forms of potassium fertilizers: potassium chloride (KCl), potassium sulfate (K_2SO_4) and potassium nitrate (KNO_3). The assumed level of potassium was kept by using different rates of fertilizers (Table 2). All treatments were fertilized with nitrogen and phosphorus, according to the annual analyses of soil and leaves. Nitrogen was used as ammonium nitrate (34%) and saltpetre (13% N, 39% K-only in combination

Table 2

Rates of potassium fertilizers in 2002-2004 (mg·kg^{-1})

Treatment	K level (mg·kg^{-1}soil)	Fertilizer form	Sum from 1999-2001	2002	2003	2004
K-1 (KCl)	120	KCl	54	125	0	0
K-2 (KCl)	160	KCl	154	170	129	0
K-3 (KCl)	200	KCl	254	100	0	75
K-1 (K_2SO_4)	120	K_2SO_4	54	36	0	75
K-2 (K_2SO_4)	160	K_2SO_4	192	40	36	0
K-3 (K_2SO_4)	200	K_2SO_4	314	186	0	0
K-1 (KNO_3) K-2 (KNO_3) K-3 (KNO_3)	120 160 200	KNO_3 KNO_3 KNO_3	54 154 254	123 177 277	94 0 0	0 0 150

with KNO_3), phosphorus as triple superphosphate (20% P), potassium as potassium chloride (60%), potassium sulfate (41%). The fertilization was applied in the middle of March, April and May.

Soil samples from the herbicide strips of each plot were collected each year in the second half of July, separately from the layers 0-20 and 20--40 cm, by using a soil drill. Soil analyses were carried out using the universal method according to NOWOSIELSKI (1974), modified for orchard soils (KOMOSA and STAFECKA, 2002). In this method, B was extracted in 0.03 M CH_3COOH, microelements Fe, Mn, Zn and Cu were extracted with Lindsay's solution, which contained in 1 dm $^{-3}$: 5 g $EDTAH_4$, 9.0 ml 25% ammonia, 4 g citric acid, 2 g $(CH_3COO)_2Ca \cdot 2H_2O$. Extractions were conducted in a 1:4 proportion of soil to extraction solution, (50 g dry weight soil and 200 cm^3 Lindsay's solution) for 30 minutes. After extraction, B was assessed by colorimetric analysis with curcuma, and Fe, Mn, Zn i Cu – by the AAS method (*Laboratory research method...* 1983).

Leaf samples were collected in the middle of July. One sample containing 100 leaves from the middle part of long-shoots (3-4 leaves per shoot) was collected from each plot. The concentration of Fe, Mn, Zn, Cu and B in the leaves was estimated. Mineralization of leaves for Fe, Mn, Zn and Cu assessment was performed in a mixture of HNO_3, $HClO_4$ and H_2SO_4 in the 10:1:1 ratio, while concentration of B was determined after dry digestion with calcium hydroxide (*Laboratory research method...* 1972).

The results were evaluated statistically using the analysis of variance. The significance of differences between means was evaluated according to *t*-Duncan's multiple range test at P=0.05.

The present study is the continuation of a previous experiment carried out in order to estimate the influence of different potassium fertilization on macro- and microelement nutrition status of young apple trees. The results, for 1991-2001 year, were published by KOMOSA and SZEWCZUK (2002). In this study, the results for the next three years are presented and concern older trees in full fruition period.

RESULTS AND DISSCUSION

The results presented in Table 3 proved that increasing potassium in arable soil layer in herbicide fallow had no influence on the content of available forms of iron, manganese, zinc, cooper and boron in soil. However, significant influence of the fertilizer forms of potassium on the content of zinc in the arable soil layer was noted (Table 3). The application of potassium sulfate caused the highest zinc content in soil, in comparison with potassium chloride and nitrate form. These differences were confirmed by the estimation of the content of available forms of microelements in the subarable

Table 3

Content of microelements in the soil layer of 0-20 cm in the herbicide strips in relation to fertilizer form and potassium level in soil (means from 2002-2004)

K level mg · kg^{-1} of soil	Fertilizer form			Mean
	KCl	K_2SO_4	KNO_3	
mg · Fe kg^{-1} of soil				
120	76.7***	74.4	76.2	75.7**
160	79.4	74.4	77.6	77.1
200	68.3	69.0	56.9	64.7
Means	74.8*	72.6	70.2	
mg · Mn kg^{-1} of soil				
120	21.1***	21.4	17.1	19.9**
160	19.2	16.8	26.5	20.8
200	25.1	19.5	11.7	18.8
Means	21.8*	19.2	18.4	
mg · Zn kg^{-1} of soil				
120	6.2 ab	17.1 cd	8.8 abc	10.7 ab
160	6.3 ab	16.0 cd	5.3 ab	9.2 a
200	6.2 ab	16.5 cd	5.2 a	9.3 a
Means	6.2 ab	15.5 cd	6.4 ab	
mg · Cu kg^{-1} of soil				
120	2.7***	2.8	2.6	2.7**
160	2.6	2.7	2.9	2.7
200	3.1	2.7	2.3	2.7
Means	2.8*	2.8	2.6	
mg · B kg^{-1} of soil				
120	0.56***	0.54	0.52	0.54**
160	0.52	0.50	0.48	0.50
200	0.62	0.50	0.46	0.53
Means	0.57*	0.51	0.49	

Means marked by the same letter are not significantly different at α=0.05.
*no significant differences between the means for the fertilizer forms
**no significant differences between the means for the potassium levels
***no significant differences for the interaction fertilizer form x potassium level

Table 4

Content of microelements in the soil layer of 20-40 cm in the herbicide strips in relation to fertilizer form and potassium level in soil (means from 2002-2004)

K level $mg \cdot kg^{-1}$ of soil	Fertilizer form			Mean
	KCl	K_2SO_4	KNO_3	
$mg \cdot Fe\ kg^{-1}$ of soil				
120	74.1***	81.4	79.0	78.2**
160	75.0	58.5	74.8	69.4
200	75.3	64.5	58.6	66.2
Means	74.8*	68.1	70.8	-
$mg \cdot Mn\ kg^{-1}$ of soil				
120	21.5***	23.4	16.7	20.5**
160	11.6	10.6	19.8	14.0
200	23.4	12.8	7.9	14.7
Means	18.8*	15.6	14.8	-
$mg \cdot Zn\ kg^{-1}$ of soil				
120	4.5ab	14.4 bc	8.0 ab	9.0ab
160	4.7ab	12.7 abc	4.4 ab	7.3a
200	4.6 ab	19.9 c	3.4 a	9.3b
Means	4.6ab	15.7c	5.3a	-
$mg \cdot Cu\ kg^{-1}$ of soil				
120	2.4***	2.5	2.3	2.4**
160	2.2	2.3	2.5	2.3
200	2.7	2.2	2.1	2.3
Means	2.4*	2.4	2.3	-
$mg \cdot B\ kg^{-1}$ of soil				
120	0.39***	0.38	0.47	0.41**
160	0.43	0.39	0.42	0.42
200	0.46	0.43	0.41	0.42
Means	0.43*	0.40	0.44	-

Means marked by the same letter are not significantly different at α=0.05.
*no significant differences between the means for the fertilizer forms
**no significant differences between the means for the potassium levels
***no significant differences for the interaction fertilizer form x potassium level

Table 5

Content of microelements in Golden Delicious apple leaves in relation to potassium level and fertilizer form in soil (means from 2002-2004)

K level $mg \cdot kg^{-1}$ of soil	Fertilizer form			Mean
	KCl	K_2SO_4	KNO_3	
$mg \cdot Fe\ kg^{-1}$ of soil				
120	69.1***	70.6	71.4	70.3**
160	70.3	74.7	68.1	71.0
200	72.4	69.6	74.3	72.1
Means	70.6*	71.6	71.3	-
$mg \cdot Mn\ kg^{-1}$ of soil				
120	120.1***	112.7	131.6	121.5 b
160	111.1	112.2	104.7	109.3 ab
200	101.5	97.7	100.0	99.7 a
Means	110.9*	107.6	112.1	-
$mg \cdot Zn\ kg^{-1}$ of soil				
120	36.0***	37.7	40.0	37.9**
160	34.8	39.9	35.4	36.7
200	35.2	47.4	35.1	39.2
Means	35.3*	41.7	36.8	-
$mg \cdot Cu\ kg^{-1}$ of soil				
120	6.7***	6.5	6.6	6.6**
160	6.8	7.0	6.4	6.7
200	6.5	6.4	6.5	6.5
Means	6.7*	6.6	6.5	-
$mg \cdot B\ kg^{-1}$ of soil				
120	40.9***	39.7	39.3	40.0 **
160	44.0	43.7	41.7	43.2
200	42.2	41.4	44.3	42.7
Means	42.4*	41.6	41.8	-

Means marked by the same letter are not significantly different at α=0.05.
*no significant differences between the means for the fertilizer forms
**no significant differences between the means for the potassium levels
***no significant differences for the interaction fertilizer form x potassium level

layer of soil in herbicide fallow strips (Table 4). Significantly the highest concentration of zinc was in the soil fertilized with the sulfate form of potassium fertilizers.

At the lower level of 120 and 160 mg K mg kg^{-1} of soil, the tendency to increase the content of iron, manganese and zinc in arable and subarable soil layer in herbicide fallow strips was noted. This relationship was not observed for copper and boron (Tables 3, 4).

Comparison with the content index for soils in apple orchards, worked out by KOMOSA and STAFECKA (2002), suggested that maintaining high level of potassium in soil (200 K $mg \cdot kg^{-1}$), impoverished the soil of iron (change for medium into low content), manganese and zinc (no change of class content). Content of copper and boron in soil was on the medium level, regardless of the levels of potassium content in soil. Reduction of the content of iron, manganese and zinc in soil, caused by the high level of potassium, can be connected with displacing these cations from sorption complex of soil and moving them into the depth of soil profile. Reduction of content of manganese in soil by the high level of potassium (200 mg $K \cdot kg^{-1}$) was confirmed by significant reduction of concentration of manganese in apple leaves.

The optimal concentration of manganese in apple leaves is 41-100 mg $Mn \cdot kg^{-1}$. In the present study, the content of manganese fell from high level of content (109.3-121.5 $mg \cdot kg^{-1}$) to the optimal level – 99.7 $mg \cdot kg^{-1}$. No significant influence of the potassium level and potassium fertilizer forms on the nutritional status of iron, zinc, copper and boron of apple trees occurred. Similar results were obtained by GĄSTOŁ and SKRZYŃSKI (2006). According to these authors, the distribution of mineral constituents in plants depended on different organs of apple tree. It is obviously attributable to the different functions of particular fruit tree organs. GĄSTOŁ and SKRZYŃSKI [2006] did not notice the influence of dwarfing methods on leaf iron, zinc and copper content. However, different levels of microelements were noted in roots, wood and bark of apple trees (GĄSTOŁ and SKRZYŃSKI 2006). The determined content of microelements in this study was low for iron and optimal for zinc, copper and boron in comparison with the optimal contents in leaves of apple trees. The low content of iron in leaves cold be caused by high $pH_{(H_2O)}$ of soil (6.98-6.99), and thus by worse uptake of iron by plants.

CONCLUSIONS

1. The increase in the content of available potassium forms from 120 to 200 $mg \cdot kg^{-1}$ of soil did not have significant influence on the content of available forms of iron, manganese, zinc, copper and boron in the herbicide fallow strips or in the arable (0-20 cm) and subarable (20-40 cm) soil layer.

2. The potassium fertilizer forms affected the zinc concentration in soil. Application of potassium sulfate resulted in an increase in the zinc concentration in arable and subarable soil layers in comparison with potassium chloride and nitrate. Different potassium fertilizers forms had no influence on the iron, manganese, copper and boron concentration in soil.

3. The increase in the content of available potassium forms from 120 to 200 mg $K \cdot kg^{-1}$ of soil reduced significantly the concentration of manganese in leaves of apple trees. No significant interaction between increasing levels of potassium content in soil and concentration of iron, cooper, zinc and boron in apple leaves was found

REFERENCES

GĄSTOŁ M., SKRZYŃSKI J. 2006. *Influence of different dwarfing methods on content of microelements in apple tree organs.* Scientific works of the Lithuanian Institute of Horticulture and Lithuanian University of Agriculture, 25(3): 262-272

JAKUBUS M., 2001. *Zawartość siarki siarczanowej w glebach uprawnych oraz wpływ wybranych czynników ja determinujących [Content of sulphate sulphur in arable soils and the influence of some factors which determine its level].* Rocz. AR Poznań, 335: 35-47 [in Polish]

KOMOSA A., STAFECKA A. 2002. *Zawartości wskaźnikowe składników pokarmowych dla gleb sadowniczych analizowanych metodą uniwersalną [Index contents of nutrients for orchard soils analysed with the universal method].* Rocz. AR Poznań, 341:, Ogrodnictwo, 35: 105-116. [in Polish]

KOMOSA A., SZEWCZUK A., 2002. *Effect of soil potassium level and different potassium fertilizer form on nutritional status, growth and field of apple trees in the first three years after planting.* J. Fruit Ornamental Plant Res., 10: 41-54.

LIPECKI M., JADCZUK E. 1998. *Wpływ dawek i sposobu nawożenia potasem na poziom odżywienia mineralnego jabłoni [Effect of rates and fertilization techniques on the mineral nutrition level of apple trees].* Zesz. Nauk. AR Kraków, 333: 499-501. [in Polish]

LESZCZYŃSKI A., SADOWSKI A. 1990. *Response of apple rootstock and of apple maiden trees to different levels of potassium supply in sand culture.* Acta Hort., 274: 277-286

Metody badań laboratoryjnych w stacjach chemiczno-rolniczych. Cz.II. *Badanie materiału roślinnego [Laboratory research methods in chemical and agricultural stations*. Part II. Analyses of plant material]. IUNG Puławy, 1972. ss. 25-83. [in Polish]

Metody badań laboratoryjnych w stacjach chemiczno-rolniczych. Cz. III. *Badanie gleb, ziem i podłoży spod warzyw i kwiatów oraz części wskaźnikowych roślin w celach diagnostycznych[Laboratory research methods in chemical and agricultural stations.* Part III. *Analyses of soils, earth and substrates under vegetables and fruit as well as index organs of plants for diagnostic purposes].* IUNG, Puławy, 1983. ss. 28-81. [in Polish]

PACHOLAK E., 1984. *Wpływ nawożenia i nawadniania na zawartość składników pokarmowych w liściach drzew i w glebie sadu jabłoniowego [Influence of fertilization and irrigation on the content of nutrients in tree leaves and in soil of an apple orchard].* Pr. Kom. Nauk Rol. Leś. PTNP 57: 233-243. [in Polish]

PIETRANEK A., JADCZUK E., *Mineral Status of 'Katja' apple trees depending on irrigation, fertlization and rootstock.* Acta Sci. Pol. Hort. Cultus, 4(1): 69-76.

SZEWCZUK A., KOMOSA A., GUDAROWSKA E. 2008. *Effect of soil potassium levels and different potassium fertilizer forms on yield and storability of 'Golden Delicious' apples*. Acta Sci. Pol. Hort. Cultus, 7(2): 53-59.

SZŰCS E., 2005. *Some aspects of integrated plant nutrition in orchard.* Acta. Sci. Pol. Hort. Cultus, 4 (1): 47-57

J. Elementol. 2009, 14(3): 563–571

MAGNESIUM CONCENTRATION IN PLASMA AND TISSUES OF PATIENTS UNDERGOING SURGERY FOR STOMACH AND LARGE INTESTINE CANCER

Monika Szewczyk, Kazimierz Pasternak, Andrzej Andrzejwski, Andrzej Dąbrowski, Grzegorz Wallner

Chair and Department of Medical Chemistry
2nd Chair and Department of General, Gastrointestinal and Oncological Surgery of the Alimentary Tract
Medical University of Lublin

Abstract

Digestive system neoplasms pose a serious health problem both in Poland and abroad. Neoplasms are frequently considered to be caused by impaired homeostasis in the human body. Development of neoplasms may be linked to disturbances in concentration of elements, including magnesium as a major intracellular cation.

The objective of this study was to evaluate the concentration of magnesium in plasma and tissue samples taken from patients suffering from neoplasms of the stomach or the large intestine.

The study involved 35 patients, including 20 affected by stomach cancer and 15 suffering from large intestine cancer. The patients were in the age rage of 36-77.

The material included blood samples taken from patients before and seven days after surgery, as well as samples of cancerous and healthy tissues. The colorimetric method with a Genesis spectrophotometer was used for determination of magnesium concentration.

A statistically significant difference was observed between plasma magnesium concentration in patients affected by stomach cancer and the normal range. Elevated values of magnesium concentration measured on the seventh day after the procedure as compared to the concentration before the procedure was noted, however, the difference was statistically insignificant. No significant differences were observed in magnesium concentration me-

Monika Szewczyk, Chair and Department of Medical Chemistry, Medical University of Lublin

asured before and after the procedure, or in comparison to the normal range in patients with large intestine cancer. Determination of tissue magnesium showed that magnesium concentration was higher in cancerous than in healthy tissue.

Obtained results demonstrate that magnesium homeostasis is impaired in patients, which may be important in the pathoghenesis of digestive system neoplasms.

Key words: magnesium, stomach cancer, large intestine cancer, hypomagnesemia, surgery.

STĘŻENIE MAGNEZU W OSOCZU I TKANKACH PACJENTÓW LECZONYCH OPERACYJNIE Z POWODU RAKA ŻOŁĄDKA I JELITA GRUBEGO

Abstrakt

Nowotwory przewodu pokarmowego stanowią istotny problem zdrowotny zarówno w Polsce, jak i na całym świecie. Przyczyny nowotworów często upatruje się w zaburzeniach homeostazy organizmu. Rozwój nowotworów może wiązać się z zaburzeniem stężenia pierwiastków, w tym również magnezu będącego najważniejszym kationem wewnątrzkomórkowym.

Celem pracy była ocena stężenia magnezu w osoczu i wycinkach tkanek nowotworowych chorych na raka żołądka i raka jelita grubego.

Badania wykonano u 35 pacjentów, w tym u 20 chorych na raka żołądka, oraz 15 chorych na raka jelita grubego. Badani byli w przedziale wiekowym od 36 do 77 lat.

Materiał do badań stanowiła krew chorych pobierana przed zabiegiem operacyjnym oraz w 7. dobie po zabiegu, a także wycinki tkanek zmienionych nowotworowo i zdrowych. Stężenie magnezu oznaczano metodą kolorymetryczną z użyciem spektrofotometru firmy Genesis.

U chorych na raka żołądka wykazano istotną statystycznie różnicę w stężeniu magnezu w osoczu w porównaniu z normą. Zaobserwowano również wyższe stężenie magnezu w 7. dobie po zabiegu w porównaniu ze stężeniem przed operacją, jednak różnica ta była nieistotna statystycznie. Natomiast w przypadku raka jelita grubego nie wykazano różnic istotnych statystycznie zarówno w stężeniu magnezu przed i po zabiegu, jak i przy porównaniu wyników stężeń magnezu z normą. Badając stężenie magnezu w tkankach, stwierdzono wyższe stężenie magnezu w tkance nowotworowej w porównaniu z tkanką zdrową.

Wyniki wskazują na istnienie zaburzeń homeostazy magnezu u chorych, co może mieć znaczenie w patogenezie nowotworów przewodu pokarmowego.

Słowa kluczowe: magnez, rak żołądka, rak jelita grubego, hipomagnezemia, leczenie operacyjne.

INTRODUCTION

Digestive system neoplasms are one of the chief causes of death from malignant tumors. The incidence rate of stomach and large intestine cancer in Poland is among the highest. Both stomach cancer and large intestine cancer are most frequently diagnosed in an advanced stage. Consequently, the efficacy of treatment and survival rate are low. Stomach cancer usually affects people in the age range of 50-60, mostly female, while the incidence of large intestine cancer among males and females is comparable (BALCERSKA et al. 2000, BORCH et al. 2000, DEHEINZELIN et al. 2000, DARADÓ et al. 2005).

Surgery is the basic and the most effective treatment of digestive system cancers. It is applied both in early and advanced stage carcinoma and involves radical removal of tumor. Hypomagnesemia frequently occurs in the post-surgery period, particularly following surgical treatment of stomach cancer (HARTGRINK et al. 2002, KIM 2002, MACHOWSKA, DUDA 2002, POPIELA 2002, BORAWSKA et al. 2005).

Advanced stage of digestive system neoplasms is usually accompanied by dysphagia and diarrhea, leading to emaciation, electrolyte disturbances and magnesium homeostasis disturbance. However, in early stages of cancerogenesis, patients have no symptoms and sings or the symptoms are non-specific (TOMATIS 2000, MACHOWSKA, DUDA 2002, WOLF et al. 2007).

Magnesium is a life essential intracellular macroelement, which is an activator of over 300 enzymes. It is a cell membrane stabilizer; it is essential to the synthesis of macroergic bonds and is active in protein synthesis as well as in nucleic acid metabolism. It plays an important role in the transportation of calcium, sodium and potassium ions. Magnesium deficiency in a period prior to a surgery may affect the patient's condition during and after the procedure. Even small changes in magnesium concentration induce changes in the cardiovascular system. Hypomagnesemia can have an inflammatory effect as it directly affects cells of the immune system, and by activating neuroendocrine mechanisms it produces an indirect effect. Low magnesium concentration causes more intensive activation of neutrophils and macrophages, increased production of inflammatory cytokines as well as excessive production of free radicals (HARTGRINK et al. 2002, HOENDEROP et al. 2005, LARSSON et al. 2005, STARZYŃSKA, WASILEWICZ 2007).

The objective of this study was to determine magnesium concentration in plasma and cancerous tissue samples taken from patients suffering from stomach or large intestine cancer.

MATERIAL AND METHODS

The study was conducted at the 2nd Chair and Department of General, Gastrointestinal and Oncological Surgery of the Alimentary Tract Medical University of Lublin in the University Hospital SPSK No.1 in Lublin. The group of patients included 35 people who underwent surgical treatment of stomach cancer (20 subjects) or large intestine cancer (15 subjects). The subjects were in the age range of 36-77. Most of them, 63%, were men. Surgical removal of cancerous changes was the chosen treatment. About 85% of operations of stomach and large intestine cancer were planned. Most of the patients were in hospital a few weeks during which they were diagnosed and medical treatment was applied. The patients did not have any symptoms and sings from the digestive system which could influence this study. No chemotherapy or radiotherapy preceded the surgical procedure. The subjects received no element supplementation. Study material included pre- and post-surgery plasma and tissue samples from the stomach and the large intestine.

The study was approved by the Bioethics Committee at the Medical University in Lublin. Approval no. KE – 0254/222/2007

Blood samples were taken before the procedure and on the seventh day after the procedure, and transferred to test tubes with the anticoagulant heparin.. Plasma was obtained by centrifuging whole blood samples at 3,500 rpm for 15 minutes.

Tissue material was obtained during surgery. Two samples were taken from each subject, one of cancerous tissue and the other one of possibly most distant healthy tissue. Tissue samples were bathed in 0.9% NaCl and stored at -40^{o}C until examination.

Tissue samples were defrozen and homogenized in Tris HCl (pH 7.4) buffer. Homogenates were centrifuged at 5,000 rpm for 15 minutes. Resulting supernatants were used for further examinations.

Magnesium concentration was determined with the colorimetric method using xylidyl blue which reacts with magnesium in alkaline solution to form a purple-coloured compound. The Cormay diagnostic kit Liquick Cor-Mg 60 was used. A single beam Genesis spectrophotometer with a wavelength at 520 nm was used to determine magnesium concentration.

The following statistical methods were used: Student's t-test, Cochran's C test and Cox test.

RESULTS AND DISCUSSION

The values of plasma magnesium concentration in subjects with stomach cancer ranged from 0.73 to 1.01 mmol l^{-1} before the surgery, and remained similar after the procedure, i.e. from 0.70 to 1.05 mmol l^{-1}. The values of plasma magnesium concentration measured before and seven days after the surgery were consistent with the normal range (Table 1). The mean magnesium concentration before the procedure was slightly lower than after the procedure.

The difference between the mean magnesium concentration in plasma of subjects suffering from stomach cancer before and after the surgery was statistically non-significant. However, there was a statistically significant difference between the values of magnesium concentration before and after the procedure versus the normal range.

Table 1

Plasma magnesium concentration in subjects

Specification	Plasma	No	From - to (mmol l^{-1})	Arithmetic mean	Median M	Standard deviation SD	Statistical significance IS pp*	Statistical significance ISn**
Stomach cancer	before	20	0.73 - 1.01	0.81	0.76	0.31	0.33	0.01
	7th day	20	0.70 - 1.05	0.94	0.86	0.24		0.01
Large intestine cancer	before	15	0.53 - 0.78	0.59	0.61	0.19	0.94	0.57
	7th day	15	0.48 - 0.62	0.51	0.48	0.06		0.67
Healthy	normal		0.60 - 1.1	0.85				

*level of statistical significance while comparing plasma Mg concentration in subjects before the surgery and on the 7th day after the surgery

**level of statistical significance while comparing plasma Mg concentration in subjects with the normal range

The values of plasma magnesium concentration in subjects with large intestine cancer were depressed (Table 1), ranging from 0.53 to 0.78 mmol l^{-1} before the surgery and from 0.48 to 0.62 after the surgery.

Similarly to stomach neoplasm, there was no statistical significance in the values of magnesium concentration before and after the surgery in subjects with neoplasm of the large intestine. Comparison of magnesium concentration before and after the procedure and the normal values did not demonstrate statistical significance.

In subjects with stomach cancer, the mean magnesium concentration reached 5.41 μg g^{-1} in healthy tissue and 6.18 μg g^{-1} in cancerous tissue. Analogously, in subjects with large intestine cancer, the mean concentration was 6.40 μg g^{-1} in healthy tissue and 6.77 μg g^{-1}. The study demonstrated that magnesium concentration was slightly higher in cancerous tissue (Table 2). The difference between magnesium concentration in cancerous and healthy tissue was statistically non-significant.

Table 2

Mean magnesium concentration in cancerous tissue

Specification	Tissue	No	From - to (μg g^{-1})	Arithmetic mean $\overline{x}$	Median M	Standard deviation SD	Statistical significance IS*
Stomach tissue	healthy	20	3.20 - 7.00	5.41	5.05	1.41	0.75
	cancerous	20	3.40 - 11.40	6.18	5.00	2.60	
Large intestine tissue	healthy	15	5.10 - 6.80	6.40	5.90	1.7	0.70
	cancerous	15	4.50 - 10.10	6.77	6.20	2.40	

*level of statistical significance by comparing Mg concentration in cancerous and healthy tissue

The etiopathogenesis of digestive system neoplasms is complex and not fully known yet. Neoplastic disease is often accompanied by disturbances in the concentration of macro- and microelements. Further disturbances may occur during treatment, especially when it involves surgery without accompanying element supplementation.

The body of a healthy adult contains approximately 24 g magnesium. More than 50% of the element is in the bones, ca 27% in the muscles and 19% in other soft tissues. Only 1% of the magnesium contained in the human body is found in blood, and its concentration in blood cells is almost three times as high as in serum. The latter ranges in a healthy person from 0.60 – 1.1 mmol l^{-1} (Głowania, Głowania 2000, Larsson et al. 2005, Lin et al. 2006).

Depressed magnesium concentration affects bone mineralization, the digestive system, the cardiovascular system, the reproductive organs, the immune system as well as the muscular and the nervous system. Low concentration of magnesium in the human body may promote oncogenic activity of carcinogenic substances leading to an increase in the number of mutations responsible for neoplasm formation (DEHEINZELIN et al. 2000, OGRODNIK et al. 2004, DAI et al. 2007).

Numerous studies have been conducted on plasma magnesium concentration in subjects with neoplasms. There have been, however, fewer studies on its concentration in cancerous tissues.

This study has demonstrated the lack of significant differences between plasma magnesium concentration measured before the surgery and on the seventh day after the procedure. In stomach cancer, pre-surgery values were consistent with the normal range. On the seventh day after the procedure, magnesium concentration was slightly elevated but still consistent with normal values.

However, magnesium concentration in subjects suffering from large intestine cancer was below the normal range or took borderline values. The results obtained on the seventh day after the procedure showed that the concentration of magnesium was slightly lower in comparison to the pre-surgery values. This confirms the results of a study reported by MACHOWSKA and DUDA, who examined plasma in subjects with stomach or large intestine cancer. They found out that in subjects who were not given magnesium intravenously its concentration decreased and half of the patients developed postoperative hypomagnesemia persisting until the examination was completed, which happened on the fourth day (MACHOWSKA, DUDA 2002).

It has been found out that in subjects with digestive neoplasms the mean magnesium concentration was higher in cancerous than in healthy tissue. Similar results were obtained by NIEDZIELSKA et al. and BORAWSKA et al. in their studies on magnesium concentration in cancerous larynx tissue (NIEDZIELSKA et al. 2000, BORAWSKA et al. 2005). Increased magnesium concentration in cancerous tissue may be indicative of intensive metabolic processes in tumorous tissue.

Yaman et al. pointed out to the lack of significant differences between magnesium concentration in healthy and cancerous stomach tissue (YAMAN 2006, YAMAN et al. 2007, 2003). Olszewski et al. obtained similar results.

To sum up, disturbances in magnesium homeostasis, or even hypomagnesemia, occur in patients who undergo surgery for malignant tumor. Low magnesium concentration is found in plasma, while its concentration in cancerous tissues, where intensive metabolic process occur, is elevated.

Surgical procedure is an additional stress for the human body, therefore some researchers recommend magnesium supplementation in the pre- and post-surgery period. MACHOWSKA and DUDA (2002) noted beneficial influence of magnesium supply on the condition of patients after the procedure.

CONCLUSIONS

1. Large intestine neoplasms are accompanied by hypomagnesemia before and after a surgery.

2. Magnesium concentration is higher in cancerous than in healthy tissue.

3. Complete evaluation of interdependences between magnesium concentration and neoplasm formation requires further studies including more subjects.

REFERENCES

BALCERSKA A., STACHOWICZ-STENCEL T., ŁYSIAK-SZYDŁOWSKA W. 2000. *Dynamika przebiegu choroby nowotworowej a stan bariery antyoksydacyjnej ustroju [Dynamics of the course of a neplastic disease and the antioxidative barrier of an organism].* Wiad. Lek., 3-4: 128-133. [in Polish]

BORAWSKA M., CZYŻEWSKA E., ŁAZARCZYK B., SOCHA K. 2005. *Wpływ nawyków żywieniowych na zawartość magnezu u ludzi z nowotworami krtani [Effect of nutritional habits on the concentration of magnesium in people suffering from laryngeal cancer].* Bromat. Chem. Toksykol., supl., 639-642. [in Polish]

BORCH K., JONSSON B., TARPILA E. 2000. *Changing pattern of histological type, location, stage and outcome of surgical treatment of gastric carcinoma.* Br. J. Sur., 87: 618-626.

DAI Q., SHRUBSOLE M., NESS R., SCHLUNDT D., CAI Q., SMALLEY W., LI M., SHYR Y., ZHENG W. 2007. *The relation of magnesium and calcium intakes and genetic polymorphism in the magnesium transporter to colonorectal neoplasia risk.* Am. J. Clin. Nutr., 86(3): 743--751.

DARADÓ A., RZETECKI T., DZIKI A., SAPOTA A. 2005. *Poziomy biologiczne kadmu, cynku, miedzi i selenu u pacjentów z nowotworem jelita grubego [Biological levels of cadmium, copper and selenium in patients with large intestine cancer].* Bromat. Chem. Toksykol., 4(38): 371-376 [in Polish]

DEHEINZELIN D., NEGRI E., TUCCI M., SALEM M., CRUZ V., OLIVIERA R., NISHIMOTO I., HOELZ C. 2000. *Hypomagnesemia in critically ill cancer patients: a prospective study of predictive factors.* Braz. J. Med. Res., 33(12): 1443-1448.

GŁOWANIA A., GŁOWANIA I. 2000. *Zastosowanie związków magnezu w praktyce lekarskiej [Use of magnesium compounds in therapeutic practice].* Med. Metabol., 1(4): 37-44. [in Polish]

HARTGRINK H., PUTTER H., KLEIN KRANENBARG E. 2002. *Value of palliative resection in gastric cancer.* Br. J. Surg., 89: 1438-1443.

HOENDEROP J., BINDELS R. 2005. *Epithelial Ca^{2+} nad Mg^{2+} channels in health and disease.* J. Am. Soc. Nephrol., 16: 15-26.

KIM J. 2002. *Current status of surgical treatment of gastric cancer.* J. Surg. Oncol., 79: 79-80.

LARSSON S., BERGKVIST L., WOLK A. 2005. *Magnesium intake in relation to risk of colonorectal cancer in women.* JAMA, 293: 86-89.

LIN J., COOK N., LEE I., MANSON J., BURING J., ZHANG S. 2006. *Total magnesium intake and colonorectal cancer incidence in women.* Cancer Epidemiol. Biomarkers Prev., 15(10): 2006-2009.

MACHOWSKA B., DUDA K. 2002. *Hipomagnezemia u chorych operowanych z powodu nowotworów złośliwych żołądka oraz jelita grubego [Hypomagnsaemia in patients undergoing surgery for malignant neoplasmia of the stomach and large intestine].* Anestezjol. Intens. Terapia, 34: 172-177. [in Polish]

NIEDZIELSKA G., CARUK K., PASTERNAK K. 2000. *Pierwiastki śladowe w tkankach krtani objętych chorobą nowotworową [Trace elements in tissues of a larynx affected by neoplasmia].* Otolaryng. Pol., 4, supl. 31: 200-2002. [in Polish]

OLSZEWSKI J., LATUSIŃSKI J., KITA A. 2003. *Porównawcza ocena stężenia pierwiastków antyoksydacyjnych w surowicy krwi i bioptatach tkankowych u chorych z brodawczakiem lub rakiem krtani [Comparative evaluation of the concentration of antioxidative elements in blood serum and tissue bioptates in patients with papilloma and laryngeal neoplasma].* Otolaryngologia, 2(2): 90-93. [in Polish]

OGRODNIK W., KACZMAREK-BORAWSKA B., PASTERNAK K., GRĄDZIEL K. 2004. *Wpływ chemioterapii zawierającej doksorubicynę na stężenie magnezu i wapnia w osoczu krwi pacjentek chorych na raka piersi [Effect of chemotherapy containing doxorubicin on levels of magnesium and calcium in blood serum of patients with breast cancer].* J. Elementol., 9(3): 452-459. [in Polish]

POPIELA T., KULIG J., KOŁODZIEJCZYK P. SIERŻĘGA M. 2002. *Long-term results of surgery for early gastric cancer.* Br. J. Surg., 89: 1035-1042.

STARZYŃSKA T., WASILEWICZ M. 2007. *Chemoprewencja raka jelita grubego [Chemoprevention of large intestine cancer].* Pol. Merk. Lek., 23: 170-173. [in Polish]

TOMATIS L. 2000. *The identification of human carcinogens and primary prevention of cancer.* Mutat. Res., 462: 407-421.

WOLF F., MAIER J., NASULEWICZ A. 2007. *Magnesium and neoplasia: From carcinogenesis to tumor growth and progression or treatment.* Arch. Biochem. Biophys., 458: 24-32.

YAMAN M., KAYA G., YEKELER H. 2007. *Distribution of trace metal concentrations in paired cancerous and non-cancerous human stomach tissues.* World J. Gastroenterol., 13(4): 612-618.

YAMAN M. 2006. *Comprehensive compassion of trace metal concentrations in cancerous and non-cancerous human tissues.* Curr. Med. Chem., 13: 2513-2525.

J. Elementol. 2009, 14(3): 573–581

ASSESSMENT OF INFANT EXPOSURE TO LEAD AND CADMIUM CONTENT IN INFANT FORMULAS

Anna Winiarska-Mieczan

Department of Nutrition, Institute of Animal Nutrition University of Life Sciences in Lublin

Abstract

Infants and little children are the highest risk group as far as the exposition to toxic metals is concerned. Newly born babies do not have effectively functioning regulatory mechanisms and the absorption of mineral elements, including toxic ones, is higher than in older children and adults. Separate, more rigorous requirements have been determined for food products for infant nutrition. Special attention is required in the case of toxic metals, including lead and cadmium, which are regarded as particularly harmful to the organism.

The objective of the present work was to establish the content of lead and cadmium in powdered milk used in the nutrition of infants in the first months of their lives. Moreover, on the basis of surveys, the popularity of using milk replacement formulas was determined, as well as factors affecting this situation. All the examined preparations were labelled as "a special nutrition product". They had been purchased in different groceries in Lublin in October 2007, all before their use-by date. The shares of Pb and Cd were marked in the samples.

It was demonstrated that only two preparations did not comply with the recommendations regarding the content of cadmium, while in the other samples the maximum value was not exceeded. It was also noted that the analysed preparations were not safe for babies as far as the content of lead was concerned. At the same time, it was observed that as many as 87% babies were breast-fed, which is a positive phenomenon, whereas 40% women used combined nutrition for their children (breast feeding alternately with powdered milk). The choice of a particular preparation resulted in the first place from the doctor's recommendations, and next from babies' preferences and friends' opinions.

Keywods: child, infant formula, cadmium, lead.

---

dr Anna Winiarska-Mieczan, Institute of Animal Nutrition, University of Life Sciences in Lublin, Akademicka 13, 20-950 Lublin, e-mail: amieczan@poczta.onet.pl

OCENA STOPNIA NARAŻENIA NIEMOWLĄT NA POBIERANIE OŁOWIU I KADMU W PREPARATACH MLEKA W PROSZKU

Abstrakt

Niemowlęta oraz małe dzieci to grupa populacji o najwyższym ryzyku narażenia na toksyczne działanie metali. U noworodków zaraz po urodzeniu brak jest sprawnie działających mechanizmów regulacyjnych, a wchłanianie składników mineralnych, w tym także pierwiastków toksycznych, jest większe niż u dzieci starszych i osób dorosłych. Ustalono odrębne, bardziej rygorystyczne wymagania dla produktów spożywczych przeznaczonych do żywienia niemowląt. Szczególnej uwagi wymagają metale szkodliwe, do których zalicza się m.in. ołów i kadm, uznawane za szczególnie niebezpieczne dla organizmu.

Celem pracy było ustalenie zawartości ołowiu i kadmu w mleku w proszku stosowanym w żywieniu niemowląt w pierwszych miesiącach życia. Ponadto na podstawie badań ankietowych określono popularność stosowania preparatów mlekozastępczych oraz czynniki wpływające na ten stan. Materiał do badań stanowiły preparaty uznanych marek, przeznaczone do żywienia niemowląt w pierwszych miesiącach życia. Wszystkie badane preparaty miały adnotację „produkt specjalnego przeznaczenia żywieniowego". Produkty zakupiono w sklepach spożywczych na terenie Lublina w październiku 2007 r., w okresie ich przydatności do spożycia. W pobranych próbach oznaczono zawartość Pb i Cd.

Jedynie dwa preparaty nie spełniały wymagań odnośnie do zawartości kadmu, w pozostałych wartość dopuszczalna nie została przekroczona. Ponadto stwierdzono, że analizowane preparaty nie są bezpieczne dla niemowląt pod względem zawartości ołowiu. Jednocześnie stwierdzono, że aż 87% dzieci było karmionych piersią, co jest zjawiskiem pozytywnym, przy czym 40% kobiet stosowało żywienie mieszane (karmienie piersią naprzemiennie z mlekiem w proszku). Największy wpływ na zakup danego preparatu miała porada lekarza, następnie upodobania dziecka oraz opinia znajomych.

Słowa kluczowe: niemowlęta, preparaty mlekozastępcze, kadm, ołów.

INTRODUCTION

Food may contain different harmful substances which, when absorbed, may present a serious hazard to health (TONG et al. 2000), especially in children, whose excretion processes are generally slower than in adults. Children have a low body weight and lower immunity. Mother's milk should not be treated as a potential source of toxic metals, since the milk producing gland creates a natural biological barrier which prevents their passing from the mother's organism into the food (GULSON et al. 1998). However, toxic metals may be passed to infants with other solid food products. Separate, more rigorous requirements have been determined for food products for infant nutrition. Special attention is required in the case of toxic metals, including lead and cadmium, which are regarded as particularly harmful to the organism. Both lead and cadmium are characterised by a high accumulation factor in living organisms. The circulation of heavy metals in the environment is linked to the food chain: soil – plant – animal – man. When metals pass to a higher link, their content accumulates increasingly (BRAD-

STREET et al. 2003). It should be noted that a positive correlation has been observed between the concentration of toxic metals in children's organisms and the incidence of autism (BRADSTREET et al. 2003). International organisations focused on children's health recommend that food products for babies and little children should be continuously monitored (UNEP/UNICEF 1999).

The aim of the present study was to determine the content of lead and cadmium in powdered milk administered in infant nutrition in the first months of the children's lives. Additionally, the popularity of replacement milk formulas was evaluated on the basis of a questionnaire survey, as well as the factors responsible for their popularity.

MATERIAL AND METHODS

The material for the examinations comprised 8 milk replacement formulas representing well-known brands, used in baby feeding in the first months of their lives (Table 1). All the examined preparations were labelled as “a special nutrition product”. They had been purchased in grocery shops in Lublin in October 2007, before their use-by date.

Table 1

Trade mark and characteristic of analysed infant formulas

Trade mark	Characteristic
A-1	starter powdered milk for babies, enriched with iron, since the day of birth
A-2	follow-on milk, with an addition of powdered rice cereal for babies, enriched, form the 4th month of life
B-1	from the day of birth until the 4th month, enriched with iron
B-2	containing a probiotic, bananas, above the 4th month
C	with a probiotic, above the 4th month
D	hypo allergic follow-on milk, enriched with iron
E-1	with rice cereal, a formula for further baby nutrition above the 4th month
E-2	hypo allergic follow-on milk for babies above 4 months of life)

The content of Pb and Cd was measured with the use of flameless AAS technique in a Varian Spectr AA-880. All chemical analyses were performed in two replications.

Questionnaire surveys were performed in Lublin and Podkarpacie regions, from October to December 2007. The questionnaire consisted of 11 questions, whose purpose was to determine the consumption volume of milk replacement formulas, depending on the place of residence (a village or a town), as well as the reasons for using and selecting a particular preparation (Table 2). Assuming that it is the mothers who most frequently buy food for their children, there were also questions about the mother’s age and educational background. The study was carried out on 100 children’s mothers.

Table 2

Questionnaire

1.	Age of children □ 1 month □ 2 months □ months □ 4 months □ 5 months □ above 5 months	7.	Trade mark of milk formulas used in the nutrition of infants □ Bebiko □ Humana □ HIPP □ NAN □ other ....
2.	Place of residence □ village □ town	8.	Used milk formulas and breast feeding simultaneously □ yes □ no
3.	Education of mother □ primary □ vocational □ college □ higher	9.	Reasons for use of breast-milk substitutes □ lack of breast milk □ supplemental feeding □ discontinuing breast feeding □ other
4.	Age of mother □ to 20 □ 20-25 □ 26-30 □ above 30	10.	Only one type of formula was used in the infant's diet □ yes □ no
5.	Number of children □ 1 □ 2 □ 3 □ more than 3	11.	Factors affecting purchase of milk formulas □ family □ friends □ doctor's recommendations □ price □ advertising □ income □ baby's preference □ trade mark □ other......................................
6.	Time of breast-feeding □ to 5 months □ above 5 months □ not at all		

RESULTS AND DISCUSSION

Newly born babies do not have effectively functioning regulatory mechanisms and the absorption of mineral elements, including toxic ones, is higher than in older children and adults (DROBNIK, LATOUR 2006). The results of marking the content of cadmium and lead in milk formulas are presented in Table 3.

Table 3

Contents of cadmium and lead in the infant formulas, mean ± SD (n=3)

Trade mark	Contents of	
	Cd ($\mu g\ g^{-1}$ fresh matter)	Pb ($\mu g\ g^{-1}$ fresh matter)
A-1	0.002	0.198
A-2	0.011	0.418
B-1	0.002	0.158
B-2	0.014	0.094
C	0.0004	0.120
D	0.002	0.450
E-1	0.002	0.161
E-2	0.002	0.289
Polish Norm (Journal of Laws 2003)	0.01	0.10

SD – standard deviation

Our studies also demonstrated that only two of the milk replacement products did not meet the requirements regarding the content of cadmium, namely A-2 and B-2. The acceptable value was not exceeded in the remaining formulas. The limit was determined by legal regulations and it amounts to 0.01 $mg \cdot kg^{-1}$ (0.01 $\mu g \cdot g^{-1}$) of the product (Journal of Laws 2003).

The results of the above studies are alarming. Cadmium is regarded as an indicator of carcinogenic processes. It also impairs kidney functions (Waalkes 2000). Moreover, a relationship between the presence of cadmium in food and calcium (Staessen et al. 1999) and iron (Ĺkesson et al. 2000) metabolism disturbances was demonstrated. Cadmium is a metal to which babies are exposed continuously, since its source may be water, air, food and cigarette smoke. Studies proved that this element accumulates in babies' organisms during their foetal lives to a much higher degree than in their mothers' organisms (Razagui, Ghribi 2005). As it was demonstrated, cadmium's half-life in the human organism is *ca* 30 years (Castelli et al. 2005), which explains why exposing children to this element is particularly hazardous.

The acceptable amount of lead was also determined by the law and it is 0.1 $mg \cdot kg^{-1}$ (0.1 $\mu g \cdot g^{-1}$) of the product (Journal of Laws 2003). Our studies revealed that the acceptable limit of lead was not exceeded only in B-2 brand (0.094 $\mu g \cdot g^{-1}$). In C formula a slightly higher amount of this element was determined, whereas in all the remaining formulas the content of lead was significantly higher than the norms. In D and A-2 preparations the limit was exceeded fourfold.

The results are highly alarming. It should be noted that food is one of the factors which most frequently put infants and little children at risk of contact with lead. Numerous studies demonstrated that food addressed to this particular group of consumers is not safe as far as the content of this toxic element is concerned (Marzec, Zaręba 2003, Winiarska-Mieczan, Gil 2007, Winiarska-Mieczan, Kwiecień 2007). Hozyasz et al. (2004) showed that the mean content of cadmium in cow milk was lower in comparison with the mean share of this element in different milk replacement mixtures, whereas the mean content of lead was lower only in starter mixtures. Guidi et al. (1996) stated that the share of lead in mixtures for babies remained within quite a wide range: from 6 $\mu g \cdot l^{-1}$ to 600 $\mu g \cdot l^{-1}$ in standard formulas based on modified cow milk, and from 2 $\mu g \cdot l^{-1}$ to 45 $\mu g \cdot l^{-1}$ in lactose-free formulas, hyper allergic (HA type) ones and those containing milk cow protein processed by hydrolysis. This provided a vital argument in favour of the necessity to increase quality requirements for manufacturers regarding raw materials and improved technological processes. What should be remembered is the results of the studies indicating possibile occurrence of high cadmium and aluminium concentrations in soy mixtures. The average content of toxic metals in cow milk is higher than in human breast milk (Hozyasz et al. 2004).

Infants and little children are the highest risk group as far as the exposition to toxic metals is concerned (Mahajan et al. 2005, Razagui, Ghribi 2005). Lead finds its way to the child's organism through the respiratory or digestive system (Tong et al. 2000). This is a highly toxic element and the resulting poisoning may lead to anaemia (Mahajan et al. 2005), dysfunctions of different organs (liver, kidneys, stomach) and convulsions (Aldous 1999). A report from 1999 drafted by UNICEF and UNEP (UNEP/UNICEF 1999) states that exposing children to the risk of contact with lead leads to impaired functioning of the nervous system, which is manifested primarily in the disorder of motor functions, and also in behaviour problems or physical hyperactivity. Such children also revealed impaired brain functions and lower IQ (Chen et al. 2005). An excessive accumulation of lead in the organism leads to death. Preventing or relieving the toxic activity of lead is reduced to enriching the diet with calcium and iron (UNEP/UNICEF 1999) and with vitamin C (Committee on Environmental Health 2005). Studies revealed a lower level of cadmium concentration in the blood of lactating mothers after enriching their diet with calcium (Hernandez-Avila et al. 2003). Experiments using radioactive lead demonstrated that in primates the level of cadmium absorption into the brain tissue is conversely proportional to their age (Willes et al. 1977). The studies performed by Tsukahara et al. (2002) demonstrated that babies with anaemia caused by iron deficiency had a higher cadmium concentration than healthy infants. The level of lead and ferritin in the blood were conversely correlated, similarly to the level of lead in the blood and haemoglobin.

The survey revealed that 87% of the children in the studied group were breast fed. According to the recommendations determined by the World Health Organisation, infants and little children should be fed breast milk exclusively until the sixth month and later they should be administered supplementary products which must be nutritionally adequate and safe while continuing breast feeding until the second year or longer (KUNACHOWICZ, KUNDZICZ 2003). Breast milk is fully adapted to feeding babies, depending on the stage of their development (PAWLUS et al. 2005). Mother's milk is a specific quality food for a baby as it is adapted to the peculiar digestion, assimilation and metabolism of the infant's organism. It contains all indispensable nutritional and protective ingredients in proper amounts and adequately composed, adjusted to the needs of the baby's organism and not burdening the digestive and excretory systems, which are not fully mature yet. Breast milk completely satisfies the demand for nutrients throughout six months, except for vitamin D (SKRAJNOWSKA 2006). Mother's milk ensures proper physical and mental development of the child. Besides, breast feeding makes the baby feel secure and contributes to the mutual emotional relationship between the mother and the child.

Our studies revealed that some mothers did not breast feed their babies at all or decided to introduce milk replacement formulas for various reasons. According to the majority of the respondents, the reason why they decided to use milk replacement preparations was to provide supplemental feeding (53.9%), 30% declared the lack of breast milk, whereas 15% used a formula after discontinuing breast feeding. 60% of the women in the studied group did not use milk formulas simultaneously with breast feeding, while 40% chose a combined diet for their children. Unquestionably, the vast majority of the surveyed women (92%) used only one type of the formula and only 8% tried more than one. The place of residence did not have any influence on the frequency of using milk formulas.

The studies demonstrated that the choice of a given formula was dictated primarily by the doctor's recommendations (66.7%), while in 25% of the cases it was the baby's preference that was decisive. The advice offered by friends was effective in above 8%. Other factors did not have any influence on the purchase of milk formulas by the questioned women. Moreover, it was observed that the products offered by well-known brands were the most popular ones. Being familiar with the brand does affect the consumers' opinion concerning its quality. The prestige of a particular brand name is one of the determinants of the product's quality (GÓRSKA-WARSEWICZ 2003).

SUMMARY

The analyses showed that only two milk replacement formulas did not satisfy the requirements regarding the share of cadmium, namely A-2 and B-2. It was also observed that the analysed preparations were not safe for babies regarding the content of lead.

It was concluded that as many as 87% of the babies were breast fed, which is a positive phenomenon. The majority of women (60%) did not administer replacement formulas and breast feeding simultaneously, while 40% chose a combined diet for their babies. The purchase of particular milk formulas was largely affected by doctors' recommendations, and in the second place by babies' preferences or friends' advice.

REFERENCES

ĹKESSON A., STĹL P., VAHTER M. 2000. *Phlebotomy increases cadmium uptake in hemochromatosis.* En. Health Perspect., 108: 289-291.

ALDOUS M.B. 1999. *Nutritional issues for infants and toddlers*. Pediatric Ann., 1(28): 101-105.

BRADSTREET J., GEIER D.A., KARTZINEL J.J., ADAMS J.B., GEIER M.R. 2003. *A case-control study of merkury burden in children with autistic spectrum disorders.* J. Am. Phys. Surg., 3: 76-79.

CASTELLI M., ROSSI B., CORSETTI F., MANTOVANI A., SPERA G., LUBRANO C., SILESTRONI L., PATRIARCA M., CHIODO F., MENDITTO A. 2005. *Levels of cadmium and lead in blood: an application of validated methods in a group of patients with endocrine/metabolic disorders from the Rome area*. Microchem. J., 79: 349-355.

CHEN A., DITRICH K.N., WARE J.H., RADCLIFFE J., ROGAN W.J. 2005. *IQ and blood lead from 2 to 7 years of age: are the effects in older children the residual of high blood lead concentrations in 2-year-olds?*. Env. Health Perspect., 5: 597-601.

COMMITTEE ON ENVIRONMENTAL HEALTH 2005. *Lead exposure in children: prevention, detection, and management.* Pediatrics, 4: 1036-1046.

DROBNIK M., LATOUR T. 2006. *Physiological importance of mineral components in natura mineral water according pharmacodynamic investigation of some therapeutic mineral waters.* J. Elementol., 11(3): 259-270.

Dziennik Ustaw 2003. The official journal of the Polish State, 4 March, Nr 37, poz. 326.

GÓRSKA-WARSEWICZ H. 2003. *Jakość produktów żywnościowych w opinii konsumentów [Quality of food products according to consumers].* Przem. Spoż., 2: 49-51. (in Polish, English abstract)

GUIDI B., RONCHI S., MATTEI F., TRIPODI A., ORI E. 1996. *Lead concentration in milk-based and special infant-feeding formulas*. Ped. Med. Chir., 18: 275-277.

GULSON B.L., JAMESON C.W., MAHAFFEY K.R., MIZON K.J., PATISON N., LAW A.J., KORSCH M.J., SALTER M.A. 1998. *Relationships of lead in breast milk to lead in blood, urine and diet of the infant and mother*. Env. Health Perspect., 106: 667-674.

HERNANDEZ-AVILA M., GONZALEZ-COSSIO T., HERNANDEZ-AVILA J.E., ROMIEU I., PETERSON K.E., ARO A., PALAZUELOS E., KAGEYAMA ESCOBAR M.L., HU H. 2003. *Dietary calcium supplements to lower blood lead levels in lactating women: a randomized placebo-controlled trial.* Epidemiology, 14: 206-212.

HOZYASZ K., RUSZCZYŃSKA A., BULSKA E. 2004. *Zawartość kadmu, ołowiu i glinu w mieszankach mlecznych dla niemowląt. Doniesienia wstępne [Content of cadmium, lead and aluminium in milk mixutres for infants. Preliminary report]*. Pediatria Pol., 79(10): 778-784. (in Polish, English abstract).

KUNACHOWICZ H., KUNDZICZ M. 2003. *Wymagania jakości zdrowotnej produktów przeznaczonych dla niemowląt i małych dzieci [Health quality requirements of food products for infants and young children]*. Bromat. Chemia Toksykol., 36(4): 295-301. (in Polish, English abstract).

MAHAJAN R.K., WALIA T.P.S., SUMANJIT. 2005. *Stripping voltammetric determination of zinc, cadmium, lead and copper in blood samples of children aged between 3 months and 6 years*. Online J. Heath Allied Sci., 1: 1-8.

MARZEC A., ZARĘBA S. 2003. *Badania zawartości metali ciężkich w niektórych produktach spożywczych stosowanych w żywieniu niemowląt i dzieci [Examination of heavy metals in some food products used in nutrition of infants and children]*. Bromat. Chemia Toksykol., 36(2): 137-142. (in Polish, English abstract).

PAWLUS B., KORDEK A., ŁONIEWSKA B. 2005. *Czy pokarm matki wcześniaka jest mniej wartościowy? [Is milk of a mother of a premature infant less valuable?]*. Żyw. Czł. Metab., 23(4): 317-324. (in Polish, English abstract).

RAZAGUI I.B., GHRIBI I. 2005. *Maternal and neonatal scalp hair concentrations of zinc, copper, cadmium, and lead: relationship to some lifestyle factors*. Biol. Trace Elem. Res., 1: 1-28.

SKRAJNOWSKA D. 2006. *Mleko kobiece – korzyści i zagrożenia dla dziecka [Women's milk – benefits and threats to a child]*. Bromat. Chemia Toksykol., 39(2): 165-171. (in Polish, English abstract).

STAESSEN J.A., ROELS H.A., EMELIANOV D., KUZNETSOVA T., THIJS L., VANGRONSVELD J., FAGARD R. 1999. *Environmental exposure to cadmium, forearm bone density, and risk of fractures: prospective population study*. Lancet, 353: 1140-1144.

TONG S., VON SCHIRNDING Y.E., PRAPAMONTOL T. 2000. *Environmental lead exposure: a public health problem of global dimensions*. Bull. World Health Organiz., 9: 1068-1077.

TSUKAHARA T., EZAKI T., MORIGUCHI J., FURUKI K., UKAI H., OKAMOTO S., SAKURAI H., IKEDA M. 2002. *Effects of iron-deficiency anemia on cadmium uptake or kidney dysfunction are essentially nil among women in general population in Japan*. Tohoku J. Exp. Med., 197(4): 243-247.

UNEP/UNICEF 1999. *Childhood lead poisoning*. Information for advocacy and action.

WAALKES M.P. 2000. *Cadmium carcinogenesis in review*. J. Inorg. Biochem., 79: 240-244.

WILLES R., LOK E., TRUELOVE J., SUNDARAM A. 1977. *Retention and tissue distribution of PB(NO) administered orally to infant and adult monkeys*. J. Toxicol. Environ. Health, 3: 395-406.

WINIARSKA-MIECZAN A., GIL G. 2007. *Ocena narażenia niemowląt na pobieranie ołowiu i kadmu z pożywieniem [Evaluation of the threat of infants' uptake of lead and cadmium from food]* . Bromat. Chemia Toksykol., 2: 137-144. (in Polish, English abstract).

WINIARSKA-MIECZAN A., KWIECIEŃ M. 2007. *Assessment of infant and preschool children exposure to lead and cadmium content in fruit-vegetable baby juices*. Pol. J. Environ. Stud., 16(3A): 313-316.

J. Elementol. 2009, 14(3): 583–591

EVALUATION OF THE MINERAL COMPOSITION OF INFANT FORMULAS

Anna Winiarska-Mieczan, Marta Tupaj

Department of Nutrition, Institute of Animal Nutrition University of Life Sciences in Lublin

Abstract

The composition of modified milk for babies is based on the model of breast milk. Milk replacement formulas are produced on the basis of cow's milk so it is necessary to modify all the nutrients in order to make them resemble most closely the model. Determination of babies' nutritional requirements is based on the knowledge of women's breast milk, which is regarded the best possible food in the first period of infant life. The concentration of mineral elements in breast milk is low and the total content of ash amounts to $0.2\ g \cdot dl^{-1}$. The content of sodium, potassium and chlorine is three times as low as in cow's milk. The source of individual elements for babies is their food, though some elements are provided in drinking water and supplements. Typically, mineral elements are absorbed more effectively in the periods of intensive growth. It should be remembered that the mineral elements found in breast milk are characterised by much higher bioavailability than those present in modified milk. The major requirement for modified milk to be registered and authorised for sale in Poland is the conformity of its mineral composition with international recommendations.

The objective of the present work was to establish the content of mineral components in powdered milk used in the nutrition of infants in the first months of their lives. All the examined preparations were labelled as "a special nutrition product". They were purchased in different groceries in Lublin in October 2007, all before their use-by date. The shares of Mg, Zn, Cu, Ca, Mn, Na, Fe and K were marked in the samples.

It was demonstrated that all the examined milk samples contained much more Ca and Cu in comparison to recommended norms, whereas they were deficient in Mg and Mn.

Key words: child, infant formula, mineral components.

dr Anna Winiarska-Mieczan, Institute of Animal Nutrition, University of Life Sciences in Lublin, Akademicka 13, 20-950 Lublin, e-mail: amieczan@poczta.onet.pl

OCENA SKŁADU MINERALNEGO MLEKA W PROSZKU

Abstrakt

Skład mleka modyfikowanego przeznaczonego dla niemowląt oparty jest na wzorcu pokarmu kobiecego. Preparaty mlekozastępcze są produkowane na bazie mleka krowiego, i dlatego konieczna jest modyfikacja wszystkich składników pokarmowych, aby je maksymalnie upodobnić do wzorca. Stężenie składników mineralnych w pokarmie kobiecym jest niskie, całkowita zawartość popiołu wynosi 0,2 g dl^{-1}. Zawartość sodu, potasu i chloru jest trzykrotnie niższa niż w mleku krowim (są to pierwiastki odpowiedzialne za osmotyczne obciążenie nerek). Źródłem poszczególnych pierwiastków dla niemowląt jest pożywienie, niektórych dostarczają woda pitna i suplementy mineralne lub witaminowo-mineralne. Zwykle składniki mineralne są wchłaniane efektywniej w okresach intensywnego wzrostu. Należy pamiętać, że składniki mineralne występujące w mleku matki charakteryzują się znacznie wyższą biodostępnością niż znajdujące się w mleku modyfikowanym. Głównym warunkiem rejestracji mleka modyfikowanego i dopuszczenia do obrotu w Polsce jest zgodność składu mineralnego i witaminowego z zaleceniami międzynarodowymi.

Celem pracy było ustalenie zawartości składników w mleku w proszku stosowanym w żywieniu niemowląt w pierwszych miesiącach życia. Materiał do badań stanowiły preparaty uznanych marek przeznaczone do żywienia niemowląt w pierwszych miesiącach życia. Wszystkie badane preparaty miały adnotację, że jest to „produkt specjalnego przeznaczenia żywieniowego". Mleka w proszku zakupiono w sklepach spożywczych na terenie Lublina w październiku 2007 r., w okresie jego przydatności do spożycia. W pobranych próbach oznaczono zawartość Mg, Zn, Cu, Ca, Mn, Na, Fe oraz K.

Wykazano, że badane próby mleka zawierały znacznie więcej Ca i Cu w porównaniu z normami, natomiast były niedoborowe pod względem zawartości Mg i Mn.

Słowa kluczowe: niemowlęta, preparaty mlekozastępcze, składniki mineralne.

INTRODUCTION

Long-term studies of the process of human growth indicate that no period is more significant to determining the health condition of an individual than the foetal stage and early childhood. Inadequate nutrition in infancy leads to development impairment. According to the latest recommendations, baby nutrition should be based on mother's breast milk and on manufactured preparations specially designed for this particular age group (ALDOUS 1999).

The composition of modified milk for infants is based on the model of human breast milk. Milk replacement formulas are manufactured on the basis of cow milk, so it is necessary to modify all nutrients in such a way that they resemble the original model to the maximum.

The determination of babies' nutritional requirements is based on the knowledge of women's breast milk which is regarded as the best possible food in the first period of infant life. The concentration of mineral elements in breast milk is low, the total content of ash amounts to 0.2 $g \cdot dl^{-1}$. The content of sodium, potassium and chlorine is three times lower in comparison to cow milk (these are the elements responsible for osmotic burden of the kidneys) (LYNCH, STOLTZFUS 2003). The source of individual elements for babies is their food, though some elements are provided in drinking water and supplements. Typically, mineral elements are absorbed more effectively in the periods of intensive growth (DROBNIK, LATOUR 2006). It should be remembered that the mineral elements found in breast milk are characterised by much higher bioavailability than those present in modified milk (SKRAJNOWSKA 2006).

The main requirement for registering and authorising the sale of modified milk in Poland is the compliance of its mineral and vitamin composition with international recommendations (STOLARCZYK 2001). Such compliance is confirmed by a certificate issued by Chief Sanitary Inspector (Główny Inspektor Sanitarny), which must be printed on the packaging of the product.

The aim of the present study was to determine the content of mineral elements, in powdered milk administered in infant nutrition in the first months of the children's lives.

MATERIALS AND METHODS

The material for the examinations was provided by milk replacement formulas representing well-known brands, used in baby feeding in the first months of their lives: A-1 (starter powdered milk for babies, enriched with iron, since the day of birth), A-2 (follow-on milk, with an addition of powdered rice cereal for babies, enriched, form the 4^{th} month of life), B-1 (from the day of birth until the 4^{th} month, enriched with iron), B-2 (containing a probiotic, bananas, above the 4^{th} month), C (with a probiotic, above the 4^{th} month), D (hypo allergic follow-on milk, enriched with iron), E-1 (with rice cereal, a formula for further baby nutrition above the 4^{th} month), E-2 (hypo allergic follow-on milk for babies above 4 months of life). All the examined preparations were labelled as "a special nutrition product". They had been purchased in grocery shops in Lublin in October 2007, before their use-by date.

The contents of dry matter and crude ash in the samples were determined with the use of the AOAC methods (1990). The content of Mg, Zn, Cu, Ca, Mn, Na, Fe and K was determined in the collected specimens, with the use of the AAS flame technique, in a Unicam 939 (AA Unicam Spectrometer).

All chemical analyses were performed in two replications. The results were statistically analysed. Arithmetic mean values and standard deviation (sd) were calculated with the use of STATISTICA 6.0 software. Taking into account energy concentration declared by the producer, proportions between individual mineral elements and energy (kcal) were calculated.

RESULTS AND DISCUSSION

The content of dry matter, crude ash and macroelements in the examined milk replacement formulas is presented in Table 1. It should be remembered that the concentration of certain mineral elements in modified milk is significantly higher in comparison with breast milk, which results from its lower assimilability (STOLARCZYK 2001).

The level of sodium in milk formulas ranged from 0.92 to 2.25 $mg \cdot g^{-1}$. The highest share of sodium was found in B-1 formula and the lowest in A-2 milk. In reference to the content of sodium calculated per 100 kcal, the highest share of this element was recorded in C (51.4 mg) and D (nearly 50 mg) preparations, as well as in B-1, B-2 and E-2. Bearing in mind the norms, it should be noted that all formula types contained an acceptable amount of sodium.

The presence of 2.411 to 4.704 mg of potassium in 1 g (from 84.68 to 169.16 $mg \cdot 100\ kcal^{-1}$) was observed in the analysed formulas. In reference to the norms, the acceptable limit of potassium was exceeded only in C preparation. Being the basic component of intracellular liquid, potassium influences the osmotic balance in the cell, participates in transmitting nerve impulses in the nervous and muscular systems, ensures the adequate course of reactions related to muscle contractions and activates a number of enzymatic reactions. Potassium takes part in carbohydrate transformations, energy transformations, systemic protein synthesis and amino acid transport in the cell (SHIMONI 2005).

It was observed that, in comparison to the norms, the content of calcium was significantly exceeded in all the analysed preparations. In formula D, the amount of this element was 3.5 times higher than the acceptable limit, in B-2 and E-1 its share was 3 times higher than the norm, whereas in C, E-2 and A-2 preparations there was 2.5-fold more calcium than the acceptable value. The demand for calcium amounts to 600 mg per day during the first six months, and 800 mg per day in the following six months (ZIEMLAŃSKI et al. 1998). According to the Polish regulations, the ratio of Ca to P in baby milk formulas cannot be lower than 1.2 or higher than 2 (Journal of Laws 2002). Calcium and phosphorus are more easily assimilable from breast milk than from milk formulas, so the content of these components in modified milk is higher than the baby's demand indicates (ALDOUS 1999).

Our studies showed that all the examined preparations contained too little magnesium, compared to the recommendations. The currently obliging norms recommend that the share of magnesium in babies' and little children's diet should range from 50 to 150 mg per day (ZIEMLAŃSKI et al. 1998). Magnesium assimilability is increased by vitamin D, sodium, galactose and animal protein. Magnesium deficiency leads to disorders in the circulation and the functioning of the heart, irritability, convulsions, behaviour disor-

Table 1

Contents of macroelements in the infant formulas, mean ± SD (n=3)

Trade mark	Dry matter (%)	Crude ash (%)	Contents of macroelements								kcal 100 g^{-1}*
			mg · g^{-1}				mg · 100 $kcal^{-1}$				
			Na	K	Ca	Mg	Na	K	Ca	Mg	
A-1	97.9±9.3	2.09±0.003	1.065±0.002	2.862±0.005	0.087 ±0.0002	0.042±0.0001	23.12	88.63	61.66	6.94	519
A-2	97.5±8.9	2.68±0.009	0.924±0.001	2.411±0.007	1.274±0.002	0.050±0.0001	27.22	84.68	119.96	7.66	496
B-1	98.1±9.0	2.63±0.02	2.247±0.005	3.313±0.01	0.098±0.0001	0.046±0.0001	45.99	99.80	76.32	7.83	511
B-2	95.0±9.0	3.79±0.04	1.551±0.006	4.331±0.02	0.167±0.001	0.616±0.002	46.22	136.55	157.56	11.13	476
C	97.0±8.9	3.58±0.03	1.632±0.005	4.704±0.02	0.141±0.001	0.067±0.0002	51.39	169.16	128.91	12.01	467
D	96.2±8.5	3.15±0.005	1.617±0.004	3.776±0.02	0.135±0.001	0.049±0.0001	49.78	138.58	165.95	11.42	463
E-1	97.3±9.3	3.15±0.02	1.500±0.004	3.173±0.009	0.148±0.002	0.059±0.0001	40.09	118.49	149.89	11.36	449
E-2	96.4±7.1	3.05±0.01	1.613±0.006	3.536±0.01	0.126±0.001	0.047±0.0001	46.74	130	122.61	9.35	460
Norms (2002)							20-60	60-145	50	25-90	

SD – standard deviation
* values as declared by the manufacturer

ders, anaemia, brittleness of the bones, higher neoplasm incidence and protein synthesis disorders (GRIFFIN et al. 2008).

The content of microelements in the s formulas is presented in Table 2.

The content of zinc ranged from 0.85 mg to 0.99 mg·100 $kcal^{-1}$. Bearing in mind the norms, it was concluded that the share of this element was adequate in all the studied preparations. The required zinc consumption in breastfed babies is 1mg per day (zinc assimilability from breast milk reaches 60%), and in children fed cow's milk or a combined diet these requirements are much higher since zinc absorption from such a diet does not exceed 40%, and is frequently at the level of 10-20%. The demand for zinc with such a diet and 20% zinc assimilability is 5-5.5 mg per day, yet a high percentage of babies fed a mixed diet or after discontinuing breast feeding receive too little zinc (MARZEC, ZARĘBA 2003a). Currently, zinc is regarded as one of the most important trace bioelements. It affects the human organism on many planes, though its role has not been fully described yet. Zinc bioavailability from a diet depends on its composition. In women's breast milk this element occurs in combination with low-molecular-weight proteins, which makes it very easily assimilable. In healthy adults zinc deficiency hardly ever occurs. However, it is frequent in new-born babies and little children. It was determined that the problem concerns about 50% of the whole population (HAMBIDGE, KREBS 2007). The digestive, central nervous, immune and bone systems all react to zinc deficiency.

A comparison of the values obtained with the norms resulted in a conclusion that the share of copper was exceeded in all the studied formulas. The demand for copper in early childhood is not high because of significant copper reserves in the liver, acquired during foetal life (SZOTOWA 1993). After birth, babies fed their mother's milk reveal negative copper balance, yet the reserves accumulated in the liver during their foetal life are sufficient to ensure the proper functioning of copper-dependent enzymes. Assuming 50% copper absorption with food and endogenic copper reserves in the liver, 40 μg·kg^{-1} per day of this element is recommended for babies during the initial three months of their lives. The daily copper recommendation for infants is 0.65mg (MARZEC, ZARĘBA 2003b).

The content of iron ranges from 0.036 mg to 0.31 mg in 1 g. It should be noted that among the preparations enriched with iron none was characterised by a high content of this component. Bearing in mind the iron content per 100 kcal, the highest amount of this element, above the limit, was observed in A-2, E-1 and D, preparations. The content of iron in B-2 and E-2 formulas also exceeded the acceptable norm. In the remaining preparations the share of this element was adequate. Iron content in breast milk is *ca* 0.3-0.5 mg·l^{-1}. The assimilability of this element from mother's milk reaches 70%, whereas in modified milk this value does not go beyond 25% (STOLARCZYK 2001). During the initial three months after birth the baby uses iron reserves accumulated in the organism, which is absolutely sufficient.

Table 2

Content of microelements in the infant formulas, mean (n=3)

Trade mark	Contents of macroelements								kcal 100 g^{-1}*
	mg·g^{-1}				mg·100 $kcal^{-1}$				
	Na	K	Ca	Mg	Na	K	Ca	Mg	
A-1	0.006	0.005	0.066	0.00	0.75	0.06	1.19	0.00	519
A-2	0.004	0.007	0.053	0.002	1.21	0.12	1.69	0.05	496
B-1	0.012	0.004	0.052	0.002	1.00	0.06	1.00	0.04	511
B-2	0.029	0.009	0.310	0.002	0.99	0.12	1.58	0.05	476
C	0.009	0.004	0.073	0.0008	0.75	0.04	1.35	0.04	467
D	0.003	0.004	0.074	0.001	0.91	0.06	1.66	0.06	463
E-1	0.005	0.006	0.036	0.002	1.02	0.102	1.69	0.04	449
E-2	0.003	0.004	0.074	0.0009	0.85	0.05	1.54	0.02	460
Norms (2002)					0.5-1.5	0.002-0.008	0.5-1.5	5-15	

* values as declared by the manufacturer

However, from the fifth month the baby must be administered iron in the diet and milk replacement formulas become its important source for children who are not breastfed (STOLARCZYK 2001). It is believed that anaemia caused by iron deficiency is the reason for mental retardation (HURTADO et al. 1990). At the same time, iron excess in the diet blocks zinc and copper absorption and stimulates the growth of certain strains of intestinal bacteria (STOLARCZYK 2001).

Our study revealed that all the examined preparations contained too little manganese, in reference to the norms. The share of this element in the analysed milk replacement formulas ranged from 0.0008 mg to 0.002 $mg \cdot g^{-1}$. Manganese deficiency is particularly hazardous to infants and little children because it can result in growth and development disorders (LJUNG, VAHTER 2007). The symptoms of manganese deficiency are rare in older children and adults because manganese is commonly present in the food. Manganese participates in carbohydrate and fat transformations, it is an activator of numerous enzymes, affects the processes of connective tissue formation and reproductive processes. If its insufficiency occurs, it may lead to disorders of the growth and development in children (LJUNG, VAHTER 2007).

SUMMARY

The source of individual elements for babies is their food, though some elements are provided in drinking water and supplements. In all the formulas the content of Ca and Cu exceeded the recommended norms. In contrast, the products were deficient in Mg and Mn. It should be remembered that the concentration of certain mineral elements in modified milk is significantly higher in comparison with breast milk, which results from its lower assimilability (SKRAJNOWSKA 2006).

REFERENCES

ALDOUS M.B. 1999. *Nutritional issues for infants and toddlers*. Pediatric Ann., 1(28): 101-105.

AOAC 1990. *Official methods of analysis (15th ed.)*. Washington, DC: Association of Official Analytical Chemists.

DROBNIK M., LATOUR T. 2006. *Physiological importance of mineral components in natura mineral water according pharmacodynamic investigation of some therapeutic mineral waters.* J. Elementol., 11(3): 259-270.

DZIENNIK USTAW 2002. The Journal of Laws of the Polish State, 31 Dec., Nr 239, poz. 2050.

GRIFFIN I.J., LYNCH M.F., HAWTHORNE K.M., CHEN Z., HAMZO M., ABRAMS S.A. 2008. *Magnesium retention in 12 to 48 month-old children*. J. Am. Coll. Nutr., 27: 349-355.

HAMBIDGE K.M., Krebs N.F. 2007. *Zinc deficiency: A special challenge*. J. Nutr., 137(4): 1101-1105.

HURTADO E.K., CLAUSSEN A.H., SCOTT K.G. 1990. *Early childhood anemia and mild or moderate mental retardation.* Am. J. Clin. Nutr., 69(1): 115-119.

LJUNG K., VAHTER M. 2007. *Time to re-evaluate the guideline value for manganese in drinking water?* Environ. Health Perspect., 115(11): 1533-1538.

LYNCH S.R., STOLTZFUS J.R. 2003. *Iron and ascorbic acid: proposed fortification levels and recommended iron compounds*. J. Nutr., 133: 2978-2984.

MARZEC A., ZARĘBA S. 2003a. *Badania zawartości metali ciężkich w niektórych produktach spożywczych stosowanych w żywieniu niemowląt i dzieci [Studies on content of heavy metals in some foodstuffs used in nutrition of infants and babies].* Bromat. Chemia Toksykol., 36(2): 137-142. (in Polish, English abstract)

MARZEC A., ZARĘBA S. 2003b. *Ocena zagrożeń żywieniowych ze strony wybranych pierwiastków pobieranych z niektórymi produktami spożywczymi stosowanymi w żywieniu niemowląt i dzieci [Evaluation of nutritional risks related to some elements ingested with certain food products used in nutrition of infants and babies].* Bromat. Chemia Toksykol., 36: 161-166. (in Polish, English abstract)

SHIMONI Y. 2005. *Dexamethasone and cardiac potassium currents in the diabetic rat*. Br. J. Pharmacol., 146(2): 280–287.

SKRAJNOWSKA D. 2006. *Mleko kobiece 0150 korzyści i zagrożenia dla dziecka [Breast milk – benefits and risks to a baby].* Bromat. Chemia Toksykol., 39(2): 165-171. (in Polish, English abstract)

STOLARCZYK A. 2001. *Witaminy i składniki mineralne w mleku modyfikowanym dla niemowląt i w wybranych preparatach leczniczych [Vitamins and minerals in modifiedmilk for infants and in some therapeutic substances].* Pediatria Współ. Gastroenter. Hepatol. Żyw. Dziecka, 3(2): 153-158. (in Polish, English abstract)

SZOTOWA W. 1993. *Karmienie naturalne i pielęgnowanie noworodka i niemowlęcia [Breasfeeding and care of an infant and baby].* Wyd. Witex, Warszawa. (in Polish)

ZIEMLAŃSKI Ś., BUŁHAK-JACHYMCZYK B., BUDZYŃSKA-TOPOLOWSKA J., PANCZENKO-KRESOWSKA B., WARTANOWICZ M. 1998. *Normy żywienia dla ludności w Polsce (energia, białko, tłuszcze, witaminy i składniki mineralne) [Nutritional norms for people in Poland (energy, protein, fats, vitamins and minerals].* Nowa Med., 4: 1-27. (in Polish, English abstract)

J. Elementol. 2009, 14(3): 593–604

CONTENT, UPTAKE AND UTILIZATION BY PLANTS OF COPPER, MANGANESE AND ZINC FROM MUNICIPAL SEWAGE SLUDGE AND WHEAT STRAW

Czesław Wołoszyk, Anna Iżewska, Ewa Krzywy-Gawrońska

Department of Environmental Chemistry Western Pomeranian University of Technology in Szczecin

Abstract

In a two-factor pot experiment, which was conducted 2004-2005, the direct and successive impact was estimated of mixed application of different doses of municipal sewage sludge (0.5, 1.0, 1.5 and 2.0% d.m. of sewage sludge relative to 6 kg d.m. soil in pot) and a constant dose of wheat straw (30 g d.m. per pot), with and without supplemental mineral fertilization with nitrogen and NPK, on the content, uptake and utilization of copper, manganese and zinc by test plants.

The soil used in the experiment was brown acid incomplete soil (good rye complex) and the test plant in the first year of research was grass – *Festulolium*, which was harvested four times, and in the second year – common sunflower and blue phacelia.

In mean object samples of *Festulolium*, common sunflower and phacelia, content of copper, manganese and zinc was marked with the ASA method after mineralization in a mixture of nitric (V) and perchloric acid (VII).

Rising doses of municipal sewage sludge with addition of a fixed dose of wheat straw, both in direct and successive effect, increased the content of copper, manganese and zinc in test plants.

The increase in the weighted mean (from four swaths) content of copper in *Festulolium,* in comparison with the control object, varied from 8.04 to 59.8%, manganese from 21.8 to 68.8% and zinc from 19.4 to 59.1%. In the second year, the mean increase in the content of copper in common sunflower from objects fertilized with sewage sludge and straw varied from 8.7 to 30.3% and in phacelia from 6.1 to 12.6%. By analogy, the mean content of manganese rose from 23.3 to 59.5% and from 5.9 to 33.1% and the content of zinc from 33.2 to 50.3% and from 15.9 to 37.9%. Mineral fertilization with N and NPK,

dr hab. Czesław Wołoszyk, prof. ZUT, Department of Environmental Chemistry, Western Pomeranian University of Technology in Szczecin, Słowackiego 17, 71-434 Szczecin, phone (091) 4496336; e-mail: czeslaw.woloszyk@zut.edu.pl

in comparison with the object without that fertilization, in both years of the experiment, increased the mean content of all microelements in test plants, with the increase being larger after NPK than N fertilization.

The uptake of microelements by plants from sewage sludge and straw, in most cases, was increasing along with the increase of the doses of sewage sludge. In the total uptake of individual microelements, about 2/3 were atken up by *Festulolium* and the remaining 1/3 by phacelia. Utilization of individual microelements from sewage sludge and straw was considerably diverse. IIn the two years, test plants utilized manganese mostly (on average 58.2%), less zinc (on average 5.54%) and to the smallest degree copper (on average 3.03%).

Key words: sewage sludge, wheat straw, test plants, copper, manganese, zinc.

ZAWARTOŚĆ, POBRANIE I WYKORZYSTANIE PRZEZ ROŚLINY TESTOWE MIEDZI, MANGANU I CYNKU Z KOMUNALNEGO OSADU ŚCIEKOWEGO I SŁOMY PSZENNEJ

Abstrakt

W dwuczynnikowym doświadczeniu wazonowym, przeprowadzonym w latach 2004--2005, oceniano wpływ bezpośredni i następczy łącznego stosowania zróżnicowanych dawek komunalnego osadu ściekowego (0,5, 1,0, 1,5 i 2,0% s.m. osadu w stosunku do 6 kg s.m. gleby w wazonie) i stałej dawki słomy pszennej (30 g s.m. na wazon), bez i z dodatkowym nawożeniem mineralnym azotem i NPK, na zawartość, pobranie i wykorzystanie miedzi, manganu i cynku przez rośliny testowe. Podłoże w doświadczeniu stanowiła gleba brunatna kwaśna, niecałkowita (kompleks żytni dobry), a rośliną testową w pierwszym roku badań była trawa – *Festulolium*, którą zebrano czterokrotnie, a w drugim roku słonecznik zwyczajny i facelia błękitna. W średnich próbkach obiektowych *Festulolium,* słonecznika i facelii oznaczono, po mineralizacji w mieszaninie kwasu azotowego (V) i chlorowego (VII), zawartość miedzi, manganu i cynku metodą ASA.

Wzrastające dawki komunalnego osadu ściekowego z dodatkiem stałej dawki słomy pszennej zarówno w działaniu bezpośrednim, jak i następczym, zwiększyły zawartość miedzi, manganu i cynku w roślinach testowych. Wzrost średniej ważonej (z czterech pokosów) zawartości miedzi w *Festulolium,* w porównaniu z obiektem kontrolnym, wahał się od 8,04 do 59,8%, manganu od 21,8 do 68,8%, a cynku od 19,4 do 59,1%. W drugim roku badań, w słoneczniku zebranym z gleby nawożonej osadem i słomą, średni wzrost zawartości miedzi wahał się od 8,7 do 30,3%, a w facelii od 6,1 do 12,6%, manganu odpowiednio od 23,3 do 59,5% i od 5,9 do 33,1%, a cynku od 33,2 do 50,3% i od 15,9 do 37,9%. Nawożenie mineralne N i NPK, w stosunku do obiektu bez tego nawożenia, w obu latach badań zwiększyło średnią zawartość wszystkich analizowanych mikroelementów w roślinach testowych, z tym że NPK w większym stopniu niż N. Pobranie mikroelementów przez rośliny z osadu i słomy, w większości przypadków, zwiększało się wraz ze wzrostem dawek osadu ściekowego. W całkowitym pobraniu poszczególnych mikroelementów udział *Festulolium* stanowił ok. 2/3, a słonecznika i facelii ok. 1/3. Wykorzystanie poszczególnych mikroelementów z osadu i słomy było znacznie zróżnicowane. W okresie dwóch lat w największym stopniurośliny testowe wykorzystały mangan (średnio 58,2%), w mniejszym cynk (średnio 5,54%), a w najmniejszym miedź (średnio 3,03%).

Słowa kluczowe: osad ściekowy, słoma pszenna, rośliny testowe, miedź, mangan, cynk.

INTRODUCTION

Environmental utilization, including agricultural use, of sewage sludge has recently increased but is still arousing many reservations, mainly due to possible microbiological and chemical contamination of soils, greasy consistency and odour of sewage sludge. Taking into consideration the fertilizing value of sewage sludge, confirmed by the research on its chemical composition (Maćkowiak 2001, Spiak, Kulczycki 2004) and impact on crop yield and soil fertility (Czekała 2000, Wołoszyk et al. 2005, 2006, Grzywnowicz 2007, Jasiewicz et al. 2007, Sulewska, Koziara 2007, Bowszys et al. 2009), it seems that sewage sludge should be used for soil and plant fertilizing. It gains more importance as production of natural fertilizers declines and significantly less mineral fertilizers are used due to their rising prices.

About 40% area of soils in Poland is poor in copper and 10% has little manganese and zinc. This should be treated as a signal for utilization of alternative sources of these microelements, for example sewage sludge. With the current production of sewage sludge (about 500 thousand t d.m. in 2006, Rocznik Statystyczny 2007) and mean content of 135 mg $Cu \cdot kg^{-1}$ d.m., 325 mg $Mn \cdot kg^{-1}$ d.m. and 1350 mg $Zn \cdot kg^{-1}$ d.m. (Maćkowiak 2001), about 68 t of copper, 163 t of manganese and 675 t of zinc annually could be received.

The aim of the research was to evaluate direct and successive impact of different doses of sewage sludge and a constant dose of wheat straw with and without supplemental mineral fertilization with nitrogen and NPK, on the content, uptake and utilization of copper, manganese and zinc by test plants.

MATERIAL AND METHODS

In 2004-2005, a pot experiment was conducted under a polyethylene roof at the Vegetation Hall of Vegetation of University of Agriculture in Szczecin. The soil used in the experiment was taken from the Ap level of a production field at the Experimental Agricultural Station in Lipnik. The soil represented acid brown incomplete soil of the grain size distribution characteristic of light, very fine sand with 12% of slit and clay (good rye complex). Potential of hydrogen of the soil before setting up the experiment was acid (pH in 1 M $KCl \cdot dm^{-3}$ – 5,31); the content of total carbon was 6.03 $g \cdot kg^{-1}$ and that of total nitrogen was 0.60 $g \cdot kg^{-1}$; the soil was rich in available phosphorus and zinc, moderately abudant in potassium, copper and manganese, poor in magnesium.

The design of the experiment included two factors. The first factor comprised 5 variants: control and 4 doses of municipal sewage sludge with addition of an equal dose of wheat straw, and the second factor consisted of a variant without mineral fertilization (0), with mineral fertilization (N) and with NPK. Doses of municipal sewage sludge were 0.5, 1.0, 1.5 and 2.0% d.m. in relation to 6.0 kg d.m. soil in a pot. Wheat straw, cut in chaff, was applied in a dose of 30 g d.m. per pot. Sewage sludge came from a mechanical and biological sewage treatment plant in Stargard Szczeciński. The content of heavy metals was lower than norms set for agricultural utilization of sewage sludge (Rozporządzenie 2002). Table 1 shows chemical composition of sewage sludge and straw.

Table 1

Chemical composition of municipal sewage sludge and wheaten straw

Specification	Dry matter ($g \cdot kg^{-1}$)	Total content					
		$g \cdot kg^{-1}$ d.m.			$mg \cdot kg^{-1}$ d.m.		
		N	P	K	Cu	Mn	Zn
Sewage sludge	175	65.6	21.6	5.01	148	349	825
Wheat straw	900	4.90	1.10	13.2	3.13	40.0	12.8

For the objects fertilized only with mineral nitrogen and NPK, the dose of nitrogen was 1 g N per pot (urea – 46% N), which was applied as 0.25 g N per pot before sowing of *Festulolium* and after harvest of the 1st, 2nd and 3rd swath. Phosphorus (double superphosphate – 20% P) in the objects with NPK was applied once as 0.164 g P and potassium (potash salt – 50% K) 0.104 g K per pot before sowing and after the 1st and the 2nd swath. The test plant was an intergenus hybrid, *Festulolium* (var. Felopa), which was sown on 28.04.2004 and harvested on 18.06 (I), 19.07 (II) 25.08 (III) and 28.09.2004 (IV). During the whole growing season, grass was watered with distilled water, keeping soil humidity at a 60% level of full water capacity. After the fourth cut of *Festulolium*, soil was mixed in pots and the stubble mulch was covered, which prepared pots to be stored until spring 2005. On 19th of April 2005 pots were placed under a polyethylene roof and after loosening the soil in pots, water solutions of mineral fertilizers were applied. On the objects with mineral fertilization, the dose of nitrogen was 0.25 g N (urea – 46% N), phosphorus – 0.164 g P (double superphosphate – 20% P) and 0.208 g K per pot (potash salt – 50% K). Test plant was common sunflower (10 seeds per pot, 7 plants were left after thinning), which was sown on 22nd of April 2005. The second dose of nitrogen (0.25 g N per pot), on the objects with N and NPK fertilization, was applied on 20th of May 2005. Common sunflower was harvested on 16th of June 2005 and fresh and dry matter yield wasdetermined. Afterwards, the soil in pots was loosened and on 21st of June 2005 blue phacelia was sown as a test plant. Twenty plants were left after the seedlings. At the same time, on the objects fertilized with N and NPK, 0.25 g N was applied per pot (water solution of urea – 46% N). Phacelia was harvested on 28th of August and fresh and dry matter yield wasdetermined. After the harvests of grass swaths, common sunflower and blue phacelia, mean object samples of individual plants were mineralized in a mixture (3:1) of nitric (V) and perchloric acid (VII) and total content of copper, manganese and zinc was determined with the ASA method. Results of the content of microelements and their uptake by plants were statistically studied with analysis of variance for a two-factor, no-replication system according to FR – Analwar – 4.3 packet. Multiple comparison of means was conducted on the basis of Tukey's procedure at p=0.01.

RESULTS AND DISCUSSION

Direct and successive impact of rising doses of municipal sewage sludge and a constant dose of wheat straw with and without mineral N and NPK fertilizing on yield was shown in previous paper (Wołoszyk et al. 2006). In direct impact all of the doses of sewage sludge (from 0.5 to 2.0% d.m. of sewage sludge in relation to 6 kg d.m. soil per pot) with addition of an equal dose of wheat straw (30 g d.m. per pot) significantly increased sum of yield of *Festulolium* from four swaths in comparison with the control object. Mean increases of dry matter yield varied from 98% to 259%. Fertilizing with mineral nitrogen, applied alongside sewage sludge and straw, in relation to objects without mineral fertilization, increased yield of *Festulolium* by about 27.4% and fertilizing with NPK – by about 38.4%. on average In successive impact of sewage sludge and straw, mean increases of sum of dry matter of sunflower and phacelia varied from 17.9% to 44.2%. However, supplemental fertilization with nitrogen increased yield of sunflower and phacelia by about 15.7% and with NPK by about 35.5%.

Total fertilization with rising doses of municipal sewage sludge and an equal dose of wheat straw increased content of copper, manganese and zinc in *Festulolium* as well as in sunflower and phacelia (Table 2). It should be mentioned that the main source of the analyzed microelements was sewage sludge, because the contribution of straw to the amount of incorporated components, with rising doses of sewage sludge, was from 2.12 to 0.53% for copper, from 11.5% to 2.86% for manganese and from 1.55% to 0.39% for zinc.

The mean weighted (from four swaths) content of copper in *Festulolium*, in most cases, was increasing along with the increase of doses of sewage sludge (Table 2). Mean increases of content of copper varied from 8.04 to 59.8%, in comparison with the control object (without sewage sludge and straw). Favourable influence of mineral fertilization on the copper content in grass was also noticed. On objects with mineral nitrogen fertiliza-

Table 2

Weighted mean (of 4 cuts) content of copper, manganese and zinc in Festulolium and content in common sunflower a blue phacelia ($mg \cdot kg^{-1} d.m.$)

Object	Without fertilization			N			NPK			Mean		
	Cu	Mn	Zn	Cu	Mn	Zn	Cu	Mn	Zn	Cu	Mn	Zn
Festulolium												
Control	3.53	120	31.8	3.85	170	33.4	4.56	220	35.2	3.98	170	33.5
Sludge 0.5%+straw	3.70	160	36.7	4.07	210	40.1	5.13	250	43.1	4.30	207	40.0
Sludge 1.0%+straw	3.96	180	44.6	4.52	220	49.9	6.65	260	53.3	5.04	220	49.2
Sludge 1.5%+straw	4.81	230	47.7	5.63	270	55.0	6.62	310	57.2	5.68	270	53.3
Sludge 2.0%+straw	5.40	250	48.0	6.39	280	54.3	7.31	330	57.3	6.36	287	53.2
Mean	4.28	188	41.8	4.89	230	46.5	6.05	274	49.2	5.07	231	45.8
$LSD_{0.01}$(for doses of sewage sludge)										n.s.	n.s.	17.0
Common sunflower												
Control	4.60	162	34.9	4.72	162	36.6	4.85	164	35.8	4.72	163	35.8
Sludge 0.5%+straw	4.90	181	39.1	5.15	208	51.2	5.36	215	52.9	5.13	201	47.7
Sludge 1.0%+straw	5.10	198	44.0	5.40	212	52.2	5.57	227	54.6	5.35	212	50.3
Sludge 1.5%+straw	5.32	227	46.8	5.55	232	53.8	5.78	254	56.8	5.55	238	52.5
Sludge 2.0%+straw	5.90	245	48.6	6.15	260	54.5	6.42	274	58.2	6.15	260	53.8
Mean	5.16	203	42.7	5.39	215	49.7	5.59	227	51.7	5.38	215	48.0
$LSD_{0.01}$(for doses of sewage sludge)										0.95	58.9	n.s.
Blue phacelia												
Control	4.40	168	34.2	4.50	170	34.9	4.45	170	33.0	4.45	169	34.0
Sludge 0.5%+straw	4.56	176	36.5	4.76	181	40.4	4.85	181	41.2	4.72	179	39.4
Sludge 1.0%+straw	4.65	191	40.2	4.82	194	43.4	4.92	207	42.9	4.79	197	42.2
Sludge 1.5%+straw	4.78	200	42.2	4.90	208	45.9	4.98	218	46.6	4.88	209	44.9
Sludge 2.0%+straw	4.90	218	45.1	5.00	224	47.4	5.15	234	48.2	5.01	225	46.9
Mean	4.66	191	39.6	4.79	195	42.4	4.87	202	42.4	4.77	196	41.4
$LSD_{0.01}$(for doses of sewage sludge)										0.57	28.5	8.19

tion, in comparison with an object without fertilization, mean content of copper was 14.2% higher and on objects with NPK fertilization it was 41.4% higher. Content of manganese in *Festulolium* was increasing along with rising doses of sewage sludge on objects without mineral fertilization as well as with nitrogen fertilization and NPK (tab. 2). Mean increases of that element on objects with sewage sludge and straw, in relation to the control, varied from 21.8 to 68.8%. The highest mean content of manganese, similarly to that of copper, was determined in grass harvested from soil fertilized with NPK (274 mg $Mn \cdot kg^{-1}$ d.m.), lower from soil with nitrogen (230 mg $Mn \cdot kg^{-1}$ d.m.) and the lowest from objects with soil without mineral fertilization (188 mg $Mn \cdot kg^{-1}$ d.m.). All of the doses of sewage sludge and straw, in comparison with the control, considerably increased the mean content of zinc in *Festulolium* (from 19.4 to 59.1%). In grass from objects with 1.5% d.m. and 2.0% d.m. doses of sewage sludge, in relation to soil mass in a pot, the content of zinc was almost identical (53.3 and 53.2 mg $Zn \cdot kg^{-1}$ d.m.) and significantly higher in relation to the control. Supplemental mineral fertilization with nitrogen and NPK, as in the case of copper and manganese, increased the content of zinc in grass suitably about 11.2 and 17.7%, but these differences were not statistically confirmed (Table 2).

As a result of the successive impact of sewage sludge and straw, the content of copper, manganese and zinc in common sunflower and blue phacelia increased (Table 2). The mean content of the analyzed microelements in sunflower was similar to the content in *Festulolium* and lower in phacelia. In turn, rising doses of sewage sludge, versus the control, increased the mean content of copper in sunflower from 8.69 to 30.3% and in phacelia from 6.07 to 12.6%, that of manganese, respectively, from 23.3 to 50.5 and from 5.92 to 33.1%, zinc – from 33.2 to 50.3% and from 15.9 to 37.9%. However, significant increase in the content of copper in sunflower and phacelia was obtained under the influence of the highest dose of sewage sludge and that of manganese as a result of 1.5% and 2.0% d.m. of sewage sludge application. However, the content of zinc in sunflower did not significantly differ. In phacelia, there was more zinc than in the control from the second dose of sewage sludge on. Mineral fertilizing with nitrogen and NPK did not cause such a considerable increase in the content of microelements in sunflower and phacelia as in the case of *Festulolium*. It was only the content of zinc in sunflower from the objects with supplemental nitrogen and NPK fertilization that increased quite considerably (on average about 16.4 and 21.1%).

Statistical analysis showed significant differences in the uptake of copper and manganese by *Festulolium* between the control and the highest dose of sewage sludge, while the uptake of zinc was significantly different after application of 1.5 and 2.0% d.m. of sewage sludge. However, the differences in the uptake of the analyzed microelements by sunflower and phacelia were not statistically confirmed (Table 3). Yet, when analyzing the mean

Table 3

Uptake of microelements by *Festulolium*, common sunflower and blue phacelia (mg from pot)

Object	Copper (Cu)				Manganese (Mn)				Zinc (Zn)			
	0	N	NPK	$\overline{x}$	0	N	NPK	$\overline{x}$	0	N	NPK	$\overline{x}$
Festulolium												
Control	0.020	0.111	0.168	0.100	0.68	4.88	8.12	4.56	0.18	0.96	1.30	0.81
Sludge 0.5%+straw	0.128	0.205	0.290	0.208	5.52	10.56	14.16	10.08	1.27	2.02	2.44	1.91
Sludge 1.0%+straw	0.207	0.322	0.463	0.331	9.42	15.68	18.10	14.40	2.33	3.56	3.71	3.20
Sludge 1.5%+straw	0.346	0.439	0.555	0.447	16.53	21.05	25.98	21.19	3.43	4.29	4.79	4.17
Sludge 2.0%+straw	0.435	0.534	0.672	0.547	20.15	23.40	30.33	24.63	3.87	4.54	5.27	4.56
Mean	0.227	0.322	0.430	0.326	10.46	15.11	19.34	14.97	2.22	3.07	3.50	2.93
$LSD_{0.01}$(for doses of sewage sludge)				0.443				19.32				2.85
Sunflower and phacelia												
Control	0.075	0.110	0.135	0.106	2.71	3.92	4.78	3.80	0.57	0.85	1.00	0.81
Sludge 0.5%+straw	0.107	0.129	0.170	0.135	4.02	5.07	6.64	5.24	0.86	1.20	1.59	1.22
Sludge 1.0%+straw	0.122	0.154	0.188	0.154	4.84	6.12	7.77	6.24	1.05	1.45	1.77	1.42
Sludge 1.5%+straw	0.142	0.164	0.187	0.164	6.02	6.91	8.20	7.04	1.25	1.57	1.80	1.54
Sludge 2.0%+straw	0.176	0.181	0.210	0.189	7.48	7.84	9.15	8.16	1.51	1.65	1.92	1.69
Mean	0.124	0.148	0.178	0.150	5.01	5.97	7.31	6.10	1.05	1.34	1.62	1.34
$LSD_{0.01}$(for doses of sewage sludge)				n.s				n.s.				n.s.
Sum of uptake by three plants												
Control	0.095	0.220	0.303	0.206	3.39	8.80	12.90	8.36	0.75	1.81	2.30	1.62
Sludge 0.5%+straw	0.235	0.333	0.460	0.343	9.54	15.63	20.80	15.32	2.12	3.22	4.03	3.13
Sludge 1.0%+straw	0.329	0.477	0.651	0.486	14.26	21.80	25.87	20.64	3.39	5.01	5.48	4.62
Sludge 1.5%+straw	0.488	0,603	0.742	0.611	22.55	27.96	34.18	28.23	4.68	5.86	6.60	5.71
Sludge 2.0%+straw	0.611	0.715	0.881	0.736	27.63	31.24	39.48	32.79	5.37	6.19	7.19	6.25
Mean	0.351	0.470	0.608	0.476	15.47	21.08	26.65	21.07	3.27	4.41	5.12	4.27
$LSD_{0.01}$(for doses of sewage sludge)				n.s.				24.17				4.00

uptake of microelements in relative numbers, there was a considerable dependence on the dose of ewage sludge and mineral fertilization was noticed.

The mean uptake of all the microelements by *Festulolium*, even at the lowest dose of sewage sludge, was over two-fold higher than in the control and from the soil with the highest dose of sewage sludge the uptake of copper and manganese was about 5-fold and zinc about 6-fold higher. From the soil fertilized with nitrogen, grass took up about 40% more microelements and from the soil with NPK – about 89.4 more copper, 84.4 more manganese and 57.6% more zinc. The total uptake of microelements by yield of sunflower and phacelia was increasing as doses of sewage sludge increased, but the increments were considerably lower than of *Festulolium*. The uptake of copper from soil with the lowest dose of sewage sludge, in comparison with the control, was about 27.4% higher, manganese about 37.9% higher and zinc about 50.6%, while from the soil with the highest dose – about 78.3, 114.7 and 108.6% higher, respectively. In the objects fertilized with nitrogen, the uptake of copper and manganese was about 19% and with NPK about 45% higher in relation to objects without fertilization, while the uptake of zinc was 27.6 and 54.3% higher.. The lowest uptake by test plants in the two years of the experiment was that of copper (on average 0.476 mg per pot), the uptake of zinc was higher (on average 4.27 mg per pot) and the highest uptake was that of manganese (on average 21.07 mg per pot; Table 3).

On the basis of the mean uptake of microelements by yield of test plants from sewage sludge and straw as well as the amount of components incorporated with those fertilizers, utilization of copper, manganese and zinc by *Festulolium*, sunflower, phacelia and total utilization by three plants was estimated (Table 4). The mean utilization of copper, manganese and zinc by test plants from individual doses of sewage sludge and the constant dose of straw was hardly diverse. The mean utilization of copper by *Festulolium* varied from 2.38 to 2.59%, manganese from 44.44 to 50.99% and zinc from 3.77 to 4.79%. Utilization of microelements by sunflower and phacelia was several-fold lower and in the case of copper varied from 0.43 to 0.64%, manganese from 9.94 to 12.34% and zinc from 0.88 to 1.63%. Lower utilization of microelements from sewage sludge and straw by sunflower and phacelia was connected with lower dry matter yield of those plants, in comparison with *Festulolium*, because the content of individual components did not differ considerably. Utilization of copper (3.03%) and zinc (5.54%) from sewage sludge over the two years should be consider as low and manganese (58.18%) as high.

The present research confirms favourable influence of municipal sewage sludge, noticed by other authors (JASIEWICZ et al. 2006, AILINACAI et al. 2007), on shaping the content of microelements in plants. It was also stated that mineral fertilization with nitrogen and NPK modified the content of microelements in plants. Positive impact of mineral fertilization with nitrogen, ap-

Table 4

Amount of microelements brought into soil, taken up and utilized by test plants

Object	Amount of microelements brought into soil with sewage sludge and straw (mg pot^{-1})			Amount of microelements taken up by plants from sewage sludge and straw (mg pot^{-1})			Utilization of micro-elements from sewage sludge and straw by test plants (%)		
	Cu	Mn	Zn	Cu	Mn	Zn	Cu	Mn	Zn
Festulolium									
Sludge 0.5%+straw	4.53	11.67	25.13	0.108	5.52	1.10	2.38	47.30	4.38
Sludge 1.0%+straw	8.97	22.14	49.88	0.231	9.84	2.39	2.58	44.44	4.79
Sludge 1.5%+straw	13.41	32.61	74.63	0.347	16.63	3.36	2.59	50.99	4.50
Sludge 2.0%+straw	17.85	43.08	99.38	0.447	20.07	3.75	2.50	46.59	3.77
Sunflower and phacelia									
Sludge 0.5%+straw	4.53	11.67	25.13	0.029	1.44	0.41	0.64	12.34	1.63
Sludge 1.0%+straw	8.97	22.14	49.88	0.048	2.44	0.61	0.53	11.02	1.22
Sludge 1.5%+straw	13.41	32.61	74.63	0.058	3.24	0.73	0.43	9.94	0.98
Sludge 2.0%+straw	17.85	43.08	99.38	0.083	4.36	0.88	0.47	10.12	0.88
Sum									
Sludge 0.5%+straw	4.53	11.67	25.13	0.137	6.96	1.51	3.02	59.64	6.01
Sludge 1.0%+straw	8.97	22.14	49.88	0.279	12.28	3.00	3.11	55.46	6.01
Sludge 1.5%+straw	13.41	32.61	74.63	0.405	19.87	4.09	3.02	60.93	5.48
Sludge 2.0%+straw	17.85	43.08	99.38	0.530	24.43	4.63	2.97	56.71	4.65

plied against the background of manure, on the content of copper, manganese and zinc in different stages of development of spring barley was confirmed by Rabikowska et al. (2000).

Low utilization of copper and zinc from municipal sewage sludge by plants in a period of three years has been proved in an earlier field study by Wołoszyk et al. (2004). High utilization of manganese from sewage sludge, especially by *Festulolium*, could be a result of high absorption of these element by plants from the soil characterized by acid reaction.

CONCLUSIONS

1. Total fertilization with rising doses of municipal sewage sludge and an equal dose of wheat straw, in comparison with the control objects, resulted in an increase in the content of copper, manganese and zinc in *Festulolium* (direct impact) as well as in sunflower and phacelia (successive impact).

2. Mineral fertilization with nitrogen and NPK, applied against the background of sewage sludge with straw, increased the content of microelements in test plants, especially in the first year of research.

3. The uptake of the analyzed microelements by test plants was increasing along with rise of the doses of sewage sludge and under the influence of mineral fertilizing. In the total uptake of individual microelements, 2/3 were taken up by *Festulolium* and 1/3 by sunflower and phacelia.

4. Utilization of individual microelements from sewage sludge and straw was quite diverse. Over the two years, manganese was utilized to the highest degree (on average 58.2%), followed by zinc (on average 5.54%) and copper (on average 3.03%).

REFERENCE

AILINACAI C., JITAREANU G., BUCUR D., AILINACAI D. 2007. *Influence of sewage sludge on maize yield and quality and soil chemical characteristics.* J. Food, Agric. Environ., 5(1): 310--313.

BOWSZYS T., WIERZBOWSKA J., BOWSZYS J. 2009. *Content and removal of Cu and Zn with harwested crops grown on soil fertilized with composted municipal sewage sludge.* J. Elementol., 14(1): 23-32.

CZEKAŁA J. 2000. *Wartość próchnicotwórcza i działanie nawozowe osadu ściekowego [Humus formation value and fertilizing activity of sewage sludge].* Fol. Univ. Agric. Stein. Agricult., 211(84): 75-80. [in Polish]

GRZYWNOWICZ I. 2007. *Dynamics of changes of mineral nitrogen form content in soil after application of sewage sludge as fertilizer.* Ecol. Chem. Eng., 14(3-4): 303-308.

JASIEWICZ CZ., ANTONKIEWICZ J., BARAN A. 2006. *Influence of organic fertilizers on heavy metal content in tall oat grass.* Ecol. Chem. Eng., 13(9): 915-923.

JASIEWICZ CZ., ANTONKIEWICZ J., MAZUR Z., MAZUR T., KRAJEWSKI W. 2007. *Agrochemical properties of soil fertilized with sewage sludge from sewage treatment plant at Olecko.* Ecol. Chem. Eng., 14(5-6): 457-463.

MAĆKOWIAK CZ. 2001. *Wartość nawozowa osadów ściekowych [Fetilizing value of sewage sludge].* Inż. Ekol., 3: 135-145. [in Polish]

RABIKOWSKA B., PISZCZ U., BELICZYŃSKA K. 2000. *Wpływ wieloletniego nawożenia obornikiem i azotem na zawartość miedzi, manganu i cynku w niektórych fazach rozwojowych jęczmienia jarego [effect of long-term fertilization with manure and nitrogen on the content of manganese and zinc in some developmental phases of spring barley].* Zesz. Prob. Post. Nauk Rol., 471: 495-504. [in Polish]

Rocznik Statystyczny. *Ochrona Środowiska [Environmental Protection.* 2007. GUS, Warszawa. [in Polish]

Rozporządzenie Ministra Środowiska z dnia 1 sierpnia 2002 r. *w sprawie komunalnych osadów ściekowych.* DzU z 2002 r. nr 134, poz. 1140. [Oridnance of the Minister for Environment of 1 August 2002 on municipal sewage sludge. Journal of Law, 2002, no 134, item 1140] [in Polish]

SPIAK Z., KULCZYCKI G. 2004. *Zawartość mikroelementów w osadach ściekowych z wybranych oczyszczalni województwa dolnośląskiego [Content of micronutrients in sewage sludge from some wastewater treatment plants in Dolnośląskie Province].* Zesz. Prob. Post. Nauk Rol., 502(2): 973-978. [in Polish]

SULEWSKA H, KOZIARA W. 2007. *Produkcja osadów ściekowych w Polsce i efekty ich stosowania w uprawie kukurydzy [Sewage sludge production in Poland and effects of sewage sludge fertilization on maize plantations].* Zesz. Prob. Post. Nauk Rol., 518: 175-183. [in Polish]

WOŁOSZYK CZ., IŻEWSKA A., KRZYWY-GAWROŃSKA E. 2004. *Zawartość, pobranie i wykorzystanie mikroelementów z kompostów przez rośliny w trzyletnim zmianowaniu [Content, uptake and utilization of micronutrients from composts by crops in a three-year rotation system].* Zesz. Prob. Post. Nauk Rol., 502: 1059-1067. [in Polish]

WOŁOSZYK CZ., KRZYWY E., IŻEWSKA A. 2005. *Ocena wartości nawozowej kompostów sporządzonych z komunalnego osadu ściekowego w trzyletnim zmianowaniu roślin [Evaluation of fertilization value of composts made from municipal sewage sludge in a three-year rotation of crops].* Frag. Agronom., 1(85): 633-642. [in Polish]

WOŁOSZYK CZ., KRZYWY E., IŻEWSKA A., KRZYWY-GAWROŃSKA E., BALCER K. 2006. *Wpływ bezpośredni i następczy komunalnego osadu ściekowego i słomy pszennej na wielkość plonu oraz na zawartość makroskładników w roślinach testowych [Direct and successive effect pf municipal sewage sludge and wheat straw on yield volume and content of macronutrients in test plants].* Zesz. Prob. Post. Nauk Rol., 512: 211-220. [in Polish]

J. Elementol. 2009, 14(3): 605–617

ACTIVITY OF DEHYDROGENASES, CATALASE AND UREASE IN COPPER POLLUTED SOIL*

Jadwiga Wyszkowska, Mirosław Kucharski, Jan Kucharski, Agata Borowik

Chair of Microbiology
University of Warmia and Mazury in Olsztyn

Abstract

Copper is a life essential element. However, in excess it can be destructive to metabolism of microbial, plant, animal and human cells. Thus, an understanding of all conditions associated with the effect produced by copper on natural environment is vital.

The purpose of the present study has been to evaluate the effect of soil contamination with copper on the activity of dehydrogenases, catalase and urease as well as to determine the tolerance of these enzymes to excessive amounts of copper in soil.

The variable factors of the experiment consisted of:

1) soil type: loamy sand and sandy loam;

2) copper pollution rate in mg kg^{-1} d.m. of soil: 0, 150, 450;

3) soil use: unseeded and seeded soil;

4) crop species: barley, spring oilseed rape and yellow lupine;

5) dates of enzymatic analyses: 25 and 50 day.

The results have revealed that copper pollution, within the rates of 150 to 450 mg kg^{-1} d.m. of soil, significantly inhibits the activity of dehydrogeanses, urease and catalase, with catalase being the most tolerant to excessive copper, unlike dehydrogeneases, which were the most sensitive enzymes. Urease was found to be intermediate in the response to copper. Dehydrogenases, urease and catalase are the least tolerant to the inhibitory effect of copper in soil under spring oilseed rape, being the most tolerant to the pollution in soil under oats. Copper produces stronger inhibitory effect on soil enzymes in unseeded than in seeded soil. The negative effect of excess copper in soil persists and, instead of diminishing, the longer copper remains in soil, the stronger effect it yields. Dehydrognases and catalase are less tolerant to copper in sandy loam than in loamy sand, unlike urease, which was more tolerant to the pollutant in loamy sand than in sandy loam. Tolerance of plants to soil contamination with copper is a species-specific trait. Among the tested crops, yellow

prof. dr. hab. Jadwiga Wyszkowska, Chair of Microbiology, University of Warmia and Mazury, pl. Łódzki 3, 10-727 Olsztyn, Poland, jadwiga.wyszkowska@uwm.edu.pl

lupine was the least tolerant whereas spring oilseed rape was the most tolerant to copper contamination.

Key words: copper, enzymatic activity, tolerance index, vulnerability index, soil contamination with copper.

AKTYWNOŚĆ DEHYDROGENAZ, KATALAZY I UREAZY W GLEBACH ZANIECZYSZCZONYCH MIEDZIĄ

Abstrakt

Miedź jest pierwiastkiem niezbędnym do prawidłowego funkcjonowania wszystkich organizmów, jednakże jej nadmiar w środowisku może działać destrukcyjnie na metabolizm komórek drobnoustrojów, roślin i zwierząt oraz ludzi. Zatem poznanie wszystkich uwarunkowań oddziaływania miedzi na środowisko przyrodnicze jest ze wszech miar uzasadnione.

Celem badań było określenie wpływu zanieczyszczenia gleby miedzią na aktywność dehydrogenaz, katalazy i ureazy oraz określenie oporności tych enzymów na nadmiar miedzi w glebie.

W doświadczeniu czynnikami zmiennymi były:

1) gatunek gleby: piasek gliniasty i glina piaszczysta;

2) stopień zanieczyszczenia miedzią w mg$\cdot$kg^{-1} s.m. gleby: 0, 150 , 450;

3) sposób użytkowania gleby: gleba nieobsiana i obsiana roślinami;

4) gatunek uprawianej rośliny: owies, rzepak jary i łubin żółty;

5) termin analiz enzymatycznych: 25. dzień i 50. dzień.

W wyniku badań stwierdzono, że zanieczyszczenie miedzią, w zakresie od 150 mg do 450 mg$\cdot$kg^{-1} gleby, hamuje istotnie aktywność dehydrogenaz, ureazy i katalazy. Przy czym najbardziej odporna na nadmiar miedzi jest katalaza, a najmniej dehydrogenazy. Pośrednie miejsce zajmuje ureaza. Dehydrogenazy, ureaza i katalaza są najbardziej oporne na inhibicyjne działanie miedzi w glebie pod uprawą rzepaku jarego, a najmniej pod uprawą owsa. Miedź silniej hamuje aktywność enzymów w glebie nieobsianej roślinami niż w glebie obsianej. Negatywne działanie nadmiaru tego pierwiastka w glebie ma charakter trwały i zamiast ustępować nasila się wraz z czasem jego zalegania w glebie. Dehydrogenazy i katalaza są bardziej oporne na działanie miedzi w glinie piaszczystej niż w piasku gliniastym, a ureaza odwrotnie – bardziej oporna w piasku gliniastym niż w glinie piaszczystej. Wrażliwość roślin na zanieczyszczenie miedzią jest cechą gatunkową. Spośród badanych roślin najbardziej wrażliwy jest łubin żółty, a najmniej rzepak jary.

Słowa kluczowe: miedź, aktywność enzymów, indeks oporności, indeks wrażliwości, zanieczyszczenie gleby miedzią.

INTRODUCTION

One of the side effects of civilization progress is excessive accumulation of toxic substances in soil environment, including such chemicals as heavy metals, which are among the most dangerous causes of degradation of natural environment. Accumulation of toxic compounds in soil is ecologically hazardous because of the risk that their remobilization may be delayed

(De Brouwere et al. 2007, Mertens et al. 2007, Oliveira, Pampulha 2006). Heavy metals cause disorders in soil metabolism (Wyszkowska et al. 2008). They depress soil fertility and activity of soil enzymes (Renella et al. 2005, Mikanova et al. 2001). They can also affect negatively the growth and development of plants (Shumaker, Begonia 2005).

Copper is classified as one of the most hazardous heavy metals, although it poses risk only when its quantities exceed natural background. It is so because copper is also a micronutrient, without which no living organism could function. On the other hand, its excess in natural environment may cause malfunctions of ecosystems (Wyszkowska et al. 2005, Wyszkowska et al. 2005a). Thus, an understanding of all conditions involved in the effects produced by copper on natural environment is important, both for expanding our knowledge and for practical purposes. Regarding soils, measurements of soil enzymatic activity is a good index of soil condition (Bielińska 2005).

The aim of the present study has been to determine the effect of soil pollution with copper on the activity of dehydrogenases, catalase and urease as well as to establish the tolerance of these enzymes on excess copper in soil. The study has been performed as part of own research project No N N305 2258 33, financed by the Ministry for Science and Higher Education.

MATERIALS AND METHODS

The experiments were conducted in a greenhouse, in polyethylene pots, with five replications. The trials were set up on soil material collected from the arable humus horizon of proper brown soils. The soils belonged to loamy sand (pH_{KCl} – 6.7, content of (in g kg^{-1}) C_{org} – 11.0, N_{og} – 0.97) and sandy loam (pH_{KCl} – 7.1, content of (in g kg^{-1}) C_{org} – 12.7, N_{og} – 1.16). The granulometric composition of the soils is presented in Table 1.

Table 1

Granulometric composition of soil

Type of soil	Percentage of fractions (d)		
	sand 2.00 ≥ d > 0.05 mm	dust 0.05 ≥ d > 0.002 mm	clay d ≤ 0.002 mm
Loamy sand	75.56	22.92	1.52
Sandy loam	47.92	48.71	3.37

The following were the variable factors of the experiments:

1) soil type: loamy sand and sandy loam;

2) soil pollution with $CuCl_2 \cdot 2H_2O$ in mg Cu kg^{-1} d.m. of soil : 0, 150, 450;

3) soil use: unseeded and seeded soil;

4) crop species: barley, spring oilseed rape and yellow lupine;

5) dates of enzymatic analyses: 25 and 50 day.

Prior to placing soil in pots (3 kg per pot), it was mixed in a polyethylene container with macronutrients and, in some objects, with copper chloride. Once in pots, the soil moisture was brought to the level of 60% capillary water capacity and in some pots, following the variable factors listed as points 3 and 4, crops were sown: cv. Kasztan oats, cv. Huzar spring oilseed rape and cv. Mister yellow lupine. After emergence, the plants were thinned and left in the pots for the following number of days: 12 for oats, 8 for rape and 5 for yellow lupine.

All the objects received identical fertilization in mg kg^{-1} soil: N × 100 (yellow lupine was not fertilized with nitrogen), P – 35, K – 100, Mg – 20. Nitrogen was applied as $CO(NH_2)_2$, phosphorus as KH_2PO_4, potassium as KH_2PO_4 + KCl and magnesium as $MgSO_4 \cdot 7H_2O$.

The plants were harvested at the flowering stage. Their yields were determined as well as the index of sensitivity to copper contamination using the formula:

$$I_K = 1 - \frac{Y_c}{Y_{nc}}$$

where:

- Y_c – is the yield of crops growing on contaminated soil,
- Y_{nc} – the yield of crops growing on uncontaminated soil,
- I_K – assumes values from +1 to -1,
- +1 – 100% inhibition of development,
- -1 – 100% stimulation of development.

On two occasions during the experiment (on days 25 and 50), soil samples from each replication in three consecutive replications were taken to determine the activity of soil enzymes: dehydrogenases 9 (EC 1.1), urease (EC 3.5.1.5) and catalase (EC 1.11.1.6). Dehydrogenases were determined with the method suggested by Öhlinger (1996), whereas urease and catalase were determined according to the procedures described by Alef & Nannpieri (1998). Additionally, resistance of enzymes to soil contamination with copper was calculated as suggested by Orwin & Wardle (2004).

The results of the experiments were processed statistically using Duncan's multiple range test. The tables show results of interaction between the following factors: crop species and soil contamination with copper, soil use and copper pollution, type of soil and copper pollution, date of analysis and soil pollution with copper. The statistical analysis was run using the software Statistica (StatSoft, Inc....2006).

RESULTS AND DISCUSSION

The activity of enzymes in soil depended on a crop species and degree of soil contamination with copper (Table 2). The crop species produced significant influence on the activity of dehydrogenases and urease in uncontaminated soils, but had no effect on the activity of catalase. In uncontaminated soil, the highest activity of dehydrogenases was observed under oats and the lowest one – under spring oilseed rape. In turn, the highest activity of urease was determined in soil under yellow lupine and the lowest one – under spring oilseed rape. The response of soil enzymes to copper pollution was evidently negative, with excess copper inhibiting most strongly the activity of dehydrogenases, followed by urease and, most weakly, catalase. The inhibition of the activity of soil enzymes was more severe as the degree of soil pollution with this metal went up. However, all the soil enzymes

Table 2

Effect of soil pollution with copper and crop species on activity of soil enzymes

Cu dose mg kg^{-1} of soil	Crop species		
	oats	spring oilseed rape	yellow lupine
Dehydrogenases , mmol TFF · kg d.m. of soil · h^{-1}			
0	19.121 ± 0.705	12.397 ± 0.547	12.657 ± 0.824
150	3.757 ± 0.045	4.387 ± 0.308	2.996 ± 0.110
450	0.650 ± 0.155	1.207 ± 0.555	1.134 ± 0.237
Average	7.842	5.997	5.596
LSD	*a* – 0.161; *b* – 0.161; *a* · *b* – 0.282		
Catalase, mol O_2 · kg^{-1} d.m. of soil · h^{-1}			
0	0.157 ± 0.004	0.153 ± 0.002	0.154 ± 0.005
150	0.127 ± 0.003	0.151 ± 0.004	0.140 ± 0.003
450	0.080 ± 0.003	0.126 ± 0.003	0.125 ± 0.005
Average	0.121	0.143	0.140
LSD	*a* – 0.002; *b* – 0.002; *a* · *b* – 0.003		
Urease, mmol N-NH_4 · kg^{-1} d.m. of soil · h^{-1}			
0	1.975 ± 0.021	1.759 ± 0.020	2.157 ± 0.057
150	0.696 ± 0.034	0.806 ± 0.017	0.909 ± 0.010
450	0.301 ± 0.014	0.693 ± 0.060	0.590 ± 0.013
Average	0.990	1.086	1.219
LSD	*a* – 0.020; *b* – 0.020; a · *b* – 0.034		

LSD for: *a* – copper rate, *b* – crop species examined proved to be more tolerant to the inhibitory influence of copper in soil under spring oilseed rape. Their tolerance was the weakest in soil under oats (Table 3). Yellow lupine cultivation acted intermediately compared to rape an oats.

Table 3

Index of resistance of enzymes to soil pollution with copper depending on crop species*

Cu dose mg kg^{-1} of soil	Crop species		
	oats	spring oilseed rape	yellow lupine
Dehydrogenases			
150	0.115 *c*	0.244 *a*	0.146 *b*
450	0.018 *f*	0.055 *d*	0.049 *e*
Average	0.066 *z*	0.149 *x*	0.098 *y*
Catalase			
150	0.672 *d*	0.899 *a*	0.722 *b*
450	0.372 *f*	0.685 *c*	0.666 *e*
Average	0.522 *z*	0.792 *x*	0.694 *y*
Urease			
150	0.307 *c*	0.415 *a*	0.348 *b*
450	0.073 f	0.318 *d*	0.226 *e*
Average	0.190 *z*	0.367 *x*	0.287 *y*

* homogenous group designated with the same letter

Leaving aside crop species, the activity of dehydrogenases was higher in cropped than in unseeded soil, regardless the degree of soil contamination with copper. In contrast, the activity of catalase and urease was higher in unseeded soil (Tabela 4). In unseeded soil, the enzymes were more tolerant to the inhibitory effect of copper (Tabela 5). The average resistance index was 0.250 for dehydrogenases, 0.697 for catalase and 0.457 for urease. In the cropped soil, the respective indices reached: 0.104, 0.669 and 0.281.

Aside the crop species, land use or the degree of copper pollution, the activity of soil enzymes was influenced by the type of soil (Tabela 6). Irrespective of the degree of copper contamination, it was found that dehydrogenases, catalase and urease were more active in sandy loam than in loamy sand. However, the resistance of the enzymes to the inhibitory effect of copper in the two types of soil was not always so unambiguous. Higher values of resistance index of dehydrogenases, by an average of 0.14, and catalase, 0.03 higher on average, were observed in sandy loam than in loamy sand (Tabela 7). On the other hand, for urease, the average resistance index was by 0.225 higher in loamy sand than in sandy loam.

Table 4

Effect of soil pollution with copper and land use on activity of soil eznymes

Cu dose mg kg^{-1} of soil	Land use	
	oats	yellow lupine
Dehydrogenases, mmol TFF · kg d.m. of soil · h^{-1}		
0	7.665 ± 0.220	14.725 ± 0.692
150	2.702 ± 0.153	3.713 ± 0.154
450	0.801 ± 0.103	0.997 ± 0.116
Average	3.723	6.478
LSD	*a* – 0.161; *b* – 0.131; a · *b* – 0.227	
Catalase, mol O_2 · kg^{-1} d.m. of soil · h^{-1}		
0	0.170 ± 0.003	0.153 ± 0.004
150	0.152 ± 0.003	0.139 ± 0.003
450	0.123 ± 0.005	0.111 ± 0.004
Average	0.148	0.134
LSD	*a* – 0.003; *b* – 0.02 ; *a* · *b* – 0.004	
Urease, mmol N-NH_4 · kg^{-1} d.m. of soil · h^{-1}		
0	2.242 ± 0.027	1.964 ± 0.032
150	1.157 ± 0.024	0.804 ± 0.020
450	0.637 ± 0.017	0.528 ± 0.021
Average	1.345	1.098
LSD	*a* – 0.020; *b* – 0.016; *a* · *b* – 0.028	

LSD for: *a* – copper rate, *b* – land use

The activity of soil enzymes varied throughout the experiment (Tabela 8). In the unpolluted soil, the activity of dehydrogenases and catalase was significantly greater on day 50 than on day 25. For urease, it was opposite – the enzyme was more active on day 25 than on day 50. The inhibitory effect of copper on these enzymes was persistent and increased as the experiment continued (Table 9). The average indices of resistance for dehydrogenases, catalase and urease were higher on day 25 than on day 50 of the trials.

Copper pollution of soil had negative influence not only on the soil enzymes but also on the test plants (Tabela 10). The crops were significantly more sensitive to higher soil pollution rates and this was a species-specific trait. Yellow lupine proved to be the most sensitive to copper pollution, unlike spring oilseed rape, which was the most tolerant species, especially when it was grown on more compact soil, i.e. on sandy loam. Oats proved to possess intermediate resistance to copper pollution, irrespective of the type of soil on which it grew.

Table 5

Index of resistance of enzymes to soil pollution with copper depending on land use*

Cu dose mg kg^{-1} of soil	Land use	
	unseeded soil	seeded soil
Dehydrogenases		
150	0.238 *b*	0.168 *c*
450	0.263 *a*	0.040 *d*
Average	0.250 *x*	0.104 *y*
Catalase		
150	0.819 *a*	0.764 *b*
450	0.576 *c*	0.574 *c*
Average	0.697 *x*	0.669 *y*
Urease		
150	0.546 *a*	0.357 *c*
450	0.369 *b*	0.206 *d*
Average	0.457 *x*	0.281 *y*

* homogenous group designated with the same letter

Table 6

Effect of soil pollution with copper and soil type on soil enzymatic activityes

Cu dose mg kg^{-1} of soil	Type of soil	
	loamy sand	sandy loam
Dehydrogenases, mmol TFF $\cdot$ kg d.m. of soil $\cdot$ h^{-1}		
0	12.789 ± 0.537	13.130 ± 0.610
150	2.645 ± 0.255	4.275 ± 0.152
450	0.290 ± 0.079	1.606 ± 0.048
Average	5.242	6.337
LSD	*a* – 0.161; *b* – 0.131; *a* · *b* – 0.227	
Catalase, mol O_2 $\cdot$ kg^{-1} d.m. of soil $\cdot$ h^{-1}		
0	0.115 ± 0.003	0.200 ± 0.004
150	0.103 ± 0.003	0.182 ± 0.003
450	0.080 ± 0.003	0.148 ± 0.005
Average	0.099	0.265
LSD	*a* – 0.003; *b* – 0.02 ; *a* · *b* – 0.004	
Urease, mmol N-NH_4 $\cdot$ kg^{-1} d.m. of soil $\cdot$ h^{-1}		
0	1.049 ± 0.028	3.017 ± 0.035
150	0.610 ± 0.018	1.174 ± 0.024
450	0.408 ± 0.012	0.703 ± 0.031
Average	0.689	1.631
LSD	*a* – 0.020; *b* – 0.016; *a* · *b* 0.028	

LSD for: *a* – copper rate, *b* – soil type

Table 7

Index of resistance of enzymes to soil pollution with copper depending on type of soil*

Cu dose mg kg^{-1} of soil	Type of soil	
	loamy sand	sandy loam
Dehydrogenases		
150	0.130 *c*	0.242 *a*
450	0.012 *d*	0.180 *b*
Average	0.071 *y*	0.211 *x*
Catalase		
150	0.773 *b*	0.783 *a*
450	0.549 *d*	0.599 *c*
Average	0.661 *y*	0.691 *x*
Urease		
150	0.539 *a*	0.268 *c*
450	0.336 *b*	0.157 *d*
Average	0.438 *x*	0.213 *y*

* homogenous group designated with the same letter

Table 8

Effect of soil pollution with copper and date of analysis on activity of soil enzymes

Cu dose mg kg^{-1} of soil	Time of analysis, days	
	25	50
Dehydrogenases , mmol TFF· d.m. of soil· h^{-1}		
0	12.133 ± 0.671	13.786 ± 0.477
150	2.950 ± 0.243	3.971 ± 0.165
450	0.978 ± 0.045	0.918 ± 0.039
Average	5.354	6.225
LSD	*a* – 0.161; *b* – 0.131; *a* · *b* – 0.227	
Catalase, mol O_2 · kg^{-1} d.m. of soil · h^{-1}		
0	0.154 ± 0.004	0.160 ± 0.004
150	0.150 ± 0.003	0.135 ± 0.003
450	0.122 ± 0.004	0.106 ± 0.004
Average	0.142	0.134
LSD	*a* – 0.003; *b* – 0.02 ; *a* · *b* – 0.004	
Urease, mmol N-NH_4 · kg^{-1} d.m. of soil · h^{-1}		
0	2.087 ± 0.023	1.979 ± 0.040
150	1.054 ± 0.020	0.729 ± 0.022
450	0.609 ± 0.013	0.502 ± 0.030
Average	1.250	1.070
LSD	*a* – 0.020; *b* – 0.016; *a* · *b* 0.028	

LSD for: *a* – copper rate, *b* – date of analysis

Table 9

Index of resistance of enzymes to copper pollution depending on the date of analysis*

Cu dose mg kg^{-1} of soil	Time of analysis, days	
	25	50
Dehydrogenases		
150	0.153 *b*	0.205 *a*
450	0.146 *c*	0.046 *d*
Average	0.149 *x*	0.125 *y*
Catalase		
150	0.852 *a*	0.704 *b*
450	0.652 *c*	0.496 *d*
Average	0.752 *x*	0.600 *y*
Urease		
150	0.486 *a*	0.322 *b*
450	0.289 c	0.204 *d*
Average	0.388 *x*	0.263 *y*

* homogenous group designated with the same letter

Whereas the inhibitory influence of soil contamination with copper was to be expected even before the experiment, it seemed more important to prove that species of crops could also modify the resistance of soil enzymes to the pollutant. The fact that spring oilseed rape and yellow lupine, unlike oats, could alleviate the negative effect of copper on soil enzymes may be linked to a more favourable effect produced by these two crops, in contrast to cereal plants, on physical and chemical properties of soil (KARLEN et al. 2003). It is interesting to find out that enzymes of the same class, i.e. dehydrogenases and catalase, respond rather differently to copper pollution of soil. Catalase is less sensitive to this pollutant than dehydrogenases, which may be connected with the specificity of dehydrogenases.

It is not quite clear why enzymes in unseeded soil were more resistant to copper pollution than those in cropped soil. What makes it even more difficult to clarify is that root secretions would typically have positive influence on soil microorganisms (DIJKSTRA et al. 2006), which is directly connected with the activity of soil enzymes.

The enzymes did not respond unambiguously to copper pollution in the test soils. It could be expected that enzymes would be more tolerant to excess copper in sandy loam, a more buffered soil, rather than in loamy sand (KARLEN et al. 2003, SCHOENHOLTZ et al. 2000, WYSZKOWSKA et al. 2005). And this dependence *did* occur in the case of dehydrogenases and catalase,

Table 10

Index of sensitivity of crops to soil pollution with copper*

Cu dose mg kg^{-1} of soil	Type of soil					
	loamy sand			loamy sand		
	crop species					
	oats	spring oilseed rape	yellow lupine	oats	spring oilseed rape	yellow lupine
150	0.104 *c*	0.019 *b*	0.275 *e*	0.015 *b*	-0.171 *a*	0.515 *f*
450	0.578 *g*	0.608 *h*	0.776 *j*	0.653 *i*	0.239 *d*	0.890 *k*
Average	0.341 *n*	0.314 *p*	0.525 *m*	0.334 *o*	0.034 *r*	0.702 *l*

* homogenous group designated with the same letter

whereas urease behaved differently. It was more resistant to copper contamination in loamy sand.

The negative effect of copper on the activity of the tested soil enzymes, like that produced by other heavy metals (WYSZKOWSKA et al. 2008, WYSZKOWSKA et al. 2005a), persisted and did not decrease as the experiment progressed. Contrary to that, the negative influence of copper grew stronger. Such an outcome is due to the character of the experiment (pot trials), when nutrients do not migrate outside the reach of the root system.

The most important test verifying the state of soil environment is a plant test. It is only partly correlated with the activity of soil enzymes. Resistance of dehydrogenases, catalase and urease to copper was higher in soil cropped with spring oilseed rape, the crop which likewise proved to be the least sensitive to copper, with the rape plants growing on a more compact soil, i.e. sandy loam, showing minimum sensitivity to this soil pollutant. No such correlation was found between resistance of soil enzymes in soil under yellow lupine versus the sensitivity of this crop to excess copper.

CONCLUSIONS

1. Copper contamination of soil from 150 to 450 mg·kg^{-1} significantly inhibit the activity of dehydrogenases, urease and catalase. Catalase is the most tolerant to excess copper, in contrast to dehydrogenases, which are the most sensitive soil enzymes. Urease is intermediately resistant to copper.

2. Dehydrogenases, urease and catalase are the least resistant to the inhibitory effect of copper in soil under spring oilseed rape. In turn, they are the most sensitive in soil cropped with oats.

3. Copper inhibited the activity of the enzymes more strongly in unseeded soil than in cropped soil. The negative effect of excessive quantities of this metal in soil is persistent and instead of disappearing gradually, it intensifies the longer the pollutant remains in soil.

4. Dehydrogenases and catalase are the more resistant to the effect of copper in sandy loam than in loamy sand, in contrast to urease, which is more tolerant to copper in loamy sand than in sandy loam.

5. Tolerance of crops to copper contamination in soil is a species-specific trait. Among the three tested crops, yellow lupine was the most sensitive whereas spring oilseed rape was the most tolerant to soil pollution with copper.

REFERENCES

ALEF K., NANNIPIERI P. (eds) 1998. *Methods in applied soil microbiology and biochemistry*. Academic Press. Harcourt Brace & Company, Publishers, London: pp. 576.

BIELIŃSKA E.J. 2005. *Determination of phosphatases acivity*. Acta Agroph., Rozprawy i Monografie, 3: 63-74.

DE BROUWERE K.D., HERTIGERS S., SMOLDERS E. 2007. *Zinc toxicity on N_2O reduction declines with time in laboratory spiked a oils and is undetectable in field contaminated soils*. Soil Biol. Biochem., 39: 3167-3176.

DIJKSTRA F.,A., CHENG W., JOHNSON D.W. 2006. *Plant biomass influences rhizosphere priming effects on soil organic matter decomposition in two differently managed soils*. Soil Biol. Biochem., 38: 2519-2526.

KARLEN D., DITZLER C.A., ANDREWS S.S. 2003. *Soil quality: why and how?* Geoderma., 114: 145-146.

MERTENS J., RUYTERS S., SPRINGAEL D., SMOLDERS E. 2007. *Resistance and resilience of zinc tolerant nitrying communities is unaffected in log-term zinc contaminated soils*. Soil Biol. Biochem., 39: 1828-1831.

MIKANOVA O., KUBAT J., MIKHAILOVSKAYA N., VOROS I., BIRO B. 2001. *Influence of heavy metal pollution on some soil biological parameters in the alluvium of the Litavka river*. Rostl. Vyr., 47(3): 117-122.

ÖHLINGER R. 1996. *Dehydrogenase activity with the substrate TTC*. In: *Methods in Soil Biology*. SCHINNER F., ÖHLINGER R., KANDELER E., MARGESIN R. (eds), Springer Verlag Berlin Heidelberg, 241-243.

OLIVEIRA A., PAMPULHA M.E. 2006. *Effects of long-term heavy metal contamination on soil microbial characteristics.* J. Bios. Bioeng., 102 (3): 157-161.

ORWIN K.H, WARDLE D.A. 2004. *New indices for quantifying the resistance and resilience of soil biota to exogenous disturbances*. Soil. Biol. Biochem., 36: 19-7-1912.

RENELLA G. MENCH M., LANDI L., NANNIPIERI P. 2005. *Microbial activity and hydrolase synthesis in long-term Cd-contaminated soils.* Soil Biol. Biochem., 37: 133-139.

SCHOENCHOLTZ S.H., VAN MIEGROET, BURGER J.A. 2000. *A review of chemical and physical properties as indicators of forest soil quality: chalelenges and opportunities*. Forest Ecol. Managem., 138: 335-356.

SHUMAKER K.L., BEGONIA G. 2005. *Heavy metal uptake, translocation, and bioaccumulation studies of Triticum aestivum cultivated in contaminated dredged materials*. Int. J. Environ. Res. Public Health., 2(2): 293-298.

StatSoft, Inc. 2006. *Statistica (data analysis software system)*, version 7.1. www.statsoft.com.

Wyszkowska J., Boros E., Kucharski J. 2008. *Enzymatic activity of nickel-contaminated soil*. J. Elementol. 13 (1): 139-151.

Wyszkowska J., Kucharski J., Boros E. 2005a. *Biochemical properties of soil contaminated with nickel and other heavy metals*. J. Elementol., 10 (3): 585-596.

Wyszkowska J., Kucharski J., Lajszner W. 2005. *Effect of soil contamination with copper on its enzymatic activity.* Pol. J. Environ. Stud., 14(5): 119-124.

J. Elementol. 2009, 14(3): 619–630

PAPER REVIEW

ROLE OF SILICON IN PLANT RESISTANCE TO WATER STRESS

Elżbieta Sacała

Chair of Plant Nutrition
Wrocław University of Environmental and Life Sciences

Abstract:

Agricultural productivity is strongly affected by different abiotic stresses, among which water stress is the major environmental constraint limiting plants growth. The primary reason for water stress is drought or high salt concentration in soil (salinity). Because both of these stress factors lead to numerous physiological and biochemical changes in plants and result in serious loss in yields, there is a pressing need for finding the effective ways for increasing crops' resistance to stress factors. One of the alternative methods involving alleviation of negative stress effects might be application of silicon as a fertiliser (root or foliar supply).

Many plants, particularly monocotyledonous species, contain large amounts of Si (up to 10% of dry mass). In spite of the high Si accumulation in plants (its amount may equal concentration of macronutrients), until now it has not been considered as an essential element for higher plants. Many reports have shown that silicon may play a very important role in increasing plant resistance to noxious environmental factors. Hence, Si is recognised as a beneficial element for plants growing under biotic and abiotic stresses. The main form of Si which is available and easily taken up by plants is monosilicic acid (H_4SiO_4). Plants take up Si from soil solution both passively and actively. Some dicotyledonous plants such as legumes tend to exclude Si from tissues – rejective uptake. These plants are unable to accumulate Si and they do not benefit from silicon. Under water stress conditions, silicon might enhance plants' resistance to stress and ameliorate growth of plants. These beneficial effects may result from better and more efficient osomoregulation, improved plant water status, reduction in water loss by transpiration, maintenance of adequate supply of essential nutrients, restriction in toxic ions uptake and efficient functioning of antioxidative mechanisms.

Based on the current knowledge and presented data, it can be concluded that the

Elżbieta Sacała, Chair of Plant Nutrition, Wrocław University of Environmental and Life Sciences, 50-357 Wrocław, Poland, ul. Grunwaldzka 53

role of Si in plants is not restricted to formation of physical or mechanical barrier (as precipitated amorphous silica) in cell walls, lumens and intercellular voids. Silicon can also modulate plants' metabolism and alter physiological activities, particularly in plants subjected to stress conditions. However, in some plants, increased silicon supply does not improve plant growth. Hence, a better understanding of the interactions between silicon application and plant responses will contribute to more efficient fertiliser practices, especially under stress conditions.

Key words: silicic acid, Si uptake, water stress, drought, salinity, resistance.

ROLA KRZEMU W ODPORNOŚCI ROŚLIN NA STRES WODNY

Abstrakt

Produktywność roślin uprawnych jest w znacznym stopniu ograniczana przez różne abiotyczne czynniki stresowe, wśród których stres wodny jest jednym z głównych problemów, na który narażone są rośliny. Stres wodny najczęściej jest spowodowany suszą glebową lub nadmiernym zasoleniem gleb. Ponieważ te czynniki powodują liczne fizjologiczne i biochemiczne zmiany w roślinach oraz prowadzą do poważnych strat plonów, konieczne jest znalezienie skutecznych sposobów zwiększenia odporności roślin na stresy. Jednym ze sposobów pozwalających na złagodzenie ujemnych skutków stresu wodnego może być zasilanie roślin krzemem (w formie oprysku lub dodatku do podłoża).

Wiele roślin, szczególnie jednoliściennych, zawiera duże ilości krzemu (do 10% s.m.). Pomimo że procentowa zawartość krzemu w roślinach może dorównywać zawartości makroelementów, to nie jest on uznawany za pierwiastek niezbędny do prawidłowego wzrostu i rozwoju roślin. Wyniki wielu badań wskazują jednak na ważną rolę krzemu w podnoszeniu odporności roślin na różne niekorzystne czynniki środowiska. Dlatego krzem jest uważany za pierwiastek wpływający korzystnie na rośliny, szczególnie poddane działaniu abiotycznych i biotycznych czynników stresowych. Główną formą krzemu dostępną dla roślin i łatwo przez nie pobieraną jest kwas ortokrzemowy (H_4SiO_4). Może on być pobierany z roztworu glebowego w sposób pasywny lub aktywny. Niektóre rośliny (głównie motylkowate) wykluczają krzem ze swoich tkanek (ang. *rejective uptake*). Rośliny te nie mają zdolności akumulowania tego pierwiastka, i w związku z tym nie mogą doświadczać jego korzystnego działania. W warunkach stresu wodnego krzem może zwiększać odporność roślin oraz poprawiać ich wzrost. Ten pozytywny wpływ może wynikać z: lepszej i bardziej sprawnej osmoregulacji, lepszego statusu wodnego, ograniczenia strat wody w procesie transpiracji, odpowiedniego zaopatrzenia w składniki mineralne, ograniczenia pobierania toksycznych jonów oraz sprawnego funkcjonowania mechanizmów antyoksydacyjnych.

Opierając się na obecnym stanie wiedzy i przedstawionych danych, można stwierdzić, że rola krzemu nie ogranicza się jedynie do tworzenia mechanicznej lub fizycznej bariery (w postaci amorficznej krzemionki) w ścianach komórkowych, przestrzeniach międzykomórkowych oraz wewnątrz komórek. Pierwiastek ten może wpływać na metaboliczną i fizjologiczną aktywność roślin, szczególnie tych, które narażone są na niekorzystne wpływy środowiskowa. Jednakże w przypadku niektórych roślin nie stwierdzono pozytywnego wpływu krzemu na ich wzrost. Dlatego zrozumienie interakcji między zastosowaniem krzemu a reakcją roślin na ten pierwiastek przyczyni się do bardziej efektywnego nawożenia roślin, szczególnie w warunkach stresowych.

Słowa kluczowe: kwas ortokrzemowy, pobieranie Si, stres wodny, susza, zasolenie, odporność.

INTRODUCTION

All terrestrial plants contain silicon (Si). In spite of the high Si accumulation in plants (its amount may equal concentration of macronutrients), until now it has not been considered as an essential element for higher plants. Many reports have shown that silicon may play a very important role in increasing plants' resistance to noxious environmental factors. Hence, Si is recognised as a beneficial element for plants growing under biotic and abiotic stresses, for example heavy metals, drought, salinity, pathogens (GRENDA and SKOWROŃSKA 2004, FAUTEX et al. 2005, GAO et al. 2006, LIANG et al. 2007, TUNA et al. 2008, DONCHEVA et al. 2009, SAVVAS et al. 2009). It is worth noting that Si is an essential trace element in animal and human nutrition. Silicon plays an important role in synthesis of glucosaminoglycans and collagen, consequently in bone formation (TANAKA 1985). High levels of soluble Si (silicic acid) may reduce bioavailability and neurotoxicity of aluminium through the formation of hydroxyaluminiosilicate, which prevents binding of aluminium to the gut (BIRCHALL 1990, EDWARDSON et al. 1993).

Plants growing in natural conditions are constantly subjected to noxious environmental factors. One of the major constraints of plants' growth and productivity is water stress. Water stress may result from water deficit in soil (drought) or excessive amount of salt (salinity), most commonly NaCl. Drought occurs in many parts of the world every year, whereas increasing salinity of soil is a growing problem, particularly in irrigated areas. Hence, our understanding of crop responses to water stress is very important, but finding effective ways for increasing crop stress tolerance seems to be crucial. One of the alternative methods involving alleviation of negative stress effects might be application of silicon as a fertiliser (root or foliar supply).

The present paper describes the results of numerous investigations conducted during last two decades on the mechanism of silicon uptake and transport in higher plants as well as its possible role in enhancing plants tolerance to salt stress and drought.

MECHANISMS OF SILICON UPTAKE

Silicon (Si) is the second, after oxygen, most abundant element in earth crust and its percentage value reaches 26%. In nature, Si does not occur as an elemental form but it is a compound of many minerals which form rocks. Silicon occurs mainly in the form of silicon dioxide (silica) and silicates that contain Si, oxygen and metals (BROGOWSKI 2000, ŘEZANKA and SIGLER 2008). Minerals containing Si are resistant to weathering processes and decomposition, hence the amount of silicon in soil solution is low (BROGOWSKI 2000). Monosilicic acid (H_4SiO_4 = $Si(OH)_4$) is amobile and soluble form of Si which is available to plants. Concentration of Si in soil solution ranges from 0.0004 to 2.0 mmol dm^{-3} but most values lie between 0.1 and 0.6 mmol dm^{-3} (EPSTEIN 1994, 1999, SOMMER et al. 2006). Uptake of Si from external solution and its transport through roots might be an active or a passive (diffusion) process (TAMAI and MA 2003, MITANI and MA 2005, MA and YAMAJI 2006). Functioning and activity of silicon transporters require energy supply. MA and YAMAJI (2006) described Si transport process in three species that evidently differ in the ability of Si accumulation: rice (high accumulation), cucumber (medium) and tomato (low Si level). These authors stated that in all the three species transport of Si is mediated by a similar transporter with K_m value of 0.15 mmol dm^{-3} but there are differences in the V_{max} values (i.e. rice > cucumber > tomato). This may suggest that among plants species there are differences in density of Si transporters. Results of investigations conducted on four different species of mono- and dicotyledonous plants (*Oryza sativa*, *Zea mays*, *Helianthus annuus*, *Benincasa hispida*) showed that both active and passive components of Si uptake system co-exist in plants (LIANG et al. 2006a). Relative contribution of these components depends on plant species and external Si concentrations. In the case of rice and maize (both gramineous species), the active component is the major mechanism responsible for Si uptake (LIANG et al. 2006a). A very important step in Si translocation is its transport from cortical cells to the xylem (xylem loading). In rice, a typical silicon accumulator, its concentration in xylem sap is high (2 mM) and process of xylem loading of Si is mediated by specific transporters (MITANI and MA 2005). Whereas in cucumber and tomato, xylem loading is a passive process, hence transport efficiency is very limited. The determined Si concentration of xylem sap in rice was 20- and 100-fold higher than that in cucumber and tomato respectively. Moreover, Si concentration in xylem of both plants was lower than in the external solution (MITANI and MA 2005). However LIANG and his colleagues (2005a) presented a contrary conclusion. They demonstrated that in *Cucumis sativus* L. silicon uptake and xylem loading are also active processes, such as in rice. Such distinct discrepancy in the results obtained might be caused by the different experimental conditions and further investigations are needed to solvethe controversy. Some dicotyledonous plants such as legumes do not accumulate Si in tissues and tend to exclude this element – rejective uptake (VAN DER VORM 1980, LIANG et al. 2005a). These plants take up Si more slowly than water and they contain less silicon than would be expected from nonselective passive uptake of silicic acid during plant growth.

Concluding, silicon is taken up in the form of uncharged molecule – silicic acid – and in plants three different modes of its uptake (active, passive, rejective) may function.

Silicon distribution in aerial parts of plants is dependent on intensity of transpiration. In the transpiration stream in xylem, silicic acid is transported to leaves and it is accumulated in older tissues (it is not mobile within the plant). In the shoot, owing to the loss of water (transpiration), silicic acid is concentrated and polymerised (MA and YAMAJI 2006). In consequence, Si forms colloidal silicic acid and finally silica gel. Silicon in the form of silica gel may amount to 90% of total Si concentration in shoots (MA and YAMAJI 2006).

SILICON ACCUMULATION AMONG PLANT SPECIES

There are substantial differences in silicon concentration in plant kingdom. The range of its concentration is 0.1-10% Si on a dry matter basis (EPSTEIN 1994, 1999). Plant species older in the evolutionary sense (diatoms, cyanosis, horsetails, ferns) contain more Si than plants that emerged later. Among higher plants, species from *Gramineae* i *Cyperaceae* families accumulate Si in large amounts and are considered as Si accumulators (higher than 1% Si on dry weight). Rice and other wetland grasses are an example of Si accumulators. Most dicotyledenous plants contain less than 1% of Si on dry matter (non-acumulators). A third distinguishable group of plants has an intermediate level of Si at about 1-3%. Among these intermediate plants, JONES and HANDRECK (1967) listed dryland grasses such as rye and oats. However, recent studies indicate that a high Si concentration is not a general feature of monocotyleonous species (HODSON et al. 2005). Within dicotyledenous plants there are considerable differences in silicon concentration. Plants from *Cucurbitaceae* and *Urticaeae* families accumulate high amounts of Si and may be classified as intermediate category. Such differences in Si concentration resulted from different abilities of plant roots to uptake and transport silicic acid (as mentioned in the previous section). The plants that take up Si only by the passive process do not accumulate this element and its concentration in plant tissues is very low. EPSTEIN (1994) published data concerning Si distribution in plant kingdom. Analyses of 175 species grown in the same soil showed that among nine examined elements (Si, Ca, Mg, K, P, Fe, Mn, B, Al) silicon was the most variable. Most analysed plants (81%) did not accumulate silicon and the mean concentration of Si was only 0.25% Si in dry weight. This information indicates that most plants, especially dicotyledonous, are unable to accumulate a large amount of Si and hence they do not benefit from silicon.

Most Si is deposited in cell walls of roots, leaves, stems and hulls, where it may form a thin layer consisting of silica gel ($SiO_2 \cdot nH_2O$). Investigations conducted by MA et al. (2003) on grains of 401 barley varieties showed that the variation in Si concentration in grains is controlled genetically. More than 80% of total Si was localised in the hull and its amount ranged between 15.343 and 27.089 mg kg^{-1} in tested varieties.

WATER STRESS

Water availability in soil is one of the major environmental factors that limit the growth of plants and the production of crops. Water deficiency may result from shortage of water in soil (drought) or problem with its uptake (physiological drought). In the latter case, water is in soil solution but plants cannot take it up because there are some physiological reasons, for example high salt concentrations (salinity), excess of water (flooding), low temperatures. All these factors consequently cause water stress and changes in cell water relations. Water potential is reduced and turgor of plant cells lowered. These changes result in disruption of most important processes and reduction in growth rates. When plants are exposed to salinity, they suffer additionally from a toxic level of salts in cells. Water deficit affects adversely photosynthesis, uptake and transport of essential nutrients and causes overproduction of ROS – reactive oxygen species ($O_2^{\cdot -}$, $^1O_2$, $OH^{\cdot}$, H_2O_2). These very reactive molecules lead to serious disorder in plant metabolism and damage in membranes (HASEGAWA et al. 2000, REDDY et al. 2004).

ROLE OF SILICON IN INCREASING PLANTS RESISTANCE TO WATER STRESS

Plants growing under natural conditions are subjected to a multitude of different stress factors through their life cycle. Cellular water deficiency may result not only from drought, salinity and low temperature but can be a secondary effect caused for example by heavy metals or high radiation. Hence, it is very important to determine mechanisms of plant resistance to water stress and also to find the ways for increasing this resistance. For overcoming the negative impact of water stress, addition of Si to the growth medium may have a beneficial effect on plants. It is worth noticing that the beneficial function of silicon does not reveal itself under optimal circumstances but mainly under stress conditions (HENRIET et al. 2006, KAYA et al. 2006, HATTORI et al. 2007). MA and YAMAJI (2006) point at the fact that silicon exerts positive effects when its concentration in plant tissues is high.

Mechanisms which are important in plant resistance to water stress and a possible role of Si in these processes may be considered at different levels (molecular, cellular, whole-plant). Essential features of plants' response to water stress are following: i) maintenance of homeostasis, including ionic balance and osmotic adjustment, ii) counteraction to damages and their prompt repair, e.g. elimination of ROS and prevention of oxidative stress, iii) detoxification of excess salts under salinity, iv) regulation and recovery of growth.

Results of experiments conducted by Kaya et al. (2006) on maize growing under water stress indicated that silicon (1 and 2 mmol dm^{-3} Na_2SiO_3) significantly improve shoot growth although it did not affect root growth. It is worthy of note that higher Si dose was more efficient than lower one, although in the case of leaf relative water content (RWC) both Si concentrations caused similar increase of this parameter in comparison to plants growing without Si. Improved plant water status (higher RWC index) may result from reduced water loss by transpiration due to deposition of Si (forming silica gel layer) on epidermal cell walls. It was surprising that both Si treatments lowered proline concentration in maize plants grown under water stress (Kaya et al. 2006). Similar response was observed in wheat growing under salinity (Tuna et al. 2008). Amino acid proline occurs widely in proteins but it may also accumulate in the cytosol in response to environmental stresses, especially under osmotic stress. Accumulated free proline contributes substantially to osmotic adjustment and may protect and stabilise sub-cellular structures (e.g. proteins and membranes).

Water stress very often leads to impairment of mineral nutrition and disruptions in ion homeostasis. Kaya et al. (2006) reported that under drought stress maize leaves contained approx. 50% less calcium than control plants while in roots its amount was higher comparing to the control. Decrease in Ca concentration in plant cells is harmful because this element plays an essential role in maintaining the structural and functional integrity of plant membranes and regulation of their permeability and selectivity. The ability of plants to maintain membrane stability is a crucial trait of stress resistance. Some investigations indicate that addition of Si may increase concentrations of Ca in plant tissues and hence restore membrane integrity in water-stressed plants (Kaya et al. 2006). Disruption of ion homeostasis may result from reduced K^+ concentrations in water-stressed plants. Potassium plays an important role in processes involving osmotic adjustment and its adequate level in plants may improve water stress tolerance. Under water-stress conditions, the presence of Si may result in better supply of K^+ (Kaya et al. 2006). This beneficial effect may be attributed to the stimulating action of Si on H^+-ATP-ase (Liang 1999).

In plants growing under salt-stress conditions, added silicon helps in maintaining an adequate supply of essential nutrients and reduces sodium uptake and its transport to shoots (Liang 1999, Tuna et al. 2008). In experiments with salt-stressed barley, Liang (1999) indicated that Si (1 mmol dm^{-3} K_2SiO_3) decreases sodium but increases potassium concentrations both in roots and shoots. Selective uptake of mineral ions is associated with the activity of H^+-ATP-ase. This membrane-located enzyme generates proton motive force that is used for ion transport inside the cell. Liang (1999) reported that under salinity (120 mmol dm^{-3} NaCl) there was a dramatic decrease in ATP-ase activity. In a salt-tolerant barley cultivar, this decline reached nearly 66% whereas in a salt-sensitive one it was 75% comparing to the control plants. In both cases, supplemental silicon resulted in a 2-fold increase in enzyme activity in comparison to plants growing without this element. It is interesting to note that salt stress caused substantial fall in potassium and calcium concentrations and added silicon led to a nearly 2-fold increase in K^+ level but had little effect on calcium content in shoots (LIANG 1999).

As mentioned above, one of the most important mechanisms of plant resistance to salinity is to control Na^+ uptake and prevent its excessive accumulation in plant tissues. LIANG (1999) and several other authors reported that addition of silicon considerably lowers concentration of potentially toxic ions in aerial parts of plants (AHMAD et al. 1992, YEO et al. 1999, GUNES et al. 2007a,b, TUNA et al. 2008, ZUCCARINI 2008). It is possible that silicon present in plant cells limits uptake of toxic ions and prevents their translocation to shoots. The beneficial effect of silicon may be related to the depression of water loss by transpiration and consequently reduced rate of passive uptake and transport of minerals (YEO et al. 1999, GAO et al. 2006, ROMERO-ARANDA et al. 2006). On the other hand, silicon deposited in the form of polymerised SiO_2 in the apoplast of roots considerably restricts ionic translocation from roots to shoots (EPSTAIN 1999, WANG 2004). However, some reports indicate that added silicon does not lower concentration of Na^+ and Cl^- (ROMERO-ARANDA et al. 2006). Maintenance of low concentration of saline ions in plant tissues is a very important mechanism of salt stress tolerance, although more crucial is the capability of plants to take up and retain water in tissues despite its low potential in external medium. This may be related to a decrease in water loss, high water use efficiency and very efficient osmotic adjustment. ROMERO-ARANDA et al. (2006) stated that silicon evidently improves water status in tomato growing under salt stress (80 mmol dm^{-3} NaCl). When salinised plants were treated with Si, their water content increased by 40% and value of water use efficiency (estimated as the ratio between net CO_2 assimilation and transpiration rate) was 17% higher than in salinised plants without silicon. Increased water content in plants growing under osmotic stress shows that processes contributed to osmotic adjustment are very effective. On the other hand, improved ability to retain water by plants treated with Si may result from a lowered transpiration rate and higher values of water use efficiency (GAO et al. 2004, 2006, ROMERO-ARANDA et al. 2006). ROMERO-ARANDA et al. (2006) maintain that beneficial effect of Si might be related to hydrophilic nature of silicon. SiO_2 nH_2O deposited in plant cells permits plants to keep water, dilute salts and protect tissues from physiological drought. In experiments with water-stressed sorghum, HATTORI et al. (2007) showed that silicon application could affect stomatal conductance through the modification of plant water status but not through any physical changes. It could be concluded that silicon facilitates water uptake and its transportation into leaves.

It is generally thought that stability and integrity of biological membranes is a crucial element of plant resistance to abiotic stress. Water stress, like other stress factors, may affect plasma membranes and cause various dysfunctions (changes in membrane permeability and fluidity, disturbance in activity of enzymes located in plasma membranes). These negative effects may result from membrane damage largely caused by membrane lipid peroxidation (oxidation of membrane bound unsaturated fatty acids). It is well known that free radicals induce peroxidation of membrane lipids and consequently increase membrane permeability to ions and electrolytes. Stability and permeability of membranes can be readily determined by measuring the electrolytes efflux from plant cells. Both drought and salinity may cause membranes damage and enhance their permeability (Bajji et al. 2001, El-Tayeb 2005). Zhu et al. (2004) reported that moderate salinity (50 mmol dm^{-3} NaCl) leads to a rise in H_2O_2 content in cucumber leaves, membrane peroxidation as well as increase in electrolyte efflux. However, silicon added to saline nutrient solution (1 mmol dm^{-3} K_2SiO_3) significantly alleviated these negative effects. Beneficial impact of Si (1 mmol dm^{-3} H_4SiO_4) on membrane fluidity, stability and functioning was also observed in salt-stressed barley (Liang et al. 2005b, Liang et al. 2006b). Similarly, Kaya et al. (2006) reported that silicon might protect cell membranes from the adverse effect induced by drought.

Reactive oxygen species (ROS) react not only with membrane lipids but may also interact non-specifically with other important compounds e.g. photosynthetic pigments, proteins, nucleic acids. For this reason, ROS concentration in plant cells must be precisely controlled and regulated. Antioxidative mechanisms that participate in the regulation and scavenging of ROS include non-enzymatic compounds (ascorbic acid, glutathione, tocopherols, carotenoids) and enzymes (superoxide dismutase, catalase, peroxidases). Efficient cooperating of these protective mechanisms is a crucial feature of plant response to water stress as well as to other stress factors (Allen 1995, Sairam and Saxena 2000). Addition of silicon can enhance activity of antioxidant enzymes and concentration of antioxidant metabolites in plants growing under water stress (Liang 1999, Al.-Aghabary et al. 2004, Zhu et al. 2004, Liang et al. 2006b, Qian et al. 2006). On the other hand, added silicon may improve concentration of chlorophyll and ultrastructure of chloroplasts preventing grannae disintegration under stress conditions (Liang 1998, Kaya et al. 2006, Qian et al. 2006, Tuna et al. 2008).

Numerous reports indicate that silicon improves growth parameters of plants growing under water stress (Zhu et al. 2004, Kaya et al. 2006, Tuna et al. 2008, Zuccarini 2008). In some cases, this beneficial effect was not observed but on the other hand added silicon may improve other processes e.g. water use efficiency (Gao et al. 2004, Hattori at al. 2007) as well as increase plant resistance to pathogens (Fauteux et al. 2005).

In conclusion, it can be stated that the role of Si in plants is not restricted to formation of a physical or mechanical barrier (as precipitated amorphous silica) in cell walls, lumens and intercellular voids. Silicon modulates plants' metabolism and alters physiological activities, particularly in plants subjected to stress conditions. There is a need for further experiments that will allow us to understand better the interactions between silicon application and plant responses. This information will be used in fertilisation practice for enhancing stress tolerance in crop systems.

REFERENCES

AHMAD R., ZAHEER S, ISMAIL S. 1992. *Role of silicon in salt tolerance of wheat (Triticum aestivum L.).* Plant Sci., 85: 43-50.

AL.-AGHABARY, ZHU K., SHI Q.H. 2004. *Influence of silicon supply on chlorophyll content, chlorophyll fluorescence, and antioxidative enzyme activities in tomato under salt stress*. J. Plant Nutr., 27: 2101-2115.

ALLEN R.D. 1995. *Dissection of oxidative stress tolerance using transgenic plants*. Plant Physiol., 107: 1049-1054.

BAJJI M., KINET J-M., LUTTS S. 2002. *The use of the electrolyte leakage method for assessing cell membrane stability as a water stress tolerance test in durum wheat*. Plant Growth Regul., 36: 61-70.

BIRCHALL J.D. 1990. *The role of silicon in biology*. Chem. Brit., 26: 141-144.

BROGOWSKI Z. 2000. *Krzem w glebie i jego rola w żywieniu roślin [Silicon in soil and its role in plant nutrition]*. Post. Nauk Rol., 6: 9-16. [in Polish]

DONCHEVA SN., POSCHENRIEDER C., STOYANOVA ZL., GEORGIEVA K., VELICHKOVA M., BARCELÓ J. 2009. *Silicon amelioration of manganese toxicity in Mn-sensitive and Mn-tolerant maize varieties*. Environ. Exp. Bot., 65: 189-197.

EDWARDSON J.A., MOORE P.B., FERRIER I.N., LILLEY J.S., NEWTON G.W.A., BARKER J., TEMPLAR J., DAY J.P. 1993. *Effect of silicon on gastrointestinal absorption of aluminium*. Lancet., 342: 211-212.

EL-TAYEB M.A. 2005. *Response of barley grains to the interactive effect of salinity and salicylic acid.* Plant Growth Regul., 45: 215-224.

EPSTEIN E. 1994. *The anomaly of silicon in plant biology*. Proc. Natl. Acad. Sci. USA, 91: 11-17.

EPSTEIN E. 1999. *Silicon*. Ann. Rev. Plant Physiol. Plant Mol. Biol., 50: 641- 664.

FAUTEUX F., RÉMUS-BOREL W., MENZIES J.G., BÉLANGER R.R. 2005. *Silicon and plant disease resistance against pathogenic fungi*. FEMS Microbiol. Lett., 249: 1-6.

GAO X., ZOU CH., WANG L., ZHANG F. 2004. *Silicon improves water use efficiency in maize plants*. J. Plant Nutr., 27: 1457-1470.

GAO X., ZOU CH., WANG L., ZHANG F. 2006. *Silicon decreases transpiration rate and conductance from stomata of maize plants*. J. Plant Nutr., 29: 1637-1647.

GRENDA A., SKOWROŃSKA M. 2004. *Nowe trendy w badaniach nad biogeochemią krzemu [New trends in studies on biogeochemistry of silicon].* Zesz. Prob. Post. Nauk Rol., 502: 781--789. [in Polish]

GUNES A., INAL A., BAGCI E.G., COBAN S., SAHIN O. 2007a. *Silicon increases boron tolerance and reduces oxidative damage of wheat grown in soil with excess of boron.* Biol. Plant., 51: 571-574.

GUNES A., INAL A., BAGCI E.G., PILBEAM D.J. 2007b. *Silicon-mediated changes of some physiological and enzymatic parameters symptomatic for oxidative stress in spinach and tomato grown in sodic-B toxic soil*. Plant Soil, 290: 103-114.

HASEGAWA P.M., BRESSAN R.A., ZHU J-K., BOHNERT H.J. 2000. *Plant cellular and molecular responses to high salinity*. Ann. Rev. Plant Physiol. Plant Mol. Biol., 551: 463-499.

HATTORI T., SONOBE K., INANAGA S., AN P., TSUJI W., ARAKI H., ENEJI A.E., MORITA S. 2007. *Short term stomatal responses to light intensity changes and osmotic stress in sorghum seedlings raised with and without silicon*. Environ. Exp. Bot., 60: 177-182.

HENRIET C., DRAYE X., OPPITZ L., SWENNEN R., DELVAUX B. 2006. *Effects, distribution and uptake of silicon in banana (Musa spp.) under controlled conditions*. Plant Soil, 287: 359-374.

HODSON M.J., WHITE P.J., MEAD A., BROADLEY M.R. 2005. *Phylogenetic variation in the silicon composition of plants*. Ann. Bot., 96: 1027-1046.

JONES L.H.P., HANDRECK K.A.1967. *Silica in soils, plants and animals*. Adv. Agron., 19: 107-149.

KAYA C., TUNA L., HIGGS D. 2006. *Effect of silicon on plant growth and mineral nutrition of maize grown under water-stress conditions*. J. Plant Nutr., 29: 1469-1480.

LIANG Y. 1998. *Effects of Si on leaf ultrastructure, chlorophyll content and photosynthetic activity in barley under salt stress*. Podosphere, 8: 289-296.

LIANG Y. 1999. *Effects of silicon on enzyme activity and sodium, potassium and calcium concentration in barley under salt stress*. Plant Soil, 209: 217-224.

LIANG Y., SI J., RÖMHELD V. 2005a. *Silicon uptake and transport is an active process in Cucumis sativus.* New Phytol., 167: 797-804.

LIANG Y., HUA H., ZHU Y-G., ZHANG J., CHENG CH., RÖMHELD V. 2006a. *Importance of plant species and external silicon concentration to active silicon uptake and transport*. New Phytol., 172: 63-72.

LIANG Y., SUN W., ZHU Y-G., CHRISTIE P. 2007. *Mechanisms of silicon alleviation of abiotic stresses in higher plants: A review*. Environ. Pollut., 147: 422-428.

LIANG Y., ZHANG W., CHEN Q., LIU Y., DING R. 2005b. *Effects of silicon on H^+-ATPase and, H^+-PPaes eactivity, fatty acids composition and fluidity of tonoplast vesicles from roots of salt-stressed barley (Hordeum vulgare L.).* Environ. Exp. Bot., 53: 29-37.

LIANG Y., ZHANG W., CHEN Q., LIU Y., DING R. 2006b. *Effect of exogenous silicon (Si) on H^+-ATPase activity, phospholipids and fluidity of plasma membrane in leaves of salt-stressed barley (Hordeum vulgare L.).* Environ. Exp. Bot., 57: 212-219.

MA J.F., HIGASHITANI A., SATO K., TAKEDA K. 2003. *Genotypic variation in silicon concentration of barley grain.* Plant Soil, 249: 383-387.

MA J.F., YAMAJI N. 2006. *Silicon uptake and accumulation in higher plants*. Trends Plant Sci., 11: 392-397.

MITANI N, MA J.F. 2005. *Uptake system of silicon in different plant species*. J. Exp. Bot., 56: 1255-1261.

QIAN Q., ZAI. W., ZHU Z., YU J. 2006. *Effects of exogenous silicon on active oxygen scavenging systems in chloroplasts of cucumber (Cucumis sativus L.) seedlings under salt stress.* J. Plant Physiol. Mol. Biol., 32: 107-112.

ŘEZANKA T., SIGLER K. 2008. *Biologically active compounds of semi-metals*. Phytochem., 69: 585-606.

REDDY A.R., CHIATANYA K.V., VIVEKANANDAN M. 2004. *Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants.* J. Plant Physiol., 161: 1189-1202.

ROMERO-ARANDA M.R., JURADO O., CUARTERO J. 2006. *Silicon alleviates the deleterious salt effect on tomato plant growth by improving plant water status*. J. Plant Physiol., 163: 847-855.

SAIRAM R.K., SAXENA D.C. 2000. *Oxidative stress and antioxidants in wheat genotypes: possible mechanism of water stress tolerance*. J. Agron. Crop Sci., 184: 55-61.

SAVVAS D., GIOTIS D., CHATZIEUSTRATIOU E., BAKEA M., PATAKIOUTAS G. 2009. *Silicon supply in soilless cultivations of zucchini alleviates stress induced by salinity and powdery mildew* infections. Environ. Exp. Bot., 65: 11-17.

SOMMER M., KACZOREK D., KUZYAKOV Y., BREUER J. 2006. *Silicon pools and fluxes in soils and landscapes – a review*. J. Plant Nutr. Soil Sci., 169: 310-329.

TAMAI K., MA J.F. 2003. *Characterization of silicon uptake by rice roots*. New Phytol., 158: 431-436.

TANAKA T. 1985. *Silicon and mammals*. Bull. Tottori Univ., 9: 1-6.

TUNA A.L., KAYA C., HIGGS D., MURILLO-AMADOR B., AYDEMIR S., GIRGIN A.R. 2008. *Silicon improves salinity tolerance in wheat plants*. Environ. Exp. Bot., 62: 10-16.

VORM P.D.J. van der. 1980. *Uptake of Si by five plant species, as influenced by variation in Si-supply*. Plant Soil, 56: 153-156.

WANG Y., STASS A., HORST W. 2004. *Apoplastic binding of aluminium is involved in silicon-induced amelioration of aluminium toxicity in maize*. Plant Physiol., 136: 3762-3770.

YEO A.R., FLOWERS S.A., RAO G., WELFARE K., SENANAYAKE N., FLOWERS T.J. 1999. *Silicon reduces sodium uptake in rice (Oreyza sativa L.) in saline conditions and this is accounted for by a reduction in the transpirational bypass flow*. Plant Cell Environ., 22: 559-565.

ZHU Z., WEI G., LI J., QIAN Q., YU I. 2004. *Silicon alleviates salt stress and increases antioxidant enzymes activity in leaves of salt-stressed of cucumber (Cucumis sativus* L.). Plant Sci., 167: 527-533.

ZUCCARINI P. 2008. *Effects of silicon on photosynthesis, water relations and nutrient uptake of Phaseolus vulgaris under NaCl stress*. Biol. Plant., 52: 157-160.

Reviewers of the Journal of Elementology Vol. 14(3), Y. 2009

Jolanta Bielińska, Anita Biesiada, Maria Brzezińska, Jacek Czekała, Zbigniew Dobrzański, Jan Kłobukowski, Jan Kryszak, Eugeniusz Kołota, Aleksandra Kwiatkowska, Bogusław Makarski, Kazimierz Pasternak, Urszula Prośba-Białczyk, Andrzej Sapek, Zofia Spiak, Lech Walasek, Małgorzata Woźniak

Regulamin ogłaszania prac w „Journal of Elementology"

1. Journal of Elementology (kwartalnik) zamieszcza na swych łamach prace oryginalne, doświadczalne, kliniczne i przeglądowe z zakresu przemian biopierwiastków i dziedzin pokrewnych.
2. W JE mogą być zamieszczone artykuły sponsorowane, przygotowane zgodnie z wymaganiami stawianymi pracom naukowym.
3. W JE zamieszczamy materiały reklamowe.
4. Materiały do wydawnictwa należy przesłać w 2 egzemplarzach. Objętość pracy oryginalnej nie powinna przekraczać 10 stron znormalizowanego maszynopisu (18 000 znaków), a przeglądowej 15 stron (27 000 znaków).
5. Układ pracy w języku angielskim: TYTUŁ PRACY, imię i nazwisko autora (-ów), nazwa jednostki, z której pochodzi praca, streszczenie w języku angielskim i polskim – minimum 250 słów. Streszczenie powinno zawierać: wstęp (krótko), cel badań, metody badań, omówienie wyników, wnioski. Przed streszczeniem w języku angielskim: Abstract (tekst streszczenia), Key words (maks 10 słów). Przed streszczeniem w języku polskim: TYTUŁ PRACY, Abstrakt, (tekst streszczenia), Słowa kluczowe: (maks, 10 słów). WSTĘP, MATERIAŁ I METODY, WYNIKI I ICH OMÓWIENIE, WNIOSKI, PIŚMIENNICTWO, U dołu pierwszej strony należy podać tytuł naukowy lub zawodowy, imię i nazwisko autora oraz dokładny adres przeznaczony do korespondencji w języku angielskim.
6. Praca powinna być przygotowana wg zasad pisowni polskiej. Jednostki miar należy podawać wg układu SI np.: mmol(+) kg^{-1}; kg ha^{-1}; mol dm^{-3}; g kg^{-1}; mg kg^{-1} (obowiązują formy pierwiastkowe).
7. W przypadku stosowania skrótu po raz pierwszy, należy podać go w nawiasie po pełnej nazwie.
8. Tabele i rysunki należy załączyć w oddzielnych plikach. U góry, po prawej stronie tabeli należy napisać Tabela i numer cyfrą arabską, również w języku angielskim, następnie tytuł tabeli w języku polskim i angielskim wyrównany do środka akapitu. Ewentualne objaśnienia pod tabelą oraz opisy tabel winny być podane w języku polskim i angielskim. Wartości liczbowe powinny być podane jako zapis złożony z 5 znaków pisarskich (np. 346,5; 46,53; 6,534; 0,653).
9. U dołu rysunku, po lewej stronie należy napisać Rys. i numer cyfrą arabską oraz umieścić podpisy i ewentualne objaśnienia w języku polskim i angielskim.
10. Piśmiennictwo należy uszeregować alfabetycznie, bez numerowania, w układzie: NAZWISKO INICJAŁ IMIENIA (KAPITALIKI), rok wydania. Tytuł pracy (kursywa). Obowiązujący skrót czasopisma, tom (zeszyt): strony od-do. np. KOWALSKA A., KOWALSKI J. 2002. *Zawartość magnezu w ziemniakach*. Przem. Spoż., 7(3): 23-27. Tytuły publikacji wyłącznie w języku angielskim z podaniem oryginalnego języka publikacji, np. (in Polish).
11. W JE można także cytować prace zamieszczone w czasopismach elektronicznych wg schematu: NAZWISKO INICJAŁ IMIENIA (KAPITALIKI), rok wydania. Tytuł pracy (kursywa). Obowiązujący skrót czasopisma internetowego oraz pełny adres strony internetowej. np. ANTONKIEWICZ J., JASIEWICZ C. 2002. *The use of plants accumulating heavy metals for detoxication of chemically polluted soils.* Electr. J. Pol. Agric. Univ., 5(1): 1-13. hyperlink "http:/www" http://www.ejpau.media.pl/series/volume5/issue1/environment/art-01.html
12. Cytując piśmiennictwo w tekście, podajemy w nawiasie nazwisko autora i rok wydania pracy (KOWALSKI 1992). W przypadku cytowania dwóch autorów, piszemy ich nazwiska rozdzielone przecinkiem i rok (KOWALSKI, KOWALSKA 1993). Jeżeli występuje większa liczba nazwisk, podajemy pierwszego autora z dodatkiem i in., np.: (KOWALSKI i in. 1994). Cytując jednocześnie kilka pozycji, należy je uszeregować od najstarszej do najnowszej, np.: (NOWAK 1978, NOWAK i in. 1990, NOWAK, KOWALSKA 2001).

13. Do artykułu należy dołączyć pismo przewodnie Kierownika Zakładu z jego zgodą na druk oraz oświadczenie Autora (-ów), że praca nie została i nie zostanie opublikowana w innym czasopiśmie bez zgody Redakcji JE.
14. Dwie kopie wydruku komputerowego pracy (Times New Roman 12 pkt. przy odstępie akapitu 1,5 - bez dyskietki) należy przesłać na adres Sekretarzy Redakcji:

dr hab. Jadwiga Wierzbowska
Uniwersytet Warmińsko-Mazurski w Olsztynie
Katedra Chemii Rolnej i Ochrony Środowiska
ul. Oczapowskiego 8, 10-744 Olsztyn-Kortowo
jadwiga.wierzbowska@uwm.edu.pl

dr Katarzyna Glińska-Lewczuk
University of Warmia and Mazury in Olsztyn
Pl. Łódzki 2, 10-759 Olsztyn, Poland
kaga@uwm.edu.pl

15. Redakcja zastrzega sobie prawo dokonania poprawek i skrótów. Wszelkie zasadnicze zmiany tekstu będą uzgadniane z Autorami.
16. Po recenzji Autor zobowiązany jest przesłać w 2 egzemplarzach poprawiony artykuł wraz z nośnikiem elektronicznym (dyskietka, CD lub e-mailem), przygotowany w dowolnym edytorze tekstu, pracującym w środowisku Windows.

Redakcja Journal of Elementology uprzejmie informuje:

Koszt wydrukowania maszynopisu (wraz z rysunkami, fotografiami i tabelami) o objętości nieprzekraczającej 6 stron formatu A4, sporządzonego wg następujących zasad:

- czcionka: Times New Roman, 12 pkt, odstęp 1,5;
- 34 wiersze na 1 stronie;
- ok. 2400 znaków (bez spacji) na 1 stronie;
- rysunki i fotografie czarno-białe;

wynosi 250 PLN + VAT.

Koszt druku każdej dodatkowej strony (wraz z rysunkami, fotografiami i tabelami) wynosi 35 PLN + VAT.

Koszt druku 1 rysunku lub fotografii w kolorze wynosi 150 PLN + VAT.

Uwaga:

Z opłaty za druk pracy zwolnieni zostaną lekarze niezatrudnieni w instytutach naukowych, wyższych uczelniach i innych placówkach badawczych.

Komitet Redakcyjny

Warunki prenumeraty czasopisma

Członkowie indywidualni PTMag - 40 PLN + 0% VAT rocznie

Osoby fizyczne 50 PLN + 0% VAT rocznie

Biblioteki i instytucje 150 PLN + 0% VAT rocznie za 1 komplet (4) egzemplarze + 10 PLN za przesyłkę

Wpłaty prosimy kierować na konto UWM w Olsztynie:
PKO S.A. I O/Olsztyn, 32124015901111000014525618
koniecznie z dopiskiem "841-2202-1121"

Guidelines for Authors „Journal of Elementology"

1. Journal of Elementology (a quarterly) publishes original scientific or clinical research as well as reviews concerning bioelements and related issues.
2. Journal of Elementology can publish sponsored articles, compliant with the criteria binding scientific papers.
3. Journal of Elementology publishes advertisements.
4. Each article should be submitted in duplicate. An original paper should not exceed 10 standard pages (18 000 signs). A review paper should not exceed 15 pages (27 000 signs).
5. The paper should be laid out as follows: **TITLE OF THE ARTICLE**, ***name and surname of the author(s)***, **the name of the scientific entity, from which the paper originates**, **INTRODUCTION**, **MAETRIAL AND METHODS**, **RESULTS AND DISCUSSION**, **CONCLUSIONS**, **REFERENCES**, abstract in the English and Polish languages, min. 250 words. Summary should contain: introduction (shortly), aim, results and conclusions. Prior to the abstract in the English language the following should be given: ***name and surname of the author(s)***, **TITLE,** Key words (max 10 words), Abstract, **TITLE**, Key words and Abstract in Polish. At the bottom of page one the following should be given: scientific or professional title of the author, name and surname of the author, detailed address for correspondence in the English and Polish languages.
6. The paper should be prepared according to the linguistic norms of the Polish and English language. Units of measurements should be given in the SI units, for example $mmol(+)\cdot kg^{-1}$; $kg\cdot ha^{-1}$; $mol\cdot dm^{-3}$; $g\cdot kg^{-1}$; $mg\cdot kg^{-1}$ (elemental forms should be used).
7. In the event of using an abbreviation, it should first be given in brackets after the full name.
8. Tables and figures should be attached as separate files. At the top, to the right of a table the following should be written: Table and table number in Arabic figures (in English and Polish), in the next lines the title of the table in English and Polish adjusted to the centre of the paragraph. Any possible explanation of the designations placed under the table as well as a description of the table should be given in English and Polish. Numerical values should consist of five signs (e.g. 346.5, 46.53, 6.534, 0.653).
9. Under a figure, on the left-hand side, the following should be written: Fig. and number in Arabic figures, description and possible explanation in Polish and English.
10. References should be ordered alphabetically but not numbered. They should be formatted as follows: Surname First Name Initial (capital letter) year of publication, Title of the paper (italics). The official abbreviated title of the journal, volume (issue): pages from – to. e.g. KOWALSKA A., KOWALSKI J. 2002. *Zawartość magnezu w ziemniakach.* Przem. Spoż., 7(3): 23-27.
11. It is allowed to cite papers published in electronic journals formatted as follows: Surname First Name Initial (capital letters) year of publication. Title of the paper (italics). The official abbreviated title of the electronic journal and full address of the website. e.g. ANTONKIEWICZ J., JASIEWICZ C. 2002. *The use of plants accumulating heavy metals for detoxication of chemically polluted soils*. Electr. J. Pol. Agric. Univ., 5(1): 1-13. hyperlink „http://www.ejpau.pl/series/volume5/issue1/environment/art-01.html" http://www.ejpau.pl/series/volume5/issue1/environment/art-01.html
12. In the text of the paper a reference should be quoted as follows: the author's name and year of publication in brackets, e.g. (KOWALSKI 1992). When citing two authors, their surnames should be separated with a comma, e.g. (KOWALSKI, KOWALSKA 1993). If there are more than two authors, the first author's name should be given followed by et al., e.g. (KOWALSKI et al. 1994). When citing several papers, these should be ordered chronologically from the oldest to the most recent one, e.g. (NOWAK 1978, NOWAK et al. 1990, NOWAK, KOWALSKA 2001).

13. A paper submitted for publication should be accompanied by a cover letter from the head of the respective institute who agrees for the publication of the paper and a statement by the author(s) confirming that the paper has not been and will not be published elsewhere without consent of the Editors of the Journal of Elementology.
14. Two computer printed copies of the manuscript (Times New Roman 12 fonts, 1.5-spaced, without a diskette) should be submitted to the Editor's Secretary:

dr hab. Jadwiga Wierzbowska
University of Warmia and Mazury in Olsztyn
ul. Michała Oczapowskiego 8, 10-719 Olsztyn
jawierz@uwm.edu.pl

dr Katarzyna Glińska-Lewczuk
University of Warmia and Mazury in Olsztyn
pl. Łódzki 2, 10-759 Olsztyn, Poland
kaga@uwm.edu.pl

15. The Editors reserve the right to correct and shorten the paper. Any major changes in the text will be discussed with the Author(s).
16. After the paper has been reviewed and accepted for publication, the Author is obliged to sent the corrected version of the article together with the diskette. The electronic version can be prepared in any word editor which is compatible with Windows software.