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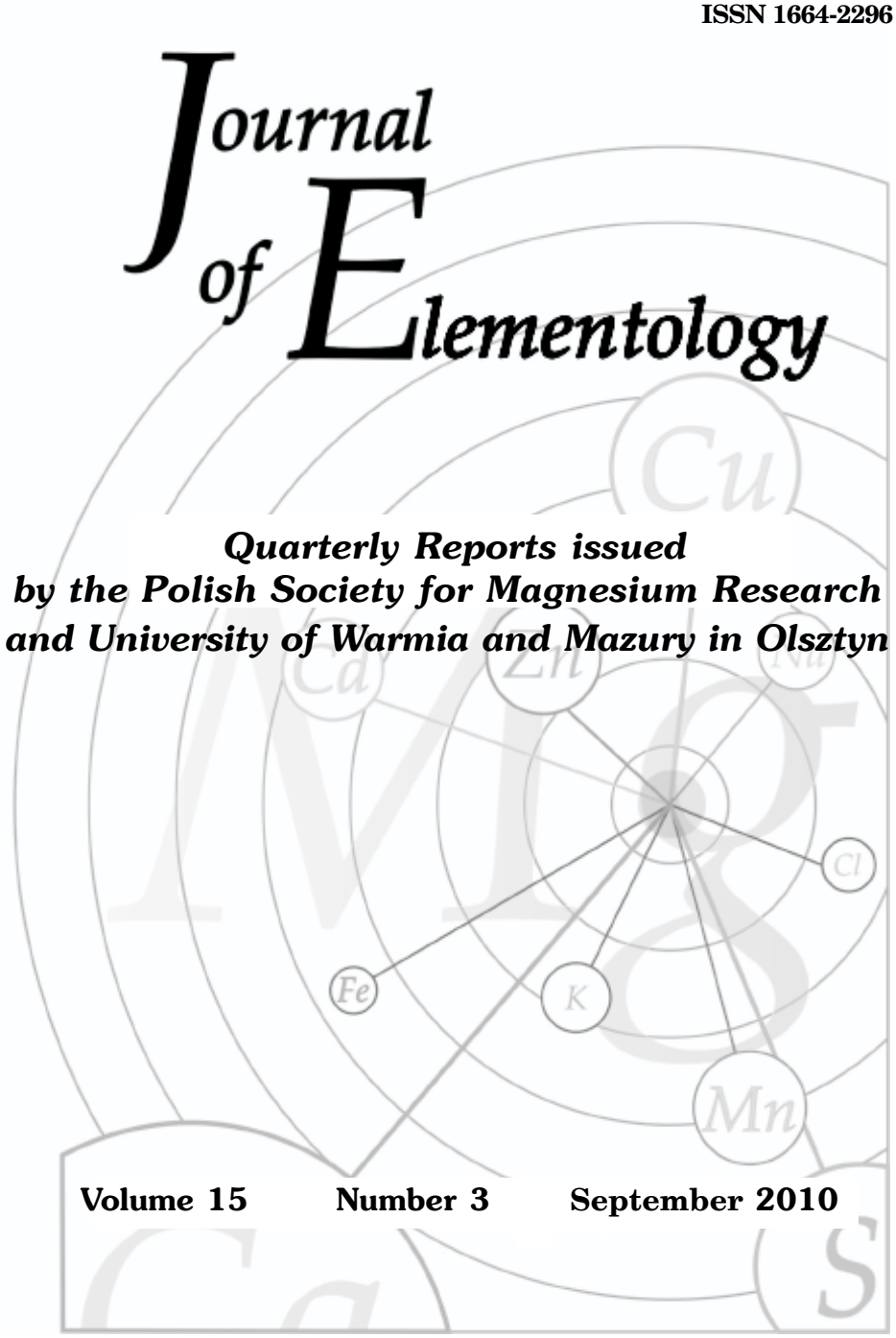

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ORIGINAL PAPERS

DIAGNOSTIC VALUES OF CALCIUM AND MAGNESIUM FORMS DETERMINED IN HUMAN SERUM AND SALIVA

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Institute for Occupational Health of AMS of Ukraine

Abstract

Calcium and magnesium are known to be necessary for the normal function of various systems in animal and human organisms. There are many diseases caused by abnormal concentration of electrolytes, e.g. arterial hypertension or nervous system diseases such as multiple sclerosis, Mb. Alzheimer or Mb. Parkinson. The mechanisms of homeostasis indicate only the ionized forms of these elements. It is known that ionized calcium serves as an endocellular intermediary in action of enzymes and hormones in cells.

Therefore, it is very important to define levels of total and ionized forms of Ca^{2+} and Mg^{2+} in blood serum and saliva by the method of atomic absorption spectrometry and to show their diagnostic value for various pathological conditions of a human body. The 39 persons, aged 21 to 47 years take part in these investigations.

The results of determinations of calcium and magnesium forms present in human serum and saliva, representing physiological states are presented. The age and daily fluctuations of Ca^{2+} and Mg^{2+} content in serum and saliva were studied by atomic absorption spectrometry. The levels of non albumin forms of these elements were found by FAAS. The significance of determination of calcium and magnesium levels in serum and saliva under various pathological conditions (arterial hypertension and osteoporosis) was shown.

Key words: calcium, magnesium, serum, saliva, forms of elements, atomic absorption spectrometry.

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WARTOŚĆ DIAGNOSTYCZNA FORM WAPNIA I MAGNEZU OZNACZONYCH W LUDZKIEJ SUROWICY KRWI I ŚLINIE

Abstrakt

Wapń i magnez to pierwiastki niezbędne do prawidłowego funkcjonowania organizmów zwierzęcych i ludzkich. Istnieje wiele chorób wywoływanych przez nieprawidłowe stężenia elektrolitów, np. nadciśnienie tętnicze lub choroby układu nerwowego, takie jak SM, choroba Alzheimera lub Parkinsona. Mechanizmy homeostazy wskazują jedynie na zjonizowane formy tych pierwiastków. Wiadomo, że zjonizowana forma wapnia służy jako międzykomórkowy pośrednik między enzymami i hormonami w komórkach. Dlatego tak ważne jest określenie zawartości całkowitych tych pierwiastków oraz jonów Ca^{2+} i Mg^{2+} w surowicy krwi oraz w ślinie za pomocą metody absorpcyjnej spektrometrii atomowej oraz wykazanie ich roli w diagnostyce różnych stanów patologicznych w organizmie człowieka. Badaniami objęto 39 osób w wieku od 21 do 47 lat.

W pracy przedstawiono wyniki oznaczeń form wapnia i magnezu występujących w ludzkiej surowicy krwi oraz ślinie odpowiadające stanom fizjologicznym. Wahania w zawartości Ca^{2+} i Mg^{2+} w surowicy i ślinie, w zależności od wieku i pory dnia, badano za pomocą aborpcyjnej spektrofotometrii atomowej. Zawartości niealbuminowych form tych pierwiastków określono za pomocą FAAS. Wykazano istotność oznaczeń zawartości wapnia i magnezu dla różnych stanów chorobowych (nadciśnienie tętnicze, osteoporoza).

Słowa kluczowe: wapń, magnez, surowica, ślina, formy pierwiastków, absorpcyjna spekrofotometria atomowa.

INTRODUCTION

The biological role of calcium and magnesium ions in vital activities of various organisms and their influence on development of pathological processes are well known (PARFENOV 1977, BAZARNOVA 1990, KONOVALOVA 2002). Calcium and magnesium participate in many processes, which sustain vital activities of a living organism. Calcium takes part in reactions of neuromuscular transmission of impulses, renders positive inotropic effect on the cardiac muscle activity, provides the control and activation of hormones and neurotransmitters, participates in blood coagulation, in the metabolism of osseous tissues. Magnesium is a physiological antagonist of calcium. Magnesium ions is an important link of neuromuscular conductivity, make depressive impact on the central nervous system, participate in heart beat and have a vasodilatation effect as a fibrinolisis promoter. Magnesium plays an important role in establishing immunity (TRAKHTENBERG 2006, KUDRIN 2006).

Mechanisms of homeostasis indicate only the ionized forms of these elements. It is known that ionized calcium serves as an endocellular intermediary of action of enzymes and hormones in cells. Ionized calcium with calmodulin influence functions of many structural elements in the cell. The role of ionized calcium as a mediator of actions of antidiuretic, adrenocorticotropic and other hormones is also important. The behaviour of ionized

forms of this macroelement in blood plasma (serum) and saliva is of interest to researchers, who study many physiological and pathological states (Jarmagomedov et al. 1978, Radishevska 1998, Zabolotski, Kulev 2007). In clinical diagnostics, determination of ionized calcium in blood plasma is useful for surgical interventions in the cardiovascular system, in hypertension, anomalies of pregnancy and diseases of the inflammatory genesis (Loskutova 2004, Kisters et al. 2005, Vereschagin et al. 2006, Typiakova 2006, Makara-Studzinska et al. 2008).

Traditionally, general behaviour of calcium and magnesium in blood serum and saliva is analyzed by photometric, potentiometric and atomic-absorption spectrometry (AAS) methods (Signifoli et al. 1989, Konovalova 2002, Kudrin, Gromova 2006, Kovalchuk et al. 2007). Concentration of ionized forms of calcium and magnesium is most often determined via potentiometry or using ion-selective electrodes (Jarmagomedov et al. 1978, Titov 1995, Typiakova 2006, Melnichenko 2008). A drawback of the latter method is the need to secure an acid-alkaline balance test, which requires a large volume of a sample (5 ml of serum for testing). Also, ion-selective membranes of electrodes are not resistant to microflora action or specific sedimentation of proteins and there are other technical defects.

In view of the above, it has been decided to assess the usefulness of the AAS method for determination of calcium and magensium forms in blood serum and magnesium. Therefore, the aim of this study was to determine levels of total and ionized forms of Ca^{2+} and Mg^{2+} in serum and saliva by the method of atomic absorption spectrometry, and to show the diagnostic importance of such determination for various pathological states of the human body.

MATERIAL AND METHODS

This investigation included 39 persons, aged 21 to 47 years. The control group covered healthy volunteers (12 patients). Experimental groups consisted of patients of the Institute Clinic, who had been diagnosed to suffer from arterial hypertension (14 patients) and osteoporosis (13 patients).

Analyses of serum and saliva were performed using the standard method of selection for a given fluid (Signifoli et al. 1989, Bazarnova 1990, Radishevska 1998). The content of Ca^{2+} and Mg^{2+} forms in serum and saliva were determined by the flame atomic absorption spectrometry (FAAS). The total content of calcium and magnesium in the investigated substrates was determined by a generally accepted method for preparation of samples (Signifoli et al. 1989, Titov 1995). The determination of non-albumin forms of metals was conducted after stabilization of proteins in serum and saliva with 5% isotonic glutaraldehyde solution (pH 7.4) (Volkova 1987). Then, the proteins were mixed with 10% nitric acid solution and the samples were

cultured with 0.1 % solution lanthanum chloride (1:10 and 1:5, respectively) (ANDRUSISHINA 2007). The results were processed statistically, using the software program Statistica ver.6. The statistical importance of intergroup differences was estimated by the Student's-test (ANTOMONOV 2006).

RESULTS AND DISCUSSION

Content of the total and ionized forms of Ca^{2+} and Mg^{2+} in serum and saliva of healthy persons (the control) is presented in Table 1.

The fluctuations in the total content of macroelements did not differ

Table 1

Content of the total and ionized forms of calcium and magnesium in serum and saliva of the control group

Biological material	Forms of chemical elements	Ranges of concentration of calcium (mmol dm^{-3})	Ranges of concentration of magnesium (mmol dm^{-3})
Blood serum	total	1.95-2.25	0.85-1.02
	ionized	0.96-1.20	0.43-0.55
Saliva	total	0.67-1.33	0.70-1.67
	ionized	0.48-0.62	0.45-0.71

from the published data (PARFENOV 1977, BAZARNOVA 1990, KUDRIN, GROMOVA 2006). The non-significant differences between the behaviour of the total forms of electrolytes in blood serum and saliva were due to the active function of salivary glands and albumin structure (ZAVIALOVA 1998, KOWALCZUK et al. 2007, NACHAROV et al. 2007). Identical percentages of the ionized forms of metals in the investigated substrates are found (50% for ionized calcium and 70% for ionized magnesium), which is in agreement with the data published in literature (JARMAGOMEDOV et al. 1998, LOSKUTOVA 2004, KUDRIN, GROMOVA 2006, TYPIAKOVA 2006). That confirms the suitability of the technique, used for preparation of samples for the FAAS determinations of the ionized forms of electrolytes.

It is known that the content of elements in serum and saliva fluctuates, depending on the time of the day, the subject's age, pH value, functional activity of the vegetative nervous system, as well as on individual daily rhythm („owls" and „early birds").

Therefore, determination of electrolytes in serum and saliva depending on the functional state of the body is important for recognition of their daily dynamics. The results are presented in Figures 1 and 2. The highest level

of Ca^{2+} in serum registered at 9.00 p.m. and in saliva – at 3.00 p.m. The lowest calcium level in serum was at 7.00 a.m. and in saliva at 11.00 p.m. At the same time, the maximum Mg^{2+} differed from Ca^{2+}, both in serum and in saliva. The highest level of Mg^{2+} in blood serum appeared at 6.00 p.m. and in saliva – at 9.00 p.m. The lowest content of that ion in serum and saliva was at 7.00 a.m. Thus, the daily changes of electrolytes, as revealed in this study, can be compared to the data reported by other authors (PARFENOV 1977, MELNICHENKO 2008), who found that release of electrolytes in the afternoon was higher than at night. This regularity is attributable to the activity of the sympathetic and parasympathetic vegetative nervous systems.

It is important that the release of Ca^{2+} and Mg^{2+} at 7:00 in the morning into biosubstrates is minimal, the fact that has to be remembered about during daily monitoring of electrolytes in human fluids. Nevertheless, the use of saliva for estimation of the body functional state has certain advantages over blood serum in that it is an adequate, noninvasive substratum.

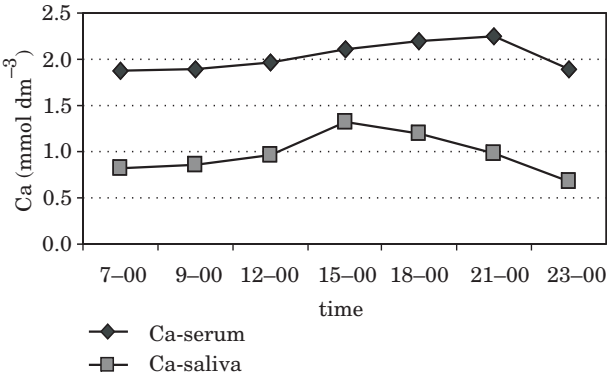

Fig. 1. Dynamics of daily fluctuations of the total calcium in human biosubstrates

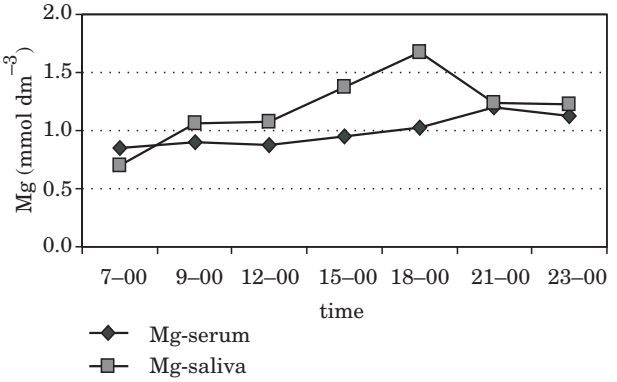

Fig. 2. Dynamics of daily fluctuations of the total magnesium in human biosubstrates

It was of interest to show the informative importance of the method of sample preparation for AAS determinations of ionized forms of Ca^{2+} and Mg^{2+}, described in this paper, for diagnosing some pathological states.

It has been demonstrated that high arterial pressure caused the decreased concentration of Mg^{2+} in cardiocytes and blood serum, whereas Ca^{2+} under such conditions either increases or remains unchanged (KISTERS et al. 2005, VERESCHAGIN et al. 2006). Normal levels of Ca^{2+} in serum are often maintained by Ca, liberated from the depot (bones), thus preventing its decrease in blood serum (BAZARNOVA 1990, ZABOLOTSKI 2007). The amount of Mg^{2+} in the heart makes up 1/5 of all magnesium in the human body. It is one of the active regulators of vascular tone; it tones the heart and causes vasodilatation. Researchers have demonstrated that 90 % of patients with myocardium heart attack have Mg^{2+} deficiency (TITOV 1995, HUNTER 2005). Therefore, it is important to determine levels of the total and ionized Ca^{2+} and Mg^{2+} in serum and saliva of patients with arterial hypertensia (AH) by the AAS method. The results are presented in Table 2.

Table 2

Content of total and ionized forms of calcium and magnesium in serum and saliva of patients with arterial hypertensia

Biological material	Treatment group	Forms of chemical elements	Calcium (mmol dm^{-3})	% of total content	Magnesium (mmol dm^{-3})	% of total content
Serum blood	control	total	1.95 ±0.07		1.02±0.02	
		ionized	0.98 ±0.11	50.26	0.77±0.07	75.49
	experiment	total	2.26±0.06*		0.74±0.02*	
		ionized	1.26±0.06*	55.75	0.50±0.02*	67.57
Saliwa	control	total	1.03 ±0.13		1.20± 0.17	
		ionized	0.51± 0.07	49.51	0.90 ±0.13	75
	experiment	total	1.30± 0.21*		0.60±0.02*	
		ionized	0.60± 0.06	46.15	0.34 ±0.13*	56.67

The growth of the total Ca^{2+} concentration in serum of patients with AH was revealed in 14.15% of cases. Thus, the ionized form of the metal did not change its level. The total Mg^{2+} in serum of patients with AH decreased by 31.49% as compared to the control group. The ionized Mg^{2+} level in serum increased by 14.51%. The imbalance between the total and ionized Ca^{2+} and Mg^{2+} in serum of patients with AH indicates the physiological antagonism between these macroelements. The increase in the ionized Mg^{2+} could be some evidence of genetic predisposition to this disease (KISTERS et al. 2005, KUDRIN, GROMOVA 2006, VERESCHAGIN et al. 2007).

At the same time, levels of the total and ionized forms of Mg^{2+} decreases by 47.86% and 37.93%, respectively. The difference between fluctuations in the forms Ca^{2+} and Mg^{2+} are the evidence that disorders of the vegetative nervous system can be accompanied by disturbances in the power exchange as well as by changes in the transmembrane ionic transport, mostly of magnesium (KISTERS et al. 2005, HUNTER 2005).

Osteoporosis is a disease which is characterised by severe destruction of organic and mineral parts of the bone tissue and by calcium loss. Osteoporosis is primary depends on the patient's age and depressed production of sex hormones. In the second, osteoporosis is caused by thyroid and parathyroid gland disfunction (calcytonin and parathyroid hormones), xenobiotics and other factors (TYPIAKOVA 2006, NACHAROV et al. 2007). Thus, in practice determination of the total Ca^{2+} and Mg^{2+} is not always effective in clinical diagnostics of the disease (KISTERS et al. 2005, MELNICHENKO 2008). Therefore, it is often necessary to monitor ionized Ca^{2+} in serum, which makes the diagnosis more complicated. Taking into account that the ratios of Ca^{2+} and Mg^{2+} in serum and saliva are identical, determination of the ionized forms of these elements in saliva can be acceptable.

It was of interest to study the suitability of the sample preparation method, discussed in this paper, for the assessment of the behaviour of the total and ionized Ca^{2+} and Mg^{2+} in patients with osteoporosis. The results are presented in Table 3. The increased total Ca^{2+} in serum appeared in 24.10% of cases; the level of ionized Ca^{2+} did not change in comparison with the control, but its share decreased by 6.46 %. In saliva of patients with osteoporosis the level of the total Ca^{2+} increased by 41.75% in comparison with the control. Thus, the level of ionized Ca^{2+} did not change considerably, although its share fell by 7.78%. The behaviour of the total Mg^{2+} changed

Table 3

Content of the total and ionized forms of calcium and magnesium in serum and saliva of patients with osteoporosis

Biological material	Treatment group	Forms of chemical elements	Calcium (mmol dm^{-3})	% of total content	Magnesium (mmol dm^{-3})	% of total content
Blood serum	control	total	1.95±0.07		1.02±0.02	
		ionized	0.98±0.11	50.26	0.77±0.07	75.49
	experiment	total	2.42±0.06*		0.74±0.02*	
		ionized	1.06±0.06	43.80	0.60±0.02*	81.08
Saliva	control	total	1.03±0.13		1.20±0.17	
		ionized	0.51±0.07	49.51	0.90±0.13	75
	experiment	total	1.46±0.20*		1.31±0.02	
		ionized	0.61±0.06	41.78	0.75±0.02*	57.25

only in blood serum, where its level decreased by 27.45%, relative to the control. Levels of ionized Mg^{2+} in serum and saliva changed differently. In blood serum, the level of ionized Mg^{2+} decreased by 22.08 %, and in saliva it increased by 16.67%. Thus, the percentages of the metals in both environments also decreased by 5.59% (in serum) and increased by 17.75% (in saliva). The results evidence the surplus of the total form of calcium and shortage of its ionized form, which can intensify physiological action of calcytonins, causing destruction of bone tissues (Zavialov, Kaxri 1998, Kudrin, Gromova 2006, Nacharov et al. 2007). Different changes in Mg^{2+} forms confirm the general deficiency of magnesium in patients with osteoporosis.

The results prove that the determined concentration of the total and ionized forms of Ca^{2+} and Mg^{2+} in the analyzed biological environments are in accordance with the "conditioned norm," in literature (Konovalova 2002, Trakhtenberg 2006). The method of preparation of blood serum and saliva samples for AAS determination of ionized Ca^{2+} and Mg^{2+} can be used as an alternative to the existing methods, used for diagnosing some diseases which are characterized by changes electrolytes, found in blood serum and saliva. Also, determination of Ca^{2+} and Mg^{2+} in saliva has certain advantages in clinical and epidemiological practice when it is necessary to sample a high volume of biomaterial and to monitor patient's health.

CONCLUSIONS

1. The deviations from the optimum level of the macroelement forms in serum and saliva in the control group testify that the method for sample preparation for the AAS determination of calcium and magnesium fractions gives results, which agree with the relevant literature data.

2. The differences in the contcentrations of Ca^{2+} and Mg^{2+} fractions in serum of patients with arterial hypertensia and osteoporosis, found in this study, confirm their diagnostic importance for evaluation of these diseases.

3. Determination of calcium and magnesium fractions in saliva gives certain advantages in clinical and epidemiological practice when it is necessary to obtain a higher volume of biomaterial and to carry out a long-term monitoring of the health status of patients.

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EFFECT OF SEWAGE SLUDGE AND FURNACE WASTE ON THE CONTENT OF SELECTED ELEMENTS IN THE SWARD OF LEGUME-GRASS MIXTURE

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Abstract

Industrial landfills resulting from operations of various industries, particularly power generation, create specific habitat conditions for flora. Among the waste which may be used for recultivation of dumping heaps there is sewage sludge, which contains a considerable amount of nutrients crucial for plants. This investigation aimed at identification of the effect of sewage sludge and furnace ashes on the content of selected elements in a mixture of grass and white clover recommended for furnace ash heap recultivation.

The experimental design comprised 6 treatments (each in four replications), which differed in a dose of the supplied sewage sludge and furnace ashes: 1) control (without waste admixture), 2) 200 t d.m. of sewage sludge, 3) 200 t d.m. of ash, 4) 150 t d.m. of sludge + 50 t d.m. of ash, 5) 50 t d.m. of ash + 150 t d.m. of ash, 6) 100 t d.m. of ash + 100 t d.m. of ash. The content of macroelements in plants depended on the treatment and ranged from 2.58-31.2 g Mg, 3.16-5.85 g Ca, 16.95-18.46 g K, 0.26-1.25 g Na and 2.27-3.37 g P kg^{-1}d.m. Plants grown exclusively on furnace ashes had the highest content of Mg, Ca and K, whereas the highest P and Na concentrations were noted in plants cultivated exclusively on sewage sludge.

While assessing the content of macroelements in the plant mixture in view of its fodder value, it was found that the content of Mg and K met the standards set for good quality feeds, the amounts of Ca and Na were below the optimum, whereas the P concentration was close to the optimum value.

Key words: sewage sludge, ash, Mg, Ca, K, Na, P, grass mixtures.

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WPŁYW OSADÓW ŚCIEKOWYCH I ODPADÓW PALENISKOWYCH NA ZAWARTOŚĆ WYBRANYCH PIERWIASTKÓW W RUNI MIESZANKI MOTYLKOWO-TRAWIASTEJ

Abstrakt

Składowiska poprzemysłowe będące efektem działalności różnych gałęzi przemysłu, zwłaszcza energetycznego, stanowią specyficzne warunki siedliskowe dla flory. Odpadem, który może być wykorzystany w rekultywacji hałd, może być osad ściekowy zawierający znaczną ilość składników pokarmowych niezbędnych dla roślin. Celem badań było poznanie wpływu osadów ściekowych i popiołów paleniskowych na zawartość wybranych pierwiastków w mieszance traw z koniczyną białą polecaną do rekultywacji hałd odpadów paleniskowych.

Schemat doświadczenia obejmował 6 obiektów (każdy w czterech powtórzeniach), różniących się dawką wprowadzonych osadów ściekowych i popiołów paleniskowych: 1) obiekt kontrolny (bez dodatku odpadów); 2) 200 t s.m. osadu ściekowego; 3) 200 t s.m. popiołu; 4) 150 t s.m. osadu + 50 t s.m. popiołu; 5) 50 t s.m. osadu + 150 t s.m. popiołu; 6) 100 t s.m. osadu + 100 t s.m. popiołu. Zawartość makroelementów w roślinach była uzależniona od obiektu i wynosiła: 2,58-3,12 g Mg, 3,16-5,85 g Ca, 16,95-18,46 g K, 026-1,25 g Na, 2,27--3,37 g P kg^{-1} s.m. Najwyższą zawartość Mg, Ca i K stwierdzono u roślin uprawianych wyłącznie na popiele paleniskowym, natomiast najwyższą zawartość P i Na – u roślin uprawianych wyłącznie na osadzie ściekowym.

Oceniając zawartość makroelementów w mieszance pod względem wartości paszowej, stwierdzono, że zawartość Mg i K w mieszance roślin odpowiadała normom stawianym paszom dobrej jakości. Zawartość Ca i Na w roślinach kształtowała się poniżej wartości optymalnej, a zawartość P w roślinach była zbliżona do wartości optymalnej.

Słowa kluczowe: osady komunalne, popiół, Mg, Ca, K, Na, P, mieszanka traw.

INTRODUCTION

Industrial waste landfills resulting from operations of various industries, particularly power generation, create specific habitat conditions for flora (Cabała, Jarząbek 1999, Gos 1999), including such unique features as the lack of humus and high alkalinity (pH 8.1-12.5), which make it difficult for plants to take up nutrients (Maciak et al. 1976a,b). Ashes contain considerable amounts of Ca, Mg, Fe, Mn, B or Na and smaller quantities of P and K whereas nitrogen occurs in heterocyclic compounds unavailable to plants. Ashes also contain remains of unburnt coal (Bereśniewicz, Nowosielski 1977). Because of these specific properties, heaps of furnace ash must be managed immediately. Various recultivation measures are undertaken to facilitate biological management of landfills (Gilewska, Przybyła 2001). During initial recultivation of an ash landfill, it is recommended to fertilize the top layer of the heap and mix it with ashes. Municipal sewage sludge containing large amounts of nutrients crucial for plants may be the type of waste used for heap recultivation (Czekała 1999, Mazur 1996). Sewage sludge used for biological recultivation of furnace waste heaps will solve the problem of their

management and at the same time will allow plants used for the recultivation to utilize the applied nutrients (KALEMBASA et al. 1987). However, it should be mentioned that beside many valuable macroelements, sewage sludge may also contain high concentrations of heavy metals, which disqualifies it as a substance useful for soilless area recultivation (ANTONKIEWICZ 2008, KALEMBASA, KALEMBASA 1997).

The research aimed at identification of the effect of sewage sludge and furnace ashes on the content of selected elements in the sward of a legume-grass mixture.

MATERIAL AND METHODS

The research was conducted in 2002-2005 as a field experiment located at the EMPOŚ Municipal and Industrial Sewage Treatment Plant in Oświęcim. A one-factor field experiment was conducted in a randomized block design on 8 m^2 plots. Sewage sludge and furnace ashes were tested in the field experiment, in which the experimental design comprised 6 treatments (each in four replications) different in a dose of the applied sewage sludge and furnace ashes (Table 1). Ash, sludge and their mixtures were applied once on the soil surface (without ploughing), two weeks prior to sowing a plant mixture.

Table 1

Design of the field experiment

No object	Combination	Doses (t ha^{-1} d.m.)	
		sludge	ash
1	control	-	-
2	sludge	200	-
3	ash	-	200
4	3/4 sludge+1/4 ash	150	50
5	1/4 sludge+3/4 ash	50	150
6	sludge + ash 1:1	100	100

Field experiment I was set up on soil of the grain size distribution of medium loam. The topsoil (0-20 cm) contained 42% sand, 7% coarse silt, 10% fine silt, 11% coarse silty clay, 12% fine silty clay and 18% colloidal clay. The soil on which the field experiment was set up had neutral pH (pH_{KCl}), whereas furnace ash pH was alkaline and municipal sewage sludge had a slightly acidic reaction (Table 2). The soil contained 8.24 g C and 0.67 g N kg^{-1}d.m. Organic carbon and total nitrogen content assessed in the

sewage sludge was respectively over 26- and 41-fold higher than in the soil, as well as over three- and two-fold higher than in the furnace ash (Table 2).

A mixture composed of the following grasses and legumes was sown for the experiment: red fescue (*Festuca rubra* L.) cv. Brudzyńska (40%), tall fescue (*Festuca arundinacea* Schreb.) cv. Skarpa (15%), Kentucky bluegrass (*Poa pratensis* L.) cv. Skiz (20%), birdsfoot (*Lotus corniculatus* L.) cv. Skrzeszowicka (10%) and white clover (*Trifolium repens* Haifa) (15%).

Table 2

Characteristics of materials used for the field experiment

Parameter		Unit	Sludge	Soil	Ash
			content		
pH	(H_2O)	pH	6.32	7.20	8.72
	(KCl 1 mol dm^{-3})		5.88	6.81	8.46
	($CaCl_2$ 0.1 mol dm^{-3})		6.22	6.91	8.47
Grain size distribution		-	-	gs*	pgmp**
Dry weight		%	19.63	-	76.12
Organic carbon		g kg^{-3} s.m.	217.0	8.24	26.1
Total nitrogen			27.51	0.67	1.91
Total phosphorus			13.70	0.265	0.96
Magnesium			0.447	0.219	0.502
Calcium			2.662	0.407	1.030
Potasssium			0.080	0.174	0.090
Sodium			0.074	0.038	0.131

* medium loam, ** strong loamy silt sand

After the harvest, the plant mixture was dried in a dryer with a forced air flow at 70ºC and the dry weight yield was determined. Samples of plant material were dry-mineralized in a muffle furnace at 450ºC and the content of Mg, Ca, K, Na and P was determined with the ICP-AES method. The paper presents the mean weighed average content of macroelements for the years 2002-2005. The concentrations of P, Mg, Ca, Na and heavy metals approximating the total in the soil (substratum) were determined after incineration of the organic matter and its digestion in a mixture of $HClO_4$ and HNO_3 (3:2) acids. As in the plant material, the content of elements in the soil filtrates was determined using the ICP-AES method (Ostrowska et al. 1991).

RESULTS AND DISCUSSION

The soil on which the experiment was set up met the standards for heavy metal concentrations, i.e. Cd, Pb, Cu, Ni, Zn and Cr, established for application of sewage sludge as a land recultivation measure for agricultural and non-agricultural purposes (*Rozporządzenie...* 2002). Sewage sludge used for the experiment met the requirements concerning heavy metal content for use in agriculture and for land recultivation for arable and non-arable purposes (*Rozporządzenie...* 2002). The content of Mg, C and Na in furnace ash used for the experiment was much higher than their soil concentrations (Table 2).

The effect of sewage sludge and furnace ashes on the crop yield of the grass-legume mixture was also presented in a monograph by ANTONKIEWICZ (2009).

A significant effect of sewage sludge and furnace ash and their mixtures on the plant mixture crop yield was found in the present field experiment. Analysis of the average yield for the investigated period showed the highest value for treatment 4, where 150 t d.m. of sludge and 50 t d.m. of ash were used per 1 ha. An increase in the plant mixture yield from this treatment was over 160% in comparison with the control. Sewage sludge applied in a dose of 200 t ha^{-1} d.m. (treatment 2) also produced a large increase (over 130% vs. the control) in the plant mixture yield. Ash and sludge mixed in a 1:1 weight ratio (100 t d.m. of sludge per 1 ha of waste; treatment 6) also led to a significant increase in yield, which was almost 100% higher in comparison with the control. High fertilizer value of sewage sludge was depressed when a large amount of ash was added, as in the combination consisting of 50 t d.m. of sludge and 150 t d.m. of ash (treatment 5). The yield obtained from this treatment was significantly higher in comparison with the control. Furnace ash used separately in a dose of 200 t ha^{-1} d.m. significantly diminished the crop yield, which corresponded to 60% of the yield obtained from the control (Table 3).

Our analysis of the chemical composition of the legume-grass sward revealed that the application of either type of waste and their mixtures led to a markedly diversified content of macroelements. The biggest differences occurred in the content of Na and Ca in the sward, while the smallest ones were found for the content of Mg. Depending on the treatment, the content of macroelements in the legume-grass sward ranged from 2.58-31.2 g Mg, 3.16-5.85 g Ca, 16.95-18.46 g K, 0.26-1.25 g Na and 2.27-3.37 g P kg^{-1}d.m. (Figure 1). The highest concentrations of calcium and potassium were determined in plants grown in the treatment where ash was used separately in a dose of 200 t d.m. ha^{-1}, while the highest content of phosphorus and sodium accumulated in plants in the treatment where sludge alone was applied, also in a dose of 200 t d.m. ha^{-1}.

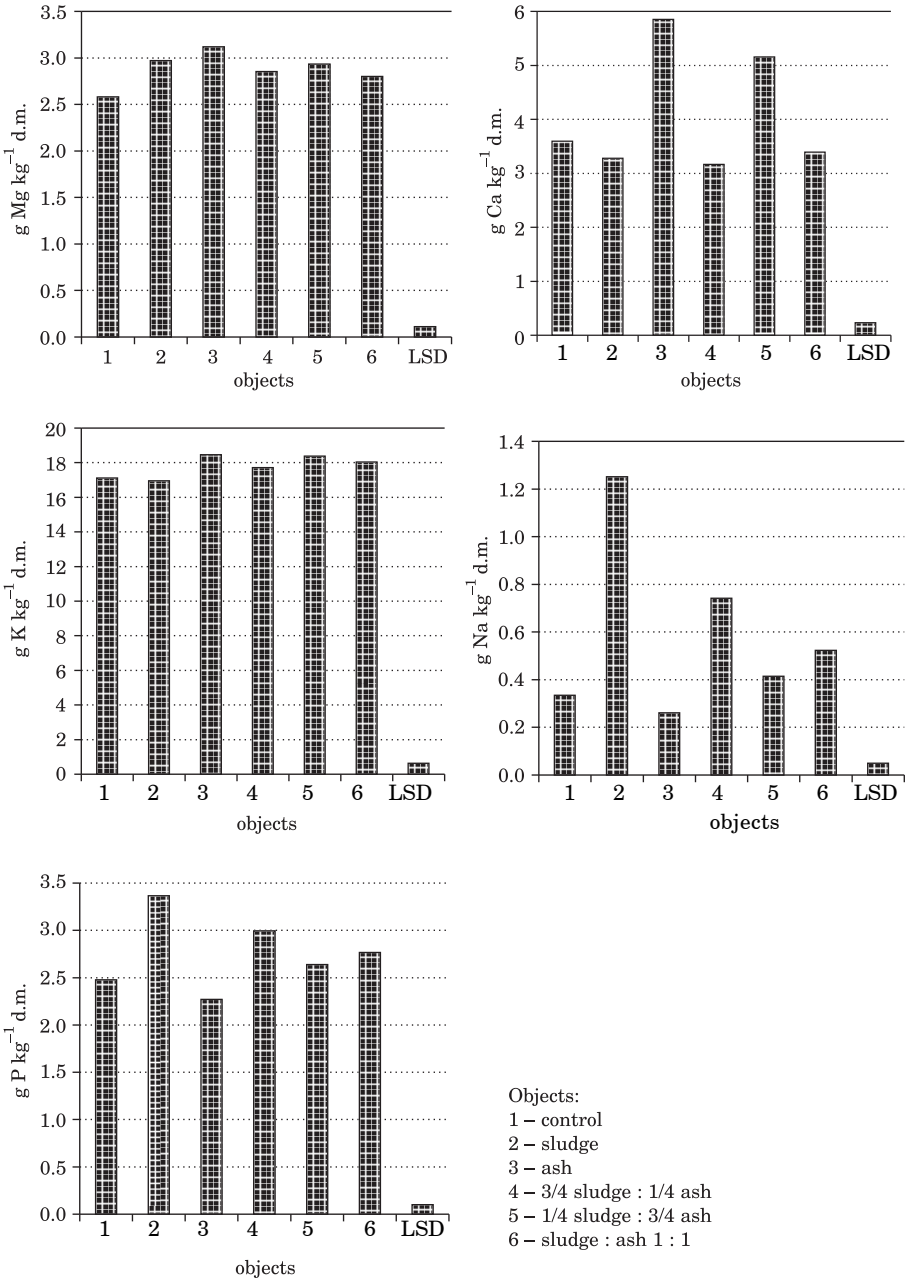

Fig. 1. Content of macronutrients in the sward of legume-grass

Table 3

Yield of plant mixture (t d.m. ha^{-1})

No object	Combination*	Year of vegetation				Average
		2002	2003	2004	2005	
1	control	0.49	4.39	4.33	4.06	3.32
2	sludge	2.45	10.22	9.41	8.56	7.66
3	ash	0.15	2.65	2.60	2.35	1.94
4	PO 3/4:1/4	2.78	11.81	10.56	9.83	8.74
5	PP 1/4:3/4	0.80	6.86	6.79	6.31	5.19
6	PO 1:1	2.26	8.21	8.20	7.73	6.60
V percent**		76.10	47.07	43.71	43.70	46.72
LSD(α=0.05)		0.17	0.42	0.25	0.25	0.302

* For experimental design see Table 1

Furnace waste used without admixtures in an amount of 200 t kg^{-1} caused a marked increase in the content of magnesium, calcium and potassium in the grass and legume mixture as compared to the control.

Moreover, the applied municipal sewage sludge and ash-sludge mixtures significantly raised the magnesium content in plants. CIEĆKO and NOWAK (1984) confirmed the results demonstrating that the biomass obtained from plants cultivated on furnace ashes was more abundant in magnesium. On the other hand, the contribution of ash in the ash-sludge mixture clearly increased the levels of calcium and potassium in the mixture of grasses and legumes. Concentrations of calcium and potassium in the sward were significantly higher in the treatments fertilized with ashes and ash-sludge mixtures than in the control. KALEMBASA and WYSOKIŃSKI (2002) reported similar results from a pot experiment, in which higher content of calcium was noted in plants cultivated on sludge-ash mixtures than in plants grown on sludge.

Furnace ash applied in the experiment caused an evident decline in the sodium and phosphorus content in the sward versus the control and sewage sludge treatment. The significantly highest sodium concentration in the mixture of grasses and legumes was determined in the object enriched with a dose of 200 t ha^{-1} d.m of sewage sludge. Also, the ash-sludge mixtures proved to be a rich source of sodium for the grass and legume mixture recommended for recultivation of post-industrial areas. The results are in accord with the research conducted by other authors (NOWAK et al. 1993), where alkalization of soil environment did not limit the potassium uptake by plants.

Fertilization with ash-sludge mixtures led to a significant increase in phosphorus concentrations in sward of the legume-grass mixture in compar-

ison with the control. Notably, the highest phosphorus content was found in plants grown in treatments fertilized only with sludge, in contrast to the control and ash-sludge mixtures. Moreover, the data shown in Figure 1 suggest that the content of phosphorus in plants decreases as a result of admixing ashes from black coal incineration to sludge as compared to fertilization with sludge alone. The results are supported by the research of KALEMBASA and WYSOKIŃSKI (2002), demonstrating that furnace ash admixture to sewage sludge decreases phosphorus content in plants. Although ashes sometimes contain large amounts of phosphorus, in an alkaline environment this component is almost completely retarded by calcium ions (MACIAK et al. 1976b).

The following concentrations of macroelements are regarded as optimal for fodder plants: 2.0 g Mg, 7.0 g Ca, 17-20 g K, 1.5-2.5 g Na and 3.0 g P kg^{-1}d.m. The municipal sewage sludge and furnace ashes applied in this experiment fully covered the plant requirements for magnesium, whereas the Ca and Na content in grass and legume mixture was below the optimum value. Potassium in plants cultivated on a substrate enriched with the above two types of waste corresponded to the optimum value, while the phosphorus content in the grass-legume mixture was close to the optimum.

CONCLUSIONS

1. Municipal sewage sludge, furnace ash and their mixtures applied in the experiment led to a significant increase in the content of Mg, Ca, K, Na and P.

2. The highest content of Mg, Ca and K was determined in the grass and legume mixture cultivated exclusively on ash, whereas the highest P and Na concentrations were found in the mixture cultivated on sewage sludge.

3. The Mg and K content in the grass-legume mixture met the standards for good quality feeds, whereas Ca, Na and P concentrations remained below the optimum value and the P content was close to the optimum.

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THE EXCHANGEABLE CATIONS IN ALLUVIAL SOILS FORMED FROM CALCAREOUS SINTER IN THE UNISŁAWSKI BASIN

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Abstract

The samples were taken from 7 profiles of alluvial soils formed from varied gytias on calcareous sinter. The research concerned determination of composition of exchangeable cations and basic saturation. In the analysed soils, lithologic discontinuities in the profile structure were observed. The calcareous sinter located in the lower part of the profile caused characteristic water properties and occurrence of gleyic process. The investigated soil profiles were varied in their morphological structure and physicochemical properties. Fluctuations in the content of carbonates, organic mater and non-carbonate mineral substance confirmed the existence of several cycles in the formation of these soils. The basic saturation (S) of the analysed soils ranged between 287.4 and 2238.7 mmol(+) kg^{-1}. The highest values were detected in gytia horizons (gyd), and the lowest ones - in gleyic horizons (G). The dominant cation in sorption complex of all horizons was calcium, and its highly differentiated content ranged from 245.3 to 2089.6 mmol(+) kg^{-1}. Magnesium was the second most abundant cation in sorption complex. The content of Mg^{2+} ranged from 19.4 mmol(+) kg^{-1} in clay-lime gytia horizons to 143.5 mmol(+) kg^{-1} in lime gytia horizons. The lowest in the content among exchangeable cations in the analysed samples was potassium (0.6-12.9 mmol(+) kg^{-1}). Our comparison of the divalent cation content (Ca^{2+}, Mg^{2+}) and monovalent cation content (Na^{+}, K^{+}) showed significant instability of the balance between these groups of cations, which was confirmed by a very wide range of the ratio (21.3-333.1) calculated between these two groups of cations. The amount of alkaline cations in the analysed soilsfollowed this order: $Ca^{2+} > Mg^{2+} > Na^{+} > K^{+}$.

Key words: soil, cation capacity, exchangeable cations.

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KATIONY WYMIENNE W GLEBACH ALUWIALNYCH WYTWORZONYCH NA MARTWICY WAPIENNEJ W BASENIE UNISŁAWSKIM

Abstrakt

W próbkach z 7 profili glebowych gleb aluwialnych, wytworzonych ze zróżnicowanych utworów gytiowych podścielonych martwicą wapienną, badano skład zasadowych kationów wymiennych oraz ich sumy. Analizowane gleby wykazały nieciągłość litologiczną w budowie profilowej i wyraźną trójczłonowość. Leżąca w spągu profilu glebowego martwica wapienna wpłynęła na gospodarkę wodną, powodując występowanie procesu glejowego. Profile glebowe wykazały duże zróżnicowanie nie tylko w budowie morfologicznej, ale także we właściwościach fizykochemicznych. Stwierdzone wahania zawartości węglanów, substancji organicznej i niewęglanowej substancji mineralnej, wskazują na występowanie wielu cykli w kształtowaniu badanych gleb. Suma zasadowych kationów wymiennych (S) kształtowała się od 287,4 do 2238,7 mmol(+) kg^{-1}. Najwyższą wartość stwierdzono w poziomach gytii detrytusowej (gyd), a najniższą w poziomach glejowych (G). Dominującym kationem wysycającym kompleks sorpcyjny był wapń, a jego ilości były zróżnicowane – od 245,3 do 2089,6 mmol(+) kg^{-1}. Drugim kationem pod względem ilości występowania w kompleksie sorpcyjnym był magnez. Zawartość magnezu w badanych glebach wynosiła od 19,4 mmol(+) kg^{-1} w poziomach gytii ilasto-wapiennej do 143,5 mmol(+) kg^{-1} w poziomach gytii wapiennej. W badanych profilach odnotowano najmniej potasu wymiennego (0,6-12,9 mmol(+) kg^{-1}). Analiza zawartości kationów dwuwartościowych (Ca^{2+}, Mg^{2+}) oraz jednowartościowych (Na^+, K^+) wykazała, że w badanych glebach występuje wyraźne zachwianie równowagi między analizowanymi kationami. Oznaką zaburzenia równowagi był szeroki zakres omawianego ilorazu – od 21,3 do 333,1. Szereg ilościowy kationów o charakterze zasadowym w analizowanych glebach układał się następująco: $Ca^{2+} > Mg^{2+} > Na^+ > K^+$.

Słowa kluczowe: gleby napływowe, pojemność sorpcyjna, kationy wymienne.

INTRODUCTION

In Poland, soils formed from alluvial deposits account for a significant part of arable lands under intensive agricultural cultivation. The soils situated in river valleys, mainly those of the Odra and the Vistula, create highly varied soil cover. Although soils of Poland have been largely studied and classified, the above areas still need far more detailed research. One such location is the Unislaw Basin, where soils of a complex structure related to some sediment accumulation cycles occur. They are: calcareous sinter in the floor, covered with a gytia layer and surface alluvial formations with flood history. The flat situation, advantageous water conditions and high nutrient abundance result in high soil fertility and encourage intensive agricultural use. However, there are no detailed and exhaustive data concerning their chemical and mineralogical composition or physicochemical properties.

Sorption properties and the exchangeable cation content play an important role in the process of leaching nutrient components from soil, thus shaping the effectiveness of fertilization, which is of importance in plant nutrition processes, especially in soils used agriculturally (HARTMAN et al. 1998, SKŁODOWSKI and ZARZYCKA 1995). The aim of the study was to evaluate the composition of cations in sorption complex of some alluvial soils.

MATERIAL AND METHODS

The research material consisted of 7 soil profiles localized in the Unislaw Basin area, which is an enlargement of the Vistula Valley, 8-9 km wide, situated north of the Fordon Brakeage (Figure 1). The dominant type of landscape, covering half the basin, is floodplain. The research showed lithologic discontinuities in the profile structures and clear trimembrality. The topmost layer consisted of alluvial material, which built the surface and

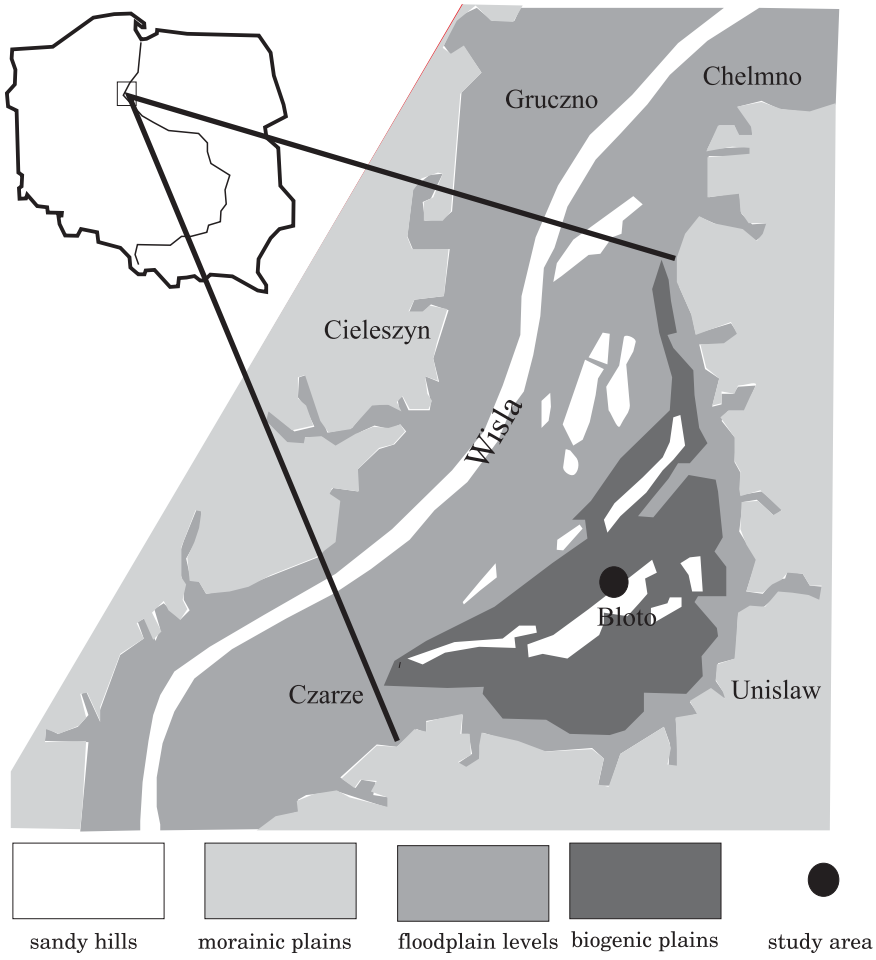

Fig. 1. Map of the analysed area (KORDOWSKI 2003)

subsurface horizons of the soil and showed high homogeneity in the granulometric and mineralogical composition. The alluvial layer was spread on gytias, differentiated in mineral and organic fractions, which in turn were deposited on the calcareous sinter lying at the depth of 100-110 cm. The calcareous sinter in the investigated area originated from the shore and is the result of the $CaCO_3$ sedimentations from the defluent waters from upland around the basin (SKOMPSKI 1961). It is most likely that the sediment of calc-sinter type characterised by very low porosity and very high hardness (SZULC 1983) consisted mainly of calcite and a small addition of quartz (Figure 2). Due to the very low porosity, the sediment is a barrier to water penetration, which becomes stagnant and therefore changes the oxy-reductive conditions. The sedimentation of gytias on the calcareous sinter occurred in standing water bodies found in this lake-rich region. Both the water level and organic matter content played an important role in this process. Different kinds of the analysed gytias were ruled out on the basis of MARKOWSKI'S triangle (1980), where the principle for the division was the carbonate content and loss during calcination as a measure of the organic matter content.

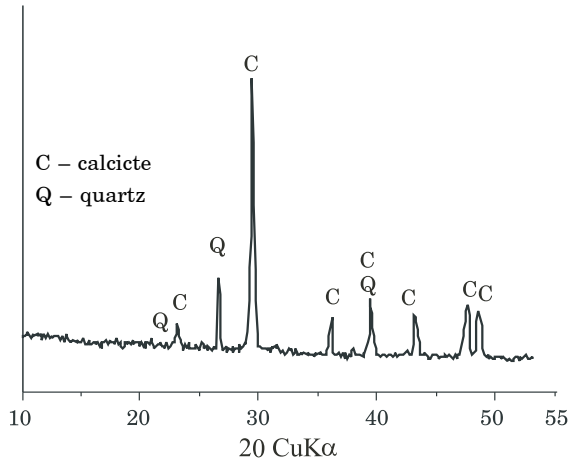

Fig. 2. Diffractogram of calcareous sinter

A great variety of factors affecting the processes of sedimentation and sedentation led to the differentiation of morphology and physicochemical properties of the formed sediments and soils lying on these sediments. Some basic physicochemical properties are given in Table 1.

Exchangeable cations (Mg^{2+}, Ca^{2+}, K^{+}, Na^{+}) in the soil samples were assayed in 0.5 M NH_4Cl (pH 8.2). Cation concentrations were determined in a filtrate by atomic absorptive spectroscopy and emissive spectroscopy using a Philips PU 9100x spectrometer (KOCIAŁKOWSKI et al. 1984). Saturation (S) with bases was also calculated.

Table 1

Selected physical and chemical properties of the analysed soils

Horizons	Content						pH 1MKCl	
	Corg (g kg^{-1})		$CaCO_3$ (%)		fraction < 0002 mm (%)			
	min	max	min	max	min	max	min	max
Surface horizons (Ap)	50.2	80.3	15.9	26.5	35.6	66.5	7.03	7.41
Subsurface horizons (Aa)	5.1	78.0	5.3	69.3	11.3	60.3	7.15	7.30
Gleyic horizons (G)	4.9	6.5	0.6	4.5	6.7	32.9	7.09	7.26
Detritus gyttia (gyd)	46.5	342.8	21.0	48.7	n.o	n.o	7.09	7.40
Detritus-calcaric gyttia (gydca)	112.9	195.2	36.7	56.5	44.1	71.3	6.88	7.16
Clay gyttia (gyi)	1.4	7.9	8.5	12.1	35.0	42.0	7.14	7.41
Clay-calcaric gyttia (gyica)	2.2	90.6	20.5	46.8	16.6	81.5	7.12	7.35
Calcaric gyttia (gyca)	9.5	59.8	51.6	76.1	12.2	78.4	7.20	7.48

n.o.– not detected

RESULTS AND DISCUSSION

Large amounts of $CaCO_3$ found in the analysed soil were determined as being either neutral or slightly alkaline in reaction. It was confirmed pH in 1M KCl ranged from 6.88 to 7.41 (Table 1). This parameter is extremely important as it influences the surface charge of soil colloids, which is reflected in the cation exchangeable capacity (JÓZEFACIUK 1998). Then, some balance between the content of exchangeable cations and the amount of soil mineral weathering products in soil solution is established in the soil (CRABTREE 1986).

Because of the high calcium carbonate content and a small share of absorbed acid cations (H^+ and Al^{3+}), the basic saturation of the analysed soils was similar to the total cations exchangeable capacity. The highest basic saturation (S) values (2238.7 mmol (+) kg^{-1}) were found in the detritus gytia horizons (gyd) enriched with calcium carbonate, while the smallest ones (287.4 mmol (+) kg^{-1}) were assayed in the gleyic horizons (G) (Table 2). The research showed significant differentiation of the bases saturation (S) within the alluvial formation consisting of the surface (Ap) and subsurface (Aa) horizons, where S values ranged from 401.1 to 1175.4 mmol (+) kg^{-1}, whereas in the Ap horizons the S variability was lower (Table 2). The high-

Table 2

Exchangeable cation content, basic saturation and ratios between cations in the analysed soils

Horizons	Exchangeable cation content mmol(+) kg^{-1}								BS* mmol(+) kg^{-1}		Ca/Mg		Na/K		Ca+Mg/K+Na	
	Ca		Mg		K		Na									
	min	max	min	max	min	max	min	max	min	max	min	max	min	max	min	max
Surface horizons (Ap)	640.9	1029.9	52.0	118.2	2.1	7.6	3.5	19.7	698.5	1175.4	12.3	8.7	1.7	2.6	123.7	145.5
Subsurface horizons (Aa)	362.7	912.7	34.2	117.9	1.0	4.6	3.2	12.5	401.1	1047.7	10.6	7.7	3.2	2.7	94.5	60.3
Gleyic horizons (G)	245.3	439.3	29.2	63.3	3.3	5.6	9.6	11.8	287.4	520.0	8.4	6.9	2.9	2.1	21.3	28.9
Detritus gyttia (gyd)	640.6	2089.6	42.3	142.4	0.6	2.0	4.3	4.7	687.8	2238.7	15.1	14.7	7.2	2.4	139.4	333.1
Detritus-calcaric gyttia (gydca)	862.8	982.4	83.7	88.1	3.0	4.0	12.0	12.2	961.5	1086.7	10.3	11.2	4.0	3.1	63.1	66.1
Clay gyttia (gyi)	450.6	498.6	42.2	45.5	3.6	5.6	8.4	10.2	504.8	550.3	10.6	11.0	2.3	1.8	41.1	34.4
Clay-calcaric gyttia (gyica)	362.7	1151.4	19.4	94.3	0.8	12.9	3.8	21.5	386.7	1280.1	18.7	12.2	4.8	1.7	83.1	36.2
Calcaric gyttia (gyca)	474.3	685.7	31.5	143.5	1.2	10.5	6.7	12.4	513.7	852.1	15.1	4.8	5.6	1.2	64.0	36.2

*BS – basic saturation

est S value was determined in the detritus gytia (2238.7 mmol (+) kg^{-1}), while in clay-lime and detritus-lime the S values were lower (1280.1 mmol (+) kg^{-1} and 1086.7 mmol (+) kg^{-1}, respectively). The lowest saturation with bases (S) was found in the gytia-clay horizons (Table 2). The investigation showed that the lower the organic matter content, the lower the bases saturation (S) values. Similar regularity for many post-bog soils was also reported by PIAŚCIK et al. (1997) and ŁACHACZ (2001).

Calcium was the dominant cation in the sorption complex of all the horizons, which is characteristic for soils of alkaline reaction. The soil content of Ca ranged within 245.3-2089.6 mmol (+) kg^{-1}. The surface horizons were characterised by the Ca content fluctuating between 640.9 and 1029.9 mmol (+) kg^{-1}, while in the subsurface ones the range was 362.7--912.7 mmol (+) kg^{-1}. When comparing specific kinds of gytia, no significant differences in Ca saturation of the sorption complex were found, except the detritus gytia in profile 5, where Ca^{2+} ion content reached the value of 2089.6 mmol (+) kg^{-1}. The lowest amounts of calcium ions were determined in the gleyic horizons, where likewise the smallest amounts of the ions (245.3 mmol (+) kg^{-1}) were assayed. Despite such considerable Ca^{2+} differentiation, its contribution in the sorption complex was found to be in a narrow range of 79.7-94.1% (Figure 3). The Ca^{2+} ion concentrations were lower than the content found by ORZECHOWSKI et al. (2005) in their studies of sorption characteristics of alluvial soil in Żuławy Wiślane.

Magnesius was the second in the sequence of the abundance of cations in the sorption complex. The content of Mg^{2+} ranged from 19.4 in the clay-lime gytia horizons to 143.5 in the lime gytia horizons. The contribution

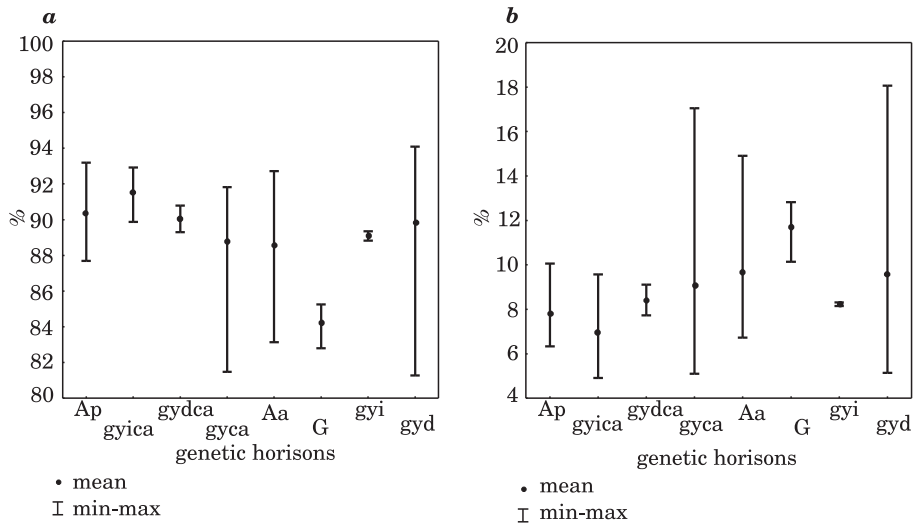

Fig. 3. Content of Ca^{2+} (*a*) and Mg^{2+} (*b*) in basic saturation (BS) in the analysed soils

of Mg^{2+} to the base saturation (S) values ranged from 4.9 to 19.4%. The base saturation analysis of the surface horizons showed that the Mg^{2+} ion content fluctuated between 52.0 mmol (+) kg^{-1} and 118.2 mmol (+) kg^{-1}, and their participation in the base saturation reached 6.3% and 10.1 %, respectively. According to the literature (NEMETH et al. 1970, LIPIŃSKI, BEDNAREK 1998, RUTKOWSKA et al. 2006), the concentration of calcium and magnesium in soil solution was observed along with an increase of particles <0.02 content, which was attrobuted to a higher amount of clay minerals which contain these elements.

Exchangeable potassium occurred in the examined profiles in small amounts (from 0.6 mmol (+) kg^{-1} to 12.9 mmol (+) kg^{-1}). The most rich in potassium were the levels of clay-lime and lime gytia, where the K^+ content was 1.9 mmol (+) kg^{-1} and 10.5 mmol (+) kg^{-1}, respectively. In the Ap horizons the K^+ affluence formed at the level of 2.1 to 7.6 mmol (+) kg^{-1}, what accounted for 0.2-0.7% of the base saturation. One should stated that most of all potassium ions were noted in the horizons which were enriched with organic matter, what may be the effect of biological accumulation of the component (ZASOŃSKI, SKIBA 1988). Similarly, a low potassium content was found by LASKOWSKI and SZOZDA (1985) in the alluvial soils along Odra.

The Na^+ ion content was lower than that of K^+ and reached 3.5 mmol (+) kg^{-1} in the Ap horizons and 21.5 mmol (+) kg^{-1} in the clay-lime gytia horizons. Our analyses of the arable horizons showed that the Na^+ ion concentrations ranged between 3.5 mmol (+) kg^{-1} and 19.7 mmol (+) kg^{-1}, which corresponded to 0.2% and 4.5 % of the total content of these ions. Similarly, SAPEK (1979) found that calcium is the basic ion saturating the exchangeable complex of organic soils. It may account for 65-100% of the total calcium content, while the respective values are lower for magnesium (23-100%), potassium (16-66%) and sodium (52-84%).

The calcium to magnesium ion concentration ratio calculated for the analysed soils was rather narrow and varied from 8.4 to 18.7. A somewhat different picture appeared in the case of the quotient of monovalent ions (Na^+/K^+), which was lower and ranged 1.2-7.2 (Table 2).

Our analysis of divalent and monovalent cation content showed that the balance between theese groups of cations in the investigated soils was significantly unstable, which is bad for plant nutrition. The indication of some disturbance in the balance of cations is a very wide range of the ratio (333.1). A wide range of the analysed quotient (from 21.3 to 333.1) should be emphasized. The lowest value of the quotient was calculated for the gleyic horizons, while the highest one – for the detritus gytia horizons.

CONCLUSIONS

1. The composition of cations saturating the exchangeable complex was mostly influenced by calcareous sinter, consisted mainly of calcite. Calcium was the dominating cation, while the potassium content was relatively low.

2. High differentiation of the absorbed exchangeable cations was probably due to a diverse exchangeable complex capacity resulting from the organic fraction content.

3. A very wide range of the divalent to monovalent cation ratio might lead to deficiency of plant-available potassium and sodium.

4. As regards the specific saturation of exchangeable complex with alkaline cations, the analysed soil requires application of physiologically acidic fertilizers.

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EFFECT OF NITROGEN FERTILIZATION ON Cu, Mn, Zn, Fe, B AND Mo AVAILABILITY IN COMMERCIALLY GROWN WHITE HEAD CABBAGE

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Abstaract

The results of three-year investigations with cv. Galaxy F_1 cabbage grown commercially in an important agricultural region of South Poland are presented. The effect of the rate of ammonium sulphate and UAN (solution of ammonium nitrate + urea), the method of application (placement and broadcast technique) and foliar fertilization (urea and Supervit K) on Cu, Mn, Zn, Fe, B and Mo concentrations in edible parts of cabbage were surveyed. Nitrogen fertilizer was applied at the rate of 120 kg N ha^{-1}. With the placement fertilization method, fertilizer was applied in rows 10 cm deep and 10 cm away from each plant when seedlings were transplanted. Foliar sprayings started at the beginning of intensive leaf growth and continued during the growing season at two-week interval. The foliar nutrition with 2% urea was carried out 3 times and 1% Supervit K was applied once. The field experiment was carried out in 2005-2007 with cv. Galaxy F_1 white cabbage on silty clay soil containing 0.91-1.02% organic carbon and soil acidity pH_{H_2O} 7.18-8.21 Micronutrient concentrations were below the lower range of the content reported for cabbage grown in non-contaminated areas. Ammonium sulphate significantly increased Mn and Fe concentrations in cabbage heads and decreased B and Mo content. However, the environmental factors considerably modified this tendency. The method of N application affected Mn content in cabbage in 2007 and Mo in 2006 and 2007. Slightly higher manganese and molybdenum concentrations for placement fertilization were noted. In 2005 and 2006, the placement fertilization at the rate of 75% N and supplemented foliar sprays increased Mo content in cabbage.

Key words: white cabbage, nitrogen fertilization, method of N application, micronutrient content.

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WPŁYW NAWOŻENIA AZOTEM NA DOSTĘPNOŚĆ Cu, Mn, Zn, Fe, B I Mo W KAPUŚCIE GŁOWIASTEJ BIAŁEJ ODM. GALAXY F_1 UPRAWIANEJ W WARUNKACH PRODUKCYJNYCH

Abstrakt

Doświadczenie z kapustą głowiastą białą odm. Galaxy F_1 prowadzono w latach 2005--2007, w prywatnym gospodarstwie warzywniczym, w Zagorzycach k. Miechowa. Badano wpływ rodzaju nawozu azotowego (siarczan amonu, RSM – roztwór saletrzano-mocznikowy), sposobu nawożenia (rzutowy, zlokalizowany) oraz dokarmiania pozakorzeniowego (mocznik i Supervit K) na stopień odżywienia roślin mikroelementami (Cu, Mn, Zn, Fe, B i Mo). Azot stosowano w dawce 120 kg N ha^{-1}. Nawożenie zlokalizowane polegało na umieszczaniu depozytów azotowych w rzędach roślin – w odległości 10 cm od rośliny i na głębokości 10 cm – w chwili sadzenia rozsady. W obiektach z dokarmianiem dolistnym opryski wykonywano 4-krotnie, rozpoczynając na początku fazy intensywnego wzrostu wegetatywnego. W pierwszym, drugim i czwartym terminie dokarmianie wykonano z użyciem 2% roztworu mocznika, natomiast w trzecim terminie stosowano 1% roztworu Supervitu K. Kapustę uprawiano na glebie ciężkiej o składzie pyłu ilastego, zawartości węgla organicznego (%C) 0,91-1,02% i odczynie pH_{H_2O} 7,18-8,21.

Rodzaj zastosowanego nawozu azotowego wpływał istotnie na zawartość Mn, Fe, B i Mo w kapuście. Istotnie więcej Mn i Fe zawierały rośliny nawożone siarczanem amonu w porównaniu z roztworem saletrzano-mocznikowym (RSM), natomiast RSM wpływał na wzrost w liściach kapusty zawartości B i Mo. Obserwowany wpływ nawozów na oznaczane w materiale roślinnym składniki zależał od warunków środowiskowych w kolejnych latach badań.

Sposób stosowania nawozów azotowych wpływał na zawartość Mn i Mo w kapuście. Lokalizowanie depozytów azotowych w pobliżu roślin miało wpływ na wzrost zawartości Mn i Mo w roślinach w porównaniu z rzutowym stosowaniem siarczanu amonu i RSM. Pozakorzeniowe dokarmianie roślin z wykorzystaniem mocznika i Supervitu K zawierającego molibden wpływało na wzrost stężenia Mo w kapuście w przypadku zlokalizowanego doglebowego nawożenia azotem.

Słowa kluczowe: kapusta biała, nawożenie azotem, metody aplikacji N, zawartość mikroelementów.

INTRODUCTION

Soils vary widely in their micronutrient content and the ability to supply micronutrients in quantities sufficient for optimal crop growth. Micronutrient availability defined as the quantity of a soil nutrient that is accessible to plant roots, depends on soil pH, organic matter content, adsorptive surfaces and other physical, chemical, and biological conditions in the rhizosphere (De Pieri et al. 1996, Gębski 1998, White, Zasoski 1999).

Nutrient interactions in crop plants are probably one of the most important factors affecting yields of annual crops. They can be measured in terms of crop growth and nutrient concentrations in plant tissues. Interactions on the root surface are usually due to the formation of chemical bonds by ions

and precipitation or complexes (MARCHNER 1995). An example of this type of a nutrient interaction is the liming of acidic soils. Massive or repeated application of lime reduces the availability of micronutrients except molybdenum. In the cabbage production, liming has been used as a control means for club root (*Plasmodiophora brassiceae*) since the early 19th century. There is a close relationship between soil pH and club root, with acidic soils generally favoring development of the disease. Due to a more rapid effect of liming, alkalinity may often lead to nutritional deficiencies (BOLAND et al. 1999).

Nitrogen fertilizers induce some direct and/or indirect changes which influence the dynamics of the availability of metals in soils. Mineral N fertilizers contain ammonium and can acidify the soil solution, thus decreasing the pH of the rhizosphere. In neutral or alkaline soils, acidification of the rhizosphere of plants fed with ammonium can enhance the uptake of micronutrients such as boron, iron, manganese, copper and zinc (JURKOWSKA et al. 1996, FAGERIA and BALIGAR 2005, DIATTA and GRZEBISZ 2006).

The objective of the present project was to assess the effect of ammonium sulphate and UAN (solution of the ammonium nitrate + urea) applied by placement and broadcast technique and additionally foliar fertilization (urea and Supervit K) on the accumulation of micronutrients in cv. Galaxy F_1 cabbage grown commercially under field conditions.

MATERIAL AND METHODS

A field experiment was carried out in 2005-2007 with cv. Galaxy F_1 white cabbage on silty clay soil containing 0.91-1.02% organic carbon and soil acidity pH_{H2O} 7.18-8.21 (Table 1). The plots were located on a private farm in Zagorzyce (50º23' and 20º04'). Farms in this area specialize in cabbage production in continuous or highly frequent cropping. In short-term crop rotation systems liming is commonly used as a measure to prevent potential damage caused by club root. Application of calcium oxide one month prior to planting is a practical means of controlling fungal disease.

Table 1

Organic carbon content (%), soil pH and soil texture before cabbage planting in 2005-2007

Year	C (%)	Sand 0-0.1 mm	Silt 0.1-0.02 mm	Clay <0.02 mm	pH_{KCl}	pH_{H_2O}
2005	0.91	15	47	38	7.70	8.21
2006	1.02	8	50	41	6.17	7.18
2007	0.98	9	55	36	7.09	7.90

Two factors were examined: the type of N fertilizer - ammonium sulphate and UAN-30 (ammonium nitrate – 42.8%, urea 32.2%, water – 25%), and the method of N application. The treatments with both fertilizers were as follows:

1) control – 100% N rate (120 kg ha^{-1}) broadcast during the planting of seedlings;
2) 75% N rate broadcast during the planting of seedlings + 25% N as top dressing;
3) 75% N rate broadcast during the planting of seedlings + foliar fertilization;
4) 75% N placement during the planting of seedlings;
5) 75% N placement during the planting of seedlings + 25% N as top dressing;
6) 75% N placement during the planting of seedlings + foliar fertilization.

Treatments were assigned the following completely randomized blocks in a split-plot arrangement with four replications. Nitrogen fertilizer was applied at the rate of 120 kg N ha^{-1} (100% N). With the placement fertilization method, fertilizer was applied in rows 10 cm deep and 10 cm away from each plant (plants were spaced 67.5 x 67.5 cm) when seedlings were transplanted. Foliar sprayings started at the beginning of intensive leaf growth and continued during the growing season in two-week intervals. The foliar nutrition with 2% urea was carried out 3 times and 1% Supervit K (% w/v: N-NH_2–4.4, N-NO_3–0.8, K–3.1, Mg–0.6, Mn–0.05, Ti–0.05, B–0.03, Fe–0.025, Mo–0.005) was applied once. Mineral fertilization with phosphorus, potassium and magnesium was adjusted to the results of chemical analysis of the soil samples. The content of soil P, K and Mg was supplemented to the level of 50, 200 and 60 mg dm^{-3}, respectively before the planting of seedlings.

The harvest was conducted in the last decade of October. Edible parts were analyzed after washing with distilled water and drying at 70°C for 48 h. The Cu, Mn, Zn, Fe, B and Mo content in the samples was determined by inductively coupled argon plasma atomic emission spectroscopy (ICP-OES) after microwave digestion with HNO_3. Soil samples were collected from a 0-30 cm surface layer. Granulometric analysis was made by the aerometric method of Prószyński and the organic carbon by Tiurin's method (Ostrowska et al. 1991). Soil pH was determined by adding deionized water and 1 M KCl at a ratio 1:2 (soil:water by volume). Total micronutrients in soil were determined by inductively coupled argon plasma atomic emission spectroscopy (ICP-OES) after microwave digestion with Aqua Regia (Houba et al.1991).

The results were subjected to a two-way factor analysis of MANOVA. The mean were separated by Fisher's LSD test ($p = 0.05$).

RESULTS AND DISCUSSION

In a current survey, the micronutrient concentration in edible parts of cabbage were below the lower range of the content reported for cabbage grown in non-contaminated areas. Low concentrations of micronutrients may indicate deficiencies that would affect crop yield.

Copper deficiency is often observed in plants growing on soils with a low total Cu and on soils high in organic matter, where Cu is bound to organic substances. Its bioavailability increases under slightly acidic conditions (CHAIGNON et al. 2002, MERCIK et al. 2004). The total Cu concentration in Polish soils range from 0.2 to 725 mg kg^{-1} dry weight (TRELAK 1997). In the present study, the total Cu concentrations in soils were low and ranged between 4.68-4.75 mg Cu kg^{-1} (Table 2). A critical deficiency level of copper in vegetative plant parts is generally in the range of 1-5 mg Cu kg^{-1} dry matter,

Table 2

Total micronutrients content (mg kg^{-1} dry weight) in soil before cabbage planting in 2005-2007

Year	Cu	Mn	Zn	Fe	B	Mo
2005	4.68	152	24.3	8184	6.02	<0.03
2006	4.74	187	25.8	6176	3.94	0.033
2007	4.75	156	32.9	6606	4.06	0.031

depending of a plant species, plant organ, development stage and nitrogen supply (MARCHNER 1995, BARKER and PILBEAM 2006). In this experiment, the copper content in plants tended to be less than the ranges reported by KABATA-PENDIAS and PENDIAS (1999) for heads of cabbage grown in non-contaminated sites (3-4 mg Cu kg^{-1} d.m.). The highest Cu concentration was detected in 2006 (2.14 ppm Cu) and the lowest one appeared in 2005 (1.58 ppm) – Table 3. When the lowest pH of soil was noticed in 2006 (pH 7.18) and in 2005 (pH 8.21), the Cu concentration in cabbage was the highest. The form of nitrogen fertilizers did not affect Cu concentrations in cabbage leaves. Similar results were presented by SMOLEŃ and SADY (2007), who proved that copper content in carrot roots was not influenced by nitrogen fertilizers. The same conclusions were drawn by CHAIGNON et al. (2002) who investigated tomato and oilseed rape and reported that the Cu bioavailability was independent of the N supply in calcareous soil The method of nitrogen application did not influence the Cu concentration in cabbage in any year during the presented study (Table 3).

Samples of soil from sites where experimental plots were located had a pH level between 7.18-8.21. Increasing the soil pH by liming usually decreases the plant availability of Mn and Zn much more than of any other

Table 3

Effect of nitrogen fertilization on Cu and Mn content in Galaxy F_1 cabbage grown in 2005-2007

Fertilizer	Application method*		Cu (mg kg^{-1} d.m.)				Mn (mg kg^{-1} d.m.)			
			2005	2006	2007	mean	2005	2006	2007	mean
$(NH_4)_2SO_4$	broadcast	1	1.45	1.90	1.83	1.73	14.2	14.8	12.6	13.9
		2	1.65	1.96	1.76	1.79	15.2	16.4	12.3	14.6
		3	1.63	2.47	2.22	2.11	15.3	15.4	13.2	14.6
	placement	4	1.38	1.94	1.92	1.75	14.3	15.1	15.8	15.1
		5	1.64	2.04	2.04	1.91	15.6	15.9	16.5	16.0
		6	1.44	1.89	2.13	1.82	14.4	15.4	14.8	14.9
UAN	broadcast	1	1.74	2.66	1.92	2.11	14.1	15.1	11.6	13.6
		2	1.69	2.39	1.70	1.93	14.9	15.5	12.2	14.2
		3	1.55	2.48	1.84	1.96	12.9	13.8	12.5	13.1
	placement	4	1.58	1.88	2.54	2.00	13.9	14.5	13.2	13.9
		5	1.67	1.98	1.83	1.83	14.9	15.0	13.5	14.5
		6	1.54	2.07	1.90	1.84	14.0	14.6	13.1	13.9
Mean for year Factor Fertilizer			1.58	2.14	1.97		14.5	15.1	13.5	
	$(NH_4)_2SO_4$		1.53	2.03	1.99	1.85	14.0	15.5	14.2	14.8
	UAN		1.63	2.24	1.95	1.94	14.1	14.7	12.7	13.8
Application method	broadcast	1	1.60	2.28	1.88	1.92	14.2	14.9	13.7	13.7
		2	1.67	2.17	1.73	1.85	15.0	15.9	14.4	14.4
		3	1.59	2.47	2.03	2.03	14.1	14.6	13.9	13.9
	placement	4	1.48	1.91	2.22	1.87	14.1	14.8	14.5	14.5
		5	1.66	2.01	1.94	1.97	15.2	15.4	15.2	15.2
		6	1.49	1.98	2.01	1.83	14.2	15.0	14.4	14.4
$LSD_{0.05}$ for:	fertilizer		ns	ns	ns		ns	ns	0.98	
	application method		ns	ns	ns		ns	ns	1.69	
	fertilizer x application		ns	ns	ns		ns	ns	ns	

*1 – 120 kg ha^{-1} N broadcast at planting of seedlings; 2 – 90 kg ha^{-1} N broadcasted at planting of seedlings + 30 kg ha^{-1} N as top dressing; 3 – 90 ha^{-1} N broadcast at planting of seedlings + + foliar fertilization; 4 – 90 kg ha^{-1} N placement at seedling planting; 5 – 90 kg ha^{-1} N placement at seedling planting + 30 kg ha^{-1} N as top dressing; 6 – 90 kg ha^{-1} N placement at seedling planting + foliar fertilization

n.s. – non – significant

mineral nutrient (CZEKAŁA et al. 1996). A critically deficient content of manganese in plants is similar among crop species, varying between 10-20 mg Mn kg^{-1} dry matter (MARCHNER 1995). In our investigations, the Mn concentration in cabbage leaves ranged from 13.5 mg to 15.1 mg kg^{-1} d.m. (Table 3). The nitrogen form and N application method affected the manganese content in plants only in 2007. A significant increase in the Mn content in cabbage fertilized with $(NH_4)_2SO_4$ was observed. However, the form of mineral nitrogen fertilizers did not affect the pH of soil measured after harvest in any year (data not published). Slightly higher manganese concentrations for placement fertilization were noticed in comparison to the N broadcast method.

The risk of Zn deficiency is high in soil after liming or in calcareous soils. In leaves, the critical deficiency levels are below 15-20 mg Zn kg^{-1} d.m. BARKER and PILBEAM (2006) report that in cabbage heads an intermediate range is 34 mg Zn kg^{-1} d.m. In this research, the Zn concentrations in cabbage varied from 12.9 mg (2007) to 14.7 mg Zn kg^{-1} d.m. (2006) – Table 4. The method of application and form of nitrogen fertilization did not affect the zinc concentrations in cabbage in any year. This result does not confirm the conclusions presented by SMOLEŃ and SADY (2007), who reported that $(NH_4)_2SO_4$ fertilization can strongly affect accumulation of heavy metals (including Zn) in yield.

In Poland, clay mineral soils have on average 0.8-2.78% of Fe in total (KABATA-PENDIAS and PENDIAS 1999). However, in a well-aerated soil of a high pH, the concentration of Fe^{2+} and Fe^{3+}in soil solution is extremely low. In the present research, samples of soil from the experimental plots contained the total Fe of 6176-8184 mg kg^{-1} dry weight (Table 2). Iron deficiency is a worldwide problem in crop production on calcareous soils. It is the major factor responsible for the so-called lime-induced chlorosis (BARKER, PILBEAM 2006). The critical deficiency content of iron in leaves is 50-150 mg Fe kg^{-1} dry matter (MARCHNER 1995). KABATA-PENDIAS, PENDIAS (1999) report that on average the concentration of Fe in edible parts of cabbage is 42 mg Fe kg^{-1} d.m. In our study, iron concentrations in cabbage were low and ranged between 24.0-28.0 mg Fe kg^{-1}d.m. (Table 4) In 2007, the $(NH_4)_2SO_4$ fertilization treatment significantly increased the iron concentration in cabbage heads compared to the UAN treatment (Table 4).

The total boron content of most agricultural soils ranges from 1-467 mg kg^{-1} dry weigh, with an average content of 9-85 mg (KABATA-PENDIAS, PENDIAS 1999). The boron concentration in the experimental soil was low and varied from 3.94 to 6.02 mg B kg^{-1} dry weight. Boron uptake is closely related to the pH. Boron adsorption to clay minerals increases sharply above pH = 6.5. In alkaline soils, low boron solubility is dictated by B adsorption to clay minerals (TYLER, OLSSON 2001). The critical deficiency range, expressed as mg B kg^{-1} dry matter, is 20-70 mg in most dicotyledonous species (in cabbage leaves, B concentration is reported above 14 ppm) (MARCHNER 1995).

Table 4

Effect of nitrogen fertilization on Zn i Fe content in Galaxy F_1 cabbage grown in 2005-2007

Fertilizer	Application method*		Zn (mg kg^{-1} d.m.)				Fe (mg kg^{-1} d.m.)			
			2005	2006	2007	mean	2005	2006	2007	mean
$(NH_4)_2SO_4$	broadcast	1	13.9	13.9	12.7	13.5	25.1	25.5	25.3	25.3
		2	15.5	15.8	12.3	14.5	27.6	27.3	23.7	26.2
		3	15.7	16.8	12.4	15.0	28.7	29.7	27.1	28.5
	placement	4	13.8	14.6	13.4	13.9	27.4	28.0	26.1	27.2
		5	16.1	14.6	13.7	14.8	27.8	28.6	25.9	27.4
		6	14.1	13.3	14.6	14.0	25.4	29.6	24.7	26.6
UAN	broadcast	1	15.0	16.9	11.6	14.5	27.7	28.7	20.7	25.7
		2	14.7	15.6	11.8	14.0	26.6	28.9	20.8	25.4
		3	13.8	14.6	12.6	13.7	24.6	26.6	21.7	24.3
	placement	4	12.3	13.3	14.2	13.3	28.6	30.5	24.0	27.7
		5	14.8	14.4	12.9	14.0	29.6	27.2	24.5	27.1
		6	13.1	12.1	12.4	12.5	23.8	24.8	24.0	24.2
Mean for year Factor Fertilizer			14.4	14.7	12.9		26.9	28.0	24.0	
	$(NH_4)_2SO_4$		14.8	14.8	13.2	14.3	27.0	28.1	25.5	26.9
	UAN		14,0	14.5	12.6	13.7	26.8	27.8	22.6	25.7
Application method	broadcast	1	14.4	15.4	12.1	14.0	26.4	27.1	23.0	25.5
		2	15.1	15.7	12.1	14.3	27.1	28.1	22.2	25.8
		3	14.7	15.7	12.5	14.3	26.7	28.2	24.4	26.4
	placement	4	13.0	14.0	13.8	13.6	28.0	29.3	25.0	27.4
		5	15.5	14.5	13.3	14.4	28.7	27.9	25.2	27.3
		6	13.6	12.7	13.5	13.3	24.6	27.2	24.3	25.4
$LSD_{0.05}$ for:	fertilizer		ns	ns	ns		ns	ns	1.99	
	application method		ns	ns	ns		ns	ns	ns	
	fertilizer x application		ns	ns	ns		ns	ns	ns	

*see Table 3

In cabbage heads 12.6-13.4 mg B kg^{-1} dry matter was detected (Table 5). In 2005 and 2006, plants fertilized with UAN solution contained significantly more B than treated with ammonium sulphate. The form of nitrogen fertilizers can affect boron accumulation in plants. WÓJCIK (2000) reported that in boron deficient soils, nitrogen added as $Ca(NO_3)_2$ and NH_4NO_3 increased

Table 5

Effect of nitrogen fertilization on B i Mo content in Galaxy F_1 cabbage grown in 2005-2007

Fertilizer	Application method *		B (mg kg^{-1} d.m.)				Mo (mg kg^{-1} d.m.)			
			2005	2006	2007	mean	2005	2006	2007	mean
$(NH_4)_2SO_4$	broadcast	1	9.9	10.1	13.7	11.2	0.049	0.042	0.089	0.060
		2	9.5	9.7	12.9	10.7	0.045	0.044	0.107	0.065
		3	9.4	9.9	14.5	11.3	0.052	0.052	0.221	0.108
	placement	4	12.1	12.7	13.5	12.8	0.073	0.073	0.088	0.078
		5	10.5	10.5	14.2	11.7	0.063	0.061	0.066	0.063
		6	11.2	12.0	13.2	12.1	0.068	0.088	0.070	0.075
UAN	broadcast	1	15.3	16.9	12.6	14.9	0.079	0.086	0.265	0.143
		2	14.7	15.8	13.0	14.5	0.073	0.081	0.126	0.093
		3	13.8	15.3	13.4	14.2	0.074	0.088	0.153	0.105
	placement	4	14.2	15.4	13.8	14.5	0.082	0.090	0.093	0.088
		5	15.6	15.5	13.3	14.8	0.094	0.083	0.075	0.084
		6	14.6	15.9	13.0	14.5	0.100	0.111	0.093	0.101
Mean for year Factor Fertilizer			12.6	13.3	13.4		0.070	0.070	0.120	
	$(NH_4)_2SO_4$		10.4	10.8	13.7	11.6	0.058	0.060	0.110	0.076
	UAN		14.7	15.8	13.2	14.6	0.084	0.090	0.130	0.101
Application method	broadcast	1	12.6	13.5	13.2	13.1	0.064	0.064	0.177	0.102
		2	12.1	12.8	13.0	12.6	0.059	0.062	0.116	0.079
		3	11.6	12.6	14.0	13.7	0.063	0.070	0.187	0.107
	placement	4	13.2	14.1	13.6	13.6	0.077	0.082	0.090	0.083
		5	13.0	13.0	13.8	13.3	0.079	0.072	0.071	0.074
		6	12.9	13.9	13.1	13.3	0.084	0.100	0.082	0.089
$LSD_{0.05}$ for:	fertilizer		0.87	1.15	ns		0.0082	0.0074	ns	
	application method		ns	ns	ns		0.0143	0.0129	ns	
	fertilizer x application		ns	ns	ns		ns	ns	ns	

*see Table 3

the availability and uptake of B by roots. This increase was attributed to the fact that nitrate inhibited boron sorption on iron and aluminum oxides and increased B in soil solution.

The molybdenum plant requirement is lower than that of the other mineral nutrients. In an aqueous solution Mo occurs as molybdate oxyanion

MoO_4^{-2}. Depending on a plant species and source of N supply, the critical deficiency levels of Mo may vary between 0.1-1.0 mg kg^{-1} (MARCHNER 1995). The molybdenum content in plant tissues increases when the soil pH rises from 5-7 because of soil liming. On the other hand, excessive Ca concentration in soil solution can reduce the availability of Mo. In the present research, molybdenum concentrations in a cabbage were below the lower range of content and varied between 0.07 to 0.120 mg Mo kg^{-1} dry matter (Table 5). In 2005 and 2006, ammonium sulphate fertilization significantly decreased the molybdenum content in cabbage. Sulphate and molybdate are strongly competing anions during the uptake by roots (BARKER and PILBEAN 2006). In the case of a wide concentration ratio of SO_4^{-2}/MoO_4^{-2} in the soil solution, sulfate containing fertilizers depresses the molybdenum uptake. In 2005 and 2006, placement fertilization at the rate of 75% N and supplemented foliar sprays increased the Mo content in cabbage.

CONCLUSIONS

In the current study, the Cu, Zn, Fe, B and Mo concentration in edible parts of cabbage was below the lower range of the content reported for cabbage grown in non-contaminated areas. The low concentration of micronutrients may indicate deficiencies that would affect cabbage crop yield in commercial production in this area. It was concluded that deficiencies of micronutrients were related to the parent material, with generally low total levels of Cu, Zn, Fe, B, and Mo leading to the deficit of these elements. WHITE, ZASOSKI (1999) report that micronutrient deficiencies in agricultural soils worldwide are disturbingly large. The problem is aggravated by the fact that many modern cultivars of major crops are highly sensitive to low micronutrient levels. The results of this study confirm other reports indicating that deficiency of micronutrients is on an increase in intensive agricultural systems characterized by large nitrogen, phosphorus and potassium fertilization rates and lime applications, inducing nutrient imbalance and increasing micronutrient demand.

The research has demonstrated that in neutral or slightly alkaline soils mineral N fertilizers containing ammonium can enhance the uptake of micronutrients such as iron and manganese. In the case of a wide concentration ratio of SO_4^{-2}/MoO_4^{-2} in the soil solution, sulfate depresses the molybdenum uptake. The UAN solution including $N\text{-}NO_3$ increased the availability and uptake of B by cabbage.

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THE EFFECT OF MAGNESIUM FERTILIZATION ON THE MACRONUTRIENT CONTENT OF PEPINO DULCE (*SOLANUM MURICATUM* AIT.) FRUIT

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Abstract

Pepino dulce (*Solanum muricatum* Ait.) of the family *Solanaceae* is native to the tropical and subtropical regions of the Andes. Pepino dulce fruit can be harvested at different stages of ripeness. As the majority of vegetables of the family *Solanaceae,* the fruit is abundant in potassium. Since there are no fertilizer recommendations for pepino dulce grown under cover, a study was launched to determine the fertilizer requirements of this vegetable. The aim of this study was to evaluate the effect of increasing magnesium rates and fruit ripeness stages on macronutrient content and ratios in the fruit of pepino dulce cv. Konsuelo. A two-factorial experiment in a completely randomized design was conducted in 2005-2007, in a tall, unheated, plastic tunnel at the Experimental Garden of the University of Warmia and Mazury in Olsztyn. Pepino dulce was propagated by cuttings taken from stock plants grown from seeds in 2004. The rooted cuttings were transferred to Kick-Brauchman pots filled with 9 dm^3 mineral soil with pH 6.8. Experimental factors were as follows: I – Mg rates: 0.5, 1.0, 1.5 g Mg $plant^{-1}$, II – fruit ripeness stages: ripe fruit showing a typical fully ripe color (yellowish-purple, yellow, cream), unripe green-colored fruit that has reached a typical form and size. The experiment was performed in four replications, and each replication comprised a pot with a single plant. Every pot was fertilized with 2 g N applied as $CO(NH_2)_2$, 3 g K applied as K_2SO_4 and increasing rates of Mg applied as $MgSO_4 \cdot 7\ H_2O$. Non-fertilized plants served as a control treatment. The plants were pruned for two stems. Fruit samples for chemical analyses were collected at full ripening (in mid-August). The concentrations of organic N, P, K, Ca and Mg in pepino fruit were determined, and the following weight ratios were calculated: Ca : P, Ca : Mg, K : Mg, K: (Ca + Mg), K : Ca. The results of chemical analyses were processed statistically by an analysis of variance (ANOVA), using Statistica 8.0 software. The highest total nitrogen and

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potassium levels were noted in the fruit of plants fertilized with the lowest magnesium rate (0.5 g Mg per plant), while the fruit of plants fertilized with the highest magnesium rate (1.5 g Mg per plant) accumulated the highest amounts of calcium and magnesium. The highest phosphorus content was reported in the fruit of non-fertilized plants. Fully ripe fruit contained significantly more nitrogen an magnesium, while unripe fruit had a higher content of phosphorus, potassium and calcium. An adequate Ca:Mg ratio, a narrow Ca:P ratio and wide K:Mg, K:(Ca + Mg) and K:Ca ratios were observed in all treatments.

Key words: *Solanum muricatum*, magnesium fertilization, macronutrients.

WPŁYW NAWOŻENIA MAGNEZEM NA ZAWARTOŚĆ MAKROELEMENTÓW W OWOCACH PEPINO (*SOLANUM MURICATUM* AIT.)

Abstrakt

Pepino (*Solanum muricatum* Ait.) należy do rodziny psiankowatych (*Solanaceae*), pochodzi z tropikalnych i subtropikalnych rejonów Andów. Owoce pepino można zbierać w różnych fazach dojrzałości. Jak w przypadku większości warzyw z rodziny *Solanaceae*, są one zasobne przede wszystkim w potas. Z powodu braku informacji o zaleceniach nawozowych do uprawy pepino pod osłonami, podjęto badania nad określeniem potrzeb nawozowych tego warzywa. Celem badań była ocena wpływu wzrastających dawek magnezu oraz stopnia dojrzałości owoców pepino na zawartość makroskładników, a także ich wzajemnych proporcji. Owoce pepino odmiany Konsuelo badano w latach 2005-2007 w wysokim nieogrzewanym tunelu foliowym, zlokalizowanym w Ogrodzie Doświadczalnym Uniwersytetu Warmińsko-Mazurskiego w Olsztynie. Pepino uprawiano z sadzonek pędowych, które pobierano z egzemplarzy uzyskanych z wysiewu nasion w 2004 r. Po ukorzenieniu sadzonki przesadzano do wazonów typu Kick-Brauchmana napełnionych 9 dm^3 gleby mineralnej o pH 6,8. Doświadczenie przeprowadzono jako dwuczynnikowe w układzie kompletnej randomizacji. Badano wpływ czynników: I – dawka Mg: 0,5; 1; 1,5 g Mg roślinę$^{-1}$; II – stopień dojrzałości owoców: dojrzałe – wybarwione (żółtofioletowe, żółte, kremowe), niedojrzałe – wyrośnięte, ale zielone. Eksperyment prowadzono w 4 powtórzeniach, powtórzenie stanowił wazon z pojedynczą rośliną. Do każdego wazonu wprowadzono: 2 g N w postaci $CO(NH_2)_2$, 3 g K w formie K_2SO_4 oraz wzrastające dawki Mg w formie $MgSO_4 \cdot 7H_2O$. Kontrolę stanowiły rośliny bez nawożenia. Rośliny prowadzono na 2 pędy. Owoce do analiz chemicznych zbierano w pełni owocowania roślin (ok. połowy sierpnia). W owocach oznaczano zawartość Nog., P, K, Ca oraz Mg. Obliczono również wagowe proporcje – Ca:P, Ca:Mg, K:Mg, K:(Ca+Mg) oraz K:Ca. Wyniki analiz chemicznych opracowano statystycznie, stosując program Statistica 8,0 i analizę wariancji ANOVA. Najwięcej azotu ogółem i potasu stwierdzono w owocach roślin nawożonych najmniejszą (0,5 g Mg na roślinę) dawką magnezu, najwięcej wapnia i magnezu – dawką maksymalną (1,5 g Mg na roślinę), natomiast najwięcej fosforu było w owocach roślin nienawożonych magnezem. Istotnie więcej azotu i magnezu zawierały owoce dojrzałe, natomiast fosforu, potasu i wapnia – owoce niedojrzałe. W każdym z wariantów doświadczenia zanotowano prawidłowe proporcje Ca : Mg, zawężone Ca : P oraz szerokie K : Mg, K : (Ca + Mg) oraz K : Ca.

Słowa kluczowe: *Solanum muricatum*, nawożenie magnezem, makroelementy.

INTRODUCTION

Pepino dulce (*Solanum muricatum* Ait.) belongs to the family *Solanaceae* and it is closely related to the tomato, pepper and potato. It is native to South America, to the tropical and subtropical regions of the Andes, where it grows at high altitudes of up to 3 000 m a.s.l. Today pepino dulce is cultivated mostly in the mountains of Latin America, in New Zealand, East Africa, East Asia, Australia, in the Canary Islands and in the Mediterranean region. It enjoys increasing popularity and continues to gain interest among gardeners throughout Europe, including in Poland. This is highly important as the typical Polish diet includes a few vegetable species only, and because eating a low variety of foods with a low nutritional value is one of the main reasons for lifestyle diseases (PROHENS et al. 1996, HEISER, ANDERSON 1999, Lost Crops of the Incas 1989, NALBORCZYK 1999, ADAMCZYK 2002).

Pepino dulce fruit can be harvested at different stages of ripeness. Unripe (green-colored) fruit resembles the cucumber in taste, flavor and aroma. It can be consumed raw or cooked. Ripe fruit can be served as a dessert. Their taste is similar to that of melons and mangos. As the majority of vegetables of the family *Solanaceae,* pepino fruit is abundant in potassium.

Macronutrients are building blocks for the human body whose healthy development is dependent upon their bioavailability. Magnesium is part of chlorophyll in green plants, and it helps activate many plant enzymes needed for growth. According to JĘDRZEJCZAK et al. (1999), vegetables are not a rich source of magnesium, compared with other edible plants. The deficiency of macroelements, in particular magnesium and calcium whose intake does not always meet the nutritional needs of humans and animals, may have serious health implications. Since there are no fertilizer recommendations for pepino dulce grown under cover, a study was launched to determine the fertilizer requirements of this vegetable.

The aim of this study was to evaluate the effect of increasing magnesium rates and fruit ripeness stages on macronutrient content and ratios in pepino dulce fruit.

MATERIALS AND METHODS

Pepino dulce cv. Konsuelo (Gavrish) was used in the study. A two-factorial experiment in a completely randomized design was conducted in 2005--2007, in a tall, unheated, plastic tunnel at the Experimental Garden of the University of Warmia and Mazury in Olsztyn. Pepino dulce was propagated by cuttings (10 cm in length) taken from stock plants grown from seeds in 2004. The rooted cuttings were transferred to Kick-Brauchman pots filled

with 9 dm^3 mineral soil with pH 6.8. Each year the cuttings were planted in a plastic tunnel between 10 and 15 May. The growing season, from taking cuttings from stock plants until the end of harvest, lasted approximately seven months. Experimental factors were as follows:

I – Mg rates: 0.5, 1.0, 1.5 g Mg $plant^{-1}$;
II – fruit ripeness stages: ripe fruit showing a typical fully ripe color (yellowish-purple, yellow, cream), unripe green-colored fruit that has reached a typical form and size.

The experiment consisted of six treatment combinations (three magnesium rates and two fruit ripeness stages). It was performed in four replications, and each replication comprised a pot with a single plant. Every pot was fertilized with 2 g N applied as $CO(NH_2)_2$, 3 g K applied as K_2SO_4 and increasing rates of Mg applied as $MgSO_4 \cdot 7H_2O$. Phosphorus fertilizers were not applied as soil was found to be rich in this element. Nitrogen, potassium and magnesium were applied as solutions. The first rate was administered three days after transferring the cuttings to pots, and the next two rates were applied at one-week intervals. Non-fertilized plants served as a control treatment.

The plants were pruned for two stems. Fruit samples for chemical analyses were collected at full ripening (in mid-August). Averaged samples from each treatment were comminuted, dried to constant weight at 65°C and ground. The weight of an individual samples of fresh plant material was 2 000 g.

The concentrations of organic nitrogen, phosphorus, potassium, calcium and magnesium in pepino fruit were determined. The content of organic N (by the Kjeldahl method), P (by the vanadium-molybdenum method), K and Ca (by flame photometry – AES) and Mg (by atomic absorption spectrometry– AAS) was estimated following wet mineralization in H_2SO_4+H_2O_2. The concentrations of total nitrogen, phosphorus, potassium, calcium and magnesium in pepino fruit were comparable in 2005, 2006 and 2007, which is why they are presented as mean values for the years of the study. The following weight ratios were also calculated: Ca : P, Ca : Mg, K : Mg, K: (Ca+Mg), K : Ca.

The results of chemical analyses were processed statistically by an analysis of variance (ANOVA), using Statistica 8.0 software.

RESULTS AND DISCUSSION

A statistical analysis showed that both experimental factors, i.e. magnesium rates and fruit ripeness stages, had a significant effect on the content of all analyzed macronutrients in pepino dulce fruit (Table 1). The total nitrogen content of the fruit varied widely, from 5.11 to 17.78 g kg^{-1} d.m. The

Table 1

The effect of magnesium rates and fruit ripeness stages on the macronutrient content of pepino dulce fruit

Mg rates in g per plant	Ripeness stage	(g kg^{-1} d.m.)				
		N_{total}	P	K	Mg	Ca
0	fully ripe	6.24	2.92	17.06	0.89	1.63
	unripe	5.11	3.03	18.22	0.85	2.00
Average		5.67	2.97	17.64	0.87	1.82
0.5	fully ripe	17.78	2.66	22.15	1.17	1.63
	unripe	12.50	3.03	21.55	0.76	2.37
Average		15.14	2.84	21.85	0.97	2.00
1.0	fully ripe	7.69	1.86	14.81	0.75	0.69
	unripe	5.90	1.96	16.00	0.71	2.48
Average		6.80	1.91	15.41	0.73	1.59
1.5	fully ripe	9.85	2.34	17.90	1.12	1.13
	unripe	9.00	2.81	25.00	1.00	3.06
Average		9.43	2.58	21.45	1.06	2.09
Average for ripeness stage	fully ripe	10.39	2.44	17.98	0.98	1.27
	unripe	8.13	2.71	20.19	0.83	2.47
Average		9.26	2.58	19.09	0.91	1.87
$LSD_{0.01}$ for						
I – fertilization		0.43	0.15	0.44	0.02	0.15
II – ripeness stage		0.30	0.11	0.31	0.01	0.11
I × II – interaction		0.60	0.16	0.63	0.03	0.22

lowest nitrogen amount was recorded in the fruit of non-fertilized plants, while the highest – in the fruit of plants fertilized with the lowest magnesium rate. Ripe fruit contained significantly more nitrogen than unripe, green-colored fruit. The phosphorus content of the analyzed plant material ranged between 1.86 and 3.03 g kg^{-1} d.m. The fruit of non-fertilized plants accumulated the largest quantity of phosphorus. Similarly as in a study by Kowalczyk, Kobryń (2002), unripe fruit was richer in phosphorus.

The potassium content of the edible parts of pepino plants was within the 14.81-25.00 g kg^{-1} d.m. range, and it was influenced by both experimental factors. The highest potassium concentrations were noted in the fruit of plants fertilized with the lowest magnesium rate (21.85 g kg^{-1} d.m.) and in unripe fruit (20.19 g kg^{-1} d.m.). Kowalczyk, Kobryń (2002) demonstrated that ripe pepino fruit had a higher potassium content, and Rubio et al. (2002) reported higher potassium concentrations in red peppers, in comparison with green. The results obtained by Bernardo et al. (2008), Flores et al. (2009) and Grembecka et al. (2008) show that green peppers contain more potassium.

In the present study, the magnesium content of pepino dulce fruit ranged from 0.71 to 1.17 g kg^{-1} d.m. Magnesium fertilization levels had a significant effect on the amount of this macronutrient accumulated in fruit. The highest magnesium concentrations were determined in the fruit of plants fertilized with the highest magnesium rate. Ripe fruit had a higher magnesium content (by 18% on average) than unripe fruit. The above results are consistent with the findings of KOWALCZYK, KOBRYŃ (2000a, 2002), REDGEWELL, TURNER (1986). KOWALCZYK, KOBRYŃ (2002) found that unripe pepino fruit contained more magnesium than ripe fruit. In a study by FLORES et al. (2009), green peppers had a higher magnesium content than red peppers, whereas BERNARDO et al. (2008) and RUBIO et al. (2002) reported that red pepper fruit contained more magnesium.

The calcium content of pepino dulce fruit was within a wide range of 0.69 to 3.06 g kg^{-1} d.m. The quantity of this element was clearly affected by both magnesium fertilization levels and fruit ripeness stages. A significantly higher calcium content was noted in pepino plants fertilized with the highest magnesium rate (2.09 g kg^{-1} d.m.) and, similarly as in an experiment by KOWALCZYK, KOBRYŃ (2002), in unripe fruit (2.47 g kg^{-1} d.m.).

The concentrations of the analyzed macronutrients in pepino dulce fruit were comparable with those reported by KOWALCZYK, KOBRYŃ (2000a,b, 2002), KOWALCZYK et al. (2004) and REDGEWELL, TURNER (1986) for the same species.

In addition to the concentrations of mineral nutrients in the edible parts of plants, also their ratios are an important indicator of nutritive value (KOTOWSKA, WYBIERALSKI 1999). According to CZAPLA, NOWAK (1995) and RADKOWSKI et al. (2005), the optimum ratios between macroelements in the diet of mammals should not be higher than: Ca : P – 2, Ca : Mg – 3, K : (Ca + Mg) – 1.6-2.2, K : Mg – 6, K : Ca – 2. KOTOWSKA and WYBIERALSKI (1999) and MATRASZEK et al. (2002) demonstrated that the above ratios may vary widely depending, among others, on the species, edible part of a plant, cultivation time and fertilization regime. Wider than optimal Ca:Mg and Ca:P ratios could be indicative of magnesium and phosphorus deficiency.

The Ca : P ratio was narrow in all collected pepino fruit samples, ranging from 0.4 to 1.3, regardless of magnesium rates and fruit ripeness. The Ca : Mg ratio in pepino fruit was adequate. In the majority of cases, the ratios of macronutrients in unripe fruit were highly satisfactory (3.0 on average). In ripe fruit the investigated ratios were narrower, within the 0.9-1.8 range. Increasing magnesium rates contributed to the widening of the above ratios in unripe fruit, and to their narrowing in ripe fruit. The K : Mg ratio in pepino fruit was very wide, ranging from 16.0 to 28.4. As regards the nutritional quality of pepino dulce, a more favorable K : Mg ratio was noted in unripe fruit. Increasing magnesium rates contributed to the narrowing of this ratio. The K : (Ca + Mg) ratio in pepino fruit ranged from 5.0 (1.0 g Mg, unripe fruit) to 10.3 (1.0 g Mg, ripe fruit). In all cases the above ratio was much wider than the optimal value. A more desirable K: (Ca + Mg)

ratio was recorded in unripe fruit. Magnesium fertilization had no significant effect on the proportions between the above macronutrients. The K : Ca varied within the widest range of 6.5 (1 g Mg, unripe fruit) to 21.5 (1 g Mg, ripe fruit). This ratio was determined primarily by the degree of fruit ripeness, while magnesium rates were of lesser importance (Table 2).

Table 2

The effect of magnesium rates and fruit ripeness stages on weight ratios between macronutrients in pepino dulce fruit

Mg rates in g per plant	Ripeness stage	K : Ca	K : Mg	Ca : P	Ca : Mg	K : (Ca+Mg)
0	fully ripe	10.5	19.2	0.6	1.8	6.8
	unripe	9.1	21.4	0.7	2.4	6.4
Average		9.7	20.3	0.6	2.1	6.6
0.5	fully ripe	13.6	18.9	0.6	1.4	7.9
	unripe	9.1	28.4	0.8	3.1	6.9
Average		10.9	22.5	0.7	2.1	7.4
1.0	fully ripe	21.5	19.7	0.4	0.9	10.3
	unripe	6.5	22.5	1.3	3.5	5.0
Average		9.7	21.1	0.8	2.2	6.6
1.5	fully ripe	15.8	16.0	0.5	1.0	8.0
	unripe	8.2	25.0	1.1	3.1	6.2
Average		10.3	20.2	0.8	2.0	6.8
Average for ripeness stage	fully ripe	14.2	18.3	0.5	1.3	8.0
	unripe	8.2	24.3	0.9	3.0	6.1
Average		10.2	21.0	0.7	2.1	6.9

CONCLUSIONS

1. Magnesium rates and fruit ripeness stages had a significant effect on the macronutrient content of pepino dulce fruit.

2. The highest total nitrogen and potassium levels were noted in the fruit of plants fertilized with the lowest magnesium rate (0.5 g Mg per plant), while the fruit of plants fertilized with the highest magnesium rate (1.5 g Mg per plant) accumulated the highest amounts of calcium and magnesium. The highest phosphorus content was reported in the fruit of non-fertilized plants.

3. Fully ripe fruit contained significantly more nitrogen an magnesium, while unripe fruit had a higher content of phosphorus, potassium and calcium.

4. An adequate Ca : Mg ratio, a narrow Ca : P ratio and wide K : Mg, K : (Ca + Mg) and K : Ca ratios were observed in all treatments.

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EFFECT OF MULTI-MICRONUTRIENT FERTILIZERS APPLIED TO FOLIAGE ON NUTRITIONAL STATUS OF WINTER OILSEED RAPE AND DEVELOPMENT OF YIELD FORMING ELEMENTS

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Abstract

Yields of oilseed rape harvested by farmers in Poland are usually much below the attainable potential of currently cultivated varieties, mainly because of the insufficient supply of nutrients during the yield foundation period, which determines the final number of secondary branches. This situation is typical of whole Poland, but may take place even on farms where high yields are harvested, for example in 2007. In 2006, 2007 and 2008, the year effect of multi-micronutrient fertilizers on yield of seeds, elements of yield structure and macronutrient content was studied. Multi-micronutrient fertilizers were applied twice over oilseed rape foliage during its pre-anthesis growth (BBCH45 and 53). In 2007, due to a drought in April, the harvested yields of seeds were below the country's average. However, in each year of the study, a significant increase in the seed yield owing to the foliar application of multi-micronutrient fertilizers was found. The increase in the yield of seeds, averaged for the three years, reached 0.486 t ha^{-1} for the NPK+MiMo treatment (full set of micronutrients) and 0.36 t ha^{-1} for the NPK + Mi treatment (without molybdenum). The increments of the oilseed rape yield resulted from an increased number of developed secondary branches. This yield-forming element was an indirect result of the application of multi-micronutrient fertilizers, which affected the nitrogen economy by oilseed rape plants during the foundation period of their growth. At the same time, the increase in seed yield was significantly modified by the total number of developed pods, which is shaped during the yield-forming period of oilseed rape crop growth. Under conditions of the study, the magnesium content in secondary branches was found to be an element significantly correcting their number, thus increasing the yield of seeds.

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Key words: oilseed rape, multi-micronutrient fertilizer, macronutrient content, yield structure elements.

WPŁYW DOLISTNEGO STOSOWANIA WIELOSKŁADNIKOWYCH NAWOZÓW MIKROLEMENTOWYCH NA STAN ODŻYWIENIA RZEPAKU OZIMEGO I WYKSZTAŁCENIE ELEMENTÓW STRUKTURY PLONU

Abstrakt

Plony rzepaku zbierane przez rolników w Polsce kształtują si,e na poziomie dużo niższym od potencjału aktualnie uprawianych odmian. Główną przyczyną jest niedostateczne odżywienie roślin w okresie budowy podstaw struktury plonu, gdy ustala się ostateczna liczba pędów bocznych. Jest to przypadek typowy w Polsce, lecz może pojawić się także w gospodarstwach zbierających, z zasady, duże plony, jaki wystąpił w trakcie badań w roku 2007. W latach 2006, 2007, 2008 badano wpływ dolistnego stosowania nawozów mikroelementowych na plon nasion, elementy struktury plonu i zawartość makroskładników. Nawozy mikroelementowe stosowano 2-krotnie w okresie przed kwitnieniem rzepaku ozimego (BBCH54 i 53). W 2007 r. z powodu suszy w okresie formowania się podstaw struktury plonu (kwiecień) plony były mniejsze od średniej krajowej. Jednakże, we wszystkich trzech latach badań, wzrost plonów w następstwie dolistnej aplikacji nawozów mikroelementowych był istotny. Wzrost plonów, średnia z lat, wyniósł 0.486 t ha^{-1} dla wariantu NPK+MiMo (pełen zestaw mikroelementów) i 0.36 dla wariantu NPK+Mi (bez molibdenu). Uzyskany wzrost plonów wynikał bezpośrednio z wytworzenia przez roślinę większej liczby pędów bocznych. Ten element struktury plonu był pośrednio następstwem działania nawozu mikroelementowego, który istotnie kształtował gospodarkę azotową rośliny w okresie budowy podstaw struktury plonu. Jednocześnie wzrost plonu nasion podlegał istotnej modyfikacji wynikającej z całkowitej liczby łuszczyn na roślinie, cechy plonu kształtowanej w okresie formowania się plonu nasion. W warunkach badań zawartość magnezu w pędach bocznych okazała się czynnikiem istotnie korygującym ich liczbę, a tym samym zwiększającym plon nasion.

Słowa kluczowe: rzepak, wieloskładnikowy nawóz mikroelementowy, zawartość makroskładników, elementy struktury plonu, zbiór.

INTRODUCTION

Oilseed rape varieties currently cultivated in Poland, with respect to a standard variety (indexing the level of attainable yield), yield at a level of 4.0 to 5.0 t ha^{-1} (COBORU 2009). However, yields harvested by farmers are much lower, ranging from 2.6 to 3.1 (GUS 2007, 2009). These two data sets clearly demonstrate that even in good years farmers in Poland are able to exploit *ca* 60% of attainable yield of oilseed rape.

The final yield of oilseed rape seeds is a result of many natural and agronomic factors affecting the plant growth during the growing season. Spring is particularly important for the growth of this crop. It is frequently assumed the final yield of seeds is a function of four main elements such as: i) number of plant per unit area; ii) number of capsules per plant,

iii) number of seeds per capsule, and iv) individual mass of a seed (expressed as 1000 seeds weight, TSW) (RATHKE 2006). It is only the first element that is established early in spring. All the other elements of yield structure are formed during anthesis and the seed filling period. Any internal or external factor disturbing carbon, nitrogen or water plant economy negatively affects plant yield structure, causing decay and drop of the youngest pods or even secondary branches (HABEKOTTE 1993).

The main cause of the shedding of the newest branches and pods is shortage of carbon due to competition between main oilseed rape plant organs, such as leaves and main branches with new developing branches as well as simultaneous in-organ competition among older and the newest developing pods. All these new, fast growing plant parts tend to have a high demand for assimilates to cover both the cost of respiration and new seed-oriented tissue ingrowths (LAWOR 2002). All these processes depend on nitrogen and carbon balance, which cannot be achieved without a sufficient supply of micronutrients (GRUSAK et al. 1999). Hence, it was assumed that application of multi-micronutrient fertilizer, containing mostly iron, manganese as well as a smaller amount of molybdenum, to oilseed rape foliage during plant growth stages preceding anthesis will be a simple agronomic measure taken to reduce loss of potentially fruit-bearing pods.

The main objective of this study has been to assess sensitivity of oilseed rape yield structure elements to the nutritional status of plant canopy at harvest and in turn the yield of seeds to an external supply of multi-micronutrient fertilizers.

MATERIAL AND METHODS

Field experiments were conducted in three consecutive growing seasons, 2006, 2007 and 2008, in Bierzglinek, a village 50 km east of Poznań (Poland; 52.40°N, 16.90°E). The field trials were established on soil classified according to the FAO as albic luvisol, originating from loamy sand lying on sandy loam or sand postglacial materials, and classified according to the Polish agronomical taxonomy to class IVb, good rye complex. The soil was agrochemically assayed in the topmost soil layer (0-30 cm) throughout the three years of the experiment, providing the following data: pH_{KCl} – 5.0 (in 2006); 5.1 (2007); 5.7 (2008); P and K (lactate buffor, pH 3.55) – 79 and 128; 77 and 101; 91 and 167 mg kg^{-1}, respectively; Mg (0.0125 M $CaCl_2$) – 101; 92 and 68 mg kg^{-1}, respectively. Each year, cereals (winter wheat or spring barley) were a preceding crop. Oilseed rape was sown at the end of August (VIII/3). Autumn dressing of P, K and Mg fertilizers was performed each year in doses based on the actual soil fertility level for each nutrient and the expected yield of seeds of 4.0 t ha^{-1}. Nitrogen dressing was conducted in ac-

cordance to soil N_{min} test (the beginning of plant spring regrowth) and fertilizers were applied in two split rates, i.e. at the beginning of spring regrowth and three weeks later. Herbicides and all other agrotechniques were applied according to standard practices.

The single-factor experiment consisted of three treatments:

1. Basic N, P, K fertilization – acronym NPK;
2. NPK + multi-micronutrient fertilizer, containing cations plus molybdenum (NPK+MiMo);
3. NPK + multi-micronutrient fertilizer without molybdenum, Mi, (NPK+Mi).

All treatments were replicated 6 times in a simple block design. Each replication was 18 m wide and 50 m long. The experiment was located on a large field characterized by uniform distribution of plants of 50(± 2) per m^2. The tested multi-microelement fertilizers were prepared at concentrations of 70 Cu, 400 Fe, 170 Mn, 150 Zn mg dm^{-3} chelated by EDTA for the Mi formulation and plus 40 g dm^{-3} of Mo as ammonium molybdate for the MiMo formulation. Both fertilizers were applied to oilseed rape foliage during two consecutive stages in the pre-anthesis growth of its canopy, accordingly to the BBCH scale, at 45 (five internodes visible) and 53 (flowers buds raised above the youngest leaves).

The average annual precipitation and average temperatures during five months of oilseed rape growth in spring were 202 mm and 13.5ºC in 2006, 282 mm and 11.5ºC in 2007 and 178.2 mm and 12.6ºC in 2008. It should be emphasized that in two years (2006 and 2008) the weather conditions for oilseed rape growth were much more suitable in comparison to 2007, characterized by a severe drought, which took place in April, i.e. during stem elongation and inflorescence emergence.

At maturity (the third decade of July), crops were harvested from an area of 80 m^2 using a combine-harvester. Total yields of seeds were adjusted to 8% moisture content. Plant samples, each containing 8 plants per replication, were taken at the stage of the physiological maturity of seeds. In the second step of our analysis, each plant was partitioned into subsamples (plant parts = elements of yield structure) and than dried (65ºC). The following elements of yield structure were determined: i) number of secondary branches, acronym SB, ii) number of pods per main branch, PMB, iii) number of pods per each secondary branch, PSB, iv) number of seeds per pod of the main branch, SMB, v) number of seeds per pod of the secondary branch, SSB, vi) weight of 1000 seeds (TSW). The content of nitrogen and other elements (as listed in Table 1) of respective plant tissues was determined by using the Kjeldahl method (Kjeltec Auto Distillation) and the flame atomic absorption spectrometry (FASS) method, respectively. Concentrations of all the investigated elements in all the tested organs are expressed on a dry matter basis.

The empirical data underwent conventional analysis of variance. The least significant difference values (LSD at $P = 0.05$) were calculated to es-

tablish the significance of mean differences. Path analysis and stepwise regression were applied to assess interrelationships between oilseed yield and its yielding components (KONYS, WIŚNIEWSKI 1984). Linear regression fit was determined for all the replications from 2006 and 2008, representing high yielding populations, by using statistical software Statistica 7. The goodness of fit was evaluated by R^2 values.

Table 1

Effect of multi-micronutrient fertilizers on macronutrient content of the main branch at harvest

Factor	Level of factor	Nutrients (g kg^{-1})				
		N	P	K	Mg	Ca
Fertilizers	NPK	8.972	2.078	16.99	1.222	7.189
	NPK+MiMo	9.333	2.172	17.24	1.344	7.550
	NPK+Mi	9.383	2.217	17.21	1.244	7.494
LSD, $P \leq 0.05$		0.027	0.010	0.013	0.007	0.021
Years	2006	9.107	2.139	17.13	1.272	7.483
	2007	9.350	2.183	1.718	1.283	7.500
	2008	9.233	2.144	17.13	1.256	7.250
LSD, $P \leq 0.05$		-	-	-	-	0.021

RESULTS AND DISCUSSION

Yield of seeds and its relationship to yield forming elements

Harvested yields of oilseed rape were variable due to the effect of year-to-year variability and the applied multi-micronutrient fertilizers (Figure 1). With respect to the first factor, yields decreased in the order: 2006 > 2008 > 2007. Yields harvested in the NPK treatment tended to be lower, corresponding to *ca* 78%, 68%, 55%, of the "standard variety yield" in the subsequent years. These results clearly indicate the size of a yield gap caused by the weather in each growing season. However, as compared to the country's average, the harvested yields were 47% and 6% higher in 2006 and 2008, being 12% lower in 2007 (HEIMANN, BRONIARZ 2009, GUS 2008, 2009). The multi-micronutrient fertilizers significantly increased the yield of oilseed rape seeds in comparison to the NPK treatment, considered as an experimental control. The highest increase, averaged for the three years to 0.486 t ha^{-1}, was attributed to the NPK+MiMo treatment. A slightly smaller increase, namely 0.362, was noted for the NPK+Mi treatment. However, irrespectively of the weather in a given growing season, both fertilizers affected the yield of seeds in the same manner. The existing yield gap was therefore

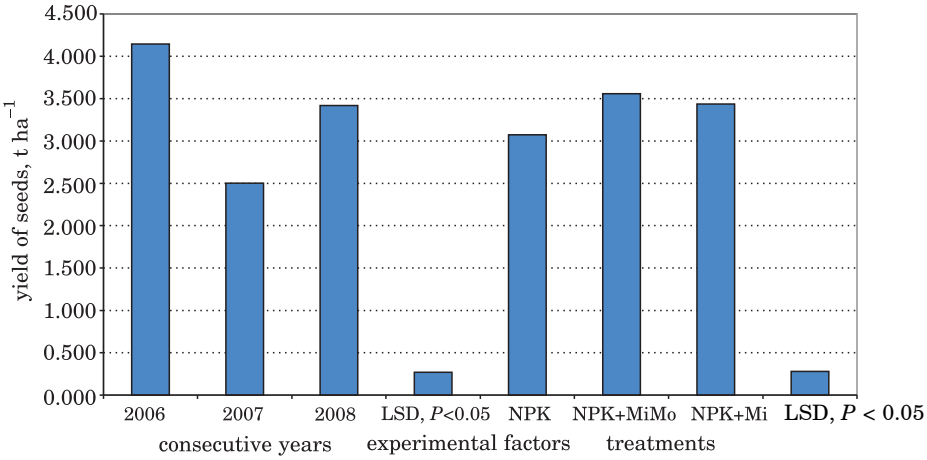

Fig. 1. Yields of oilseed rape in consecutive growing seasons and in response to the multi-micronutrient fertilizers

significantly covered owing to the application of both fertilizers, especially the MiMo variant. In 2006, the above gap diminished to 9%; but in 2008 it reached 21% and in 2007 it was the worst, namely 38%. Considering the effect of the MiMo fertilizer, in relative terms, the highest yield gap cover was noticed in 2007 (+17%), which indirectly suggests the stabilization effect of the applied multi-micronutrient fertilizers on oilseed rape growth and yielding.

The yield increase determined in our experiment was validated by an evaluation of the sensitivity of yield structure elements to the applied multi-micronutrient fertilizers (Table 2). The first yield structure component was the number of secondary branches (SB), which depends on plant density and water and nitrogen supply from the stage of rosette up to full flowering (Habekotte 1993, Rathke et al. 2006). The influence of initial characteristics of the plant canopy, i.e. number of plants per unit area, was eliminated by locating the experiments on part of a field with a constant number of plants, i.e. *ca* 50±2 averaged for the three years. Both factors, independently of each other, affected however the number of secondary branches (SB). The effect of year-to-year variability was significant in the two following years compared to 2006. The fFertilizers applied to oilseed rape foliage increased significantly the number of branches compared to the NPK object. The number of pods developed on the main branch (PMB) was significantly affected only by the applied fertilizers, where the effect was much stronger than in the case of secondary branches. The number of pods per secondary branch (PSB) was much lower than on the main branch and the response to micronutrient fertilizers was small and insignificant. The total number of pods per plant (TPP) showed a strong response to both tested fertilizers. However, a significant response was attributed only to the NPK+MiMo ferti-

Table 2

Effect of multi-micronutrient fertilizers on yield-forming elements of oilseed rape

Factor	Level of factor	Elements of yield structure						
		SB*	PMB	PSB	TPP	SMB	SSB	TSW
Fertilizers	NPK	5.806	38.89	28.17	166.7	14.36	12.12	4.122
	NPK+MiMo	6.706	46.61	33.72	226.9	16.39	12.57	4.422
	NPK+Mi	6.578	45.39	31.17	207.5	15.01	12.32	4.367
LSD, $P \leq 0.05$		0.383	3.807	-	51.19	0.818	-	0.195
Years	2006	7.039	44.56	34.61	247.3	16.12	13.13	4.739
	2007	6.033	43.50	27.00	164.33	14.97	12.02	4.133
	2008	6.017	42.83	31.44	189.5	14.67	11.87	4.039
LSD, $P \leq 0.05$		0.370	-	-	-	0.791	0.698	0.188

*Acronyms: SB – number of secondary branches; PMB – number of pods per main branch; PSB – number of pods per secondary branch; TPP – total number of pods per plant SMB – number of seeds per pod of the main branch; SSB – number of seeds per pod of the secondary branch; TSW – thousand weight of seeds, g

lizer as compared to the NPK control. Plants fertilized with NPK+MiMo developed 36% more pods than in the NPK treatment.

The response of both vegetative oilseed rape organs and finally the yield of seeds to the applied multi-micronutrient fertilizers indirectly indicates improved nitrogen economy in the whole plant during its consecutive stages of growth, i.e. i) yield foundation and ii) yield formation (Habekotte 1993, Malagoli et al. 2005).

The number of seeds per pod is considered as one of the most important indices of oilseed rape growth conditions during the flowering and seed filling phases (Rathke et al. 2006). With respect of the main branch, a significant effect of both factors was noted. The effect of the applied multi-micronutrient fertilizers followed the same pattern as determined for the total number of pods. The number of seeds in a pod on a secondary branch was much lower than on the main branch. However, similarly to the main branch, the seasonal effect was significant, demonstrating much worse conditions in 2007 and 2008 in comparison to 2006. The weight of seeds is the last yield-forming element, generally highly sensitive to internal factors, i.e. plant nutritional status, and external factors, i.e. weather conditions (Rathke et al. 2006). The effect of the applied fertilizers showed the same pattern as for all the previously described yield structure elements, but its percentage increase in comparison to the NPK treatment was much lower. At the same time, the seasonal effect was much stronger, again indicating much worse growth conditions during the seed filling period in 2007 and 2008.

Taking into account all the elements of yield structure, one can find much similarity of the harvested yields to the total number of pods per plant (TPP). However, the conducted regression analyses showed slightly more complicated patterns due to different sets of data affecting yield of seeds depending on the applied multi-micronutrient fertilizer type. In the NPK treatment, as evidenced by the conducted stepwise regression, the yield of harvested seeds was significantly correlated to two elements, as seen in the equation:

$$Y = -1.773 + 0.120\ PMB + 0.826\ TSW, \text{ for } R^2 = 74.12\% \quad (1)$$

where:

Y – yield of seeds, t ha^{-1};
PMB – number of pods per main branch;
TSW – weight of a thousand seeds, g.

This equation is however only partly supported by the simultaneously conducted path analysis, which showed a decisive role of TSW evidenced by the path coefficients for the total number of pods per plant (TPP) – Figure 2a. In the NPK+MiMo treatment, the relationship showed a much more complicated pattern. The final yield of seeds (Y) was correlated to three yield elements such as i) number of secondary branches (SB), ii) number of pods per secondary branch (PSB) and iii) number of seeds per pod on a secondary branch (SSB). All of these elements, as presented below, affected significantly and positively the final yield of seeds:

$$Y = -1.382 + 0.435\ SB + 0.018\ PSB + 0.140\ SSB \quad \text{for } R^2 = 87.07\% \quad (2)$$

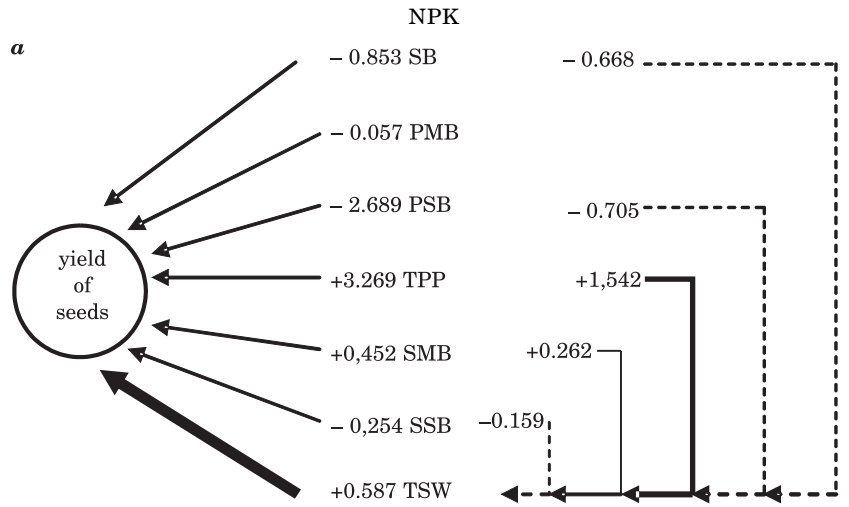

Fig. 2. Path diagram: relationships between yield-forming elements and yield of oilseeds rape

The path analysis, conducted simultaneously, indicated the number of secondary branches (SB) as the main single yield structure element directly affecting the yield of seeds and only slightly balanced via indirect effects of the other elements (Figure 2b). The most negative, but moderate effect has been attributed to the total number of pods per plant (TPP).

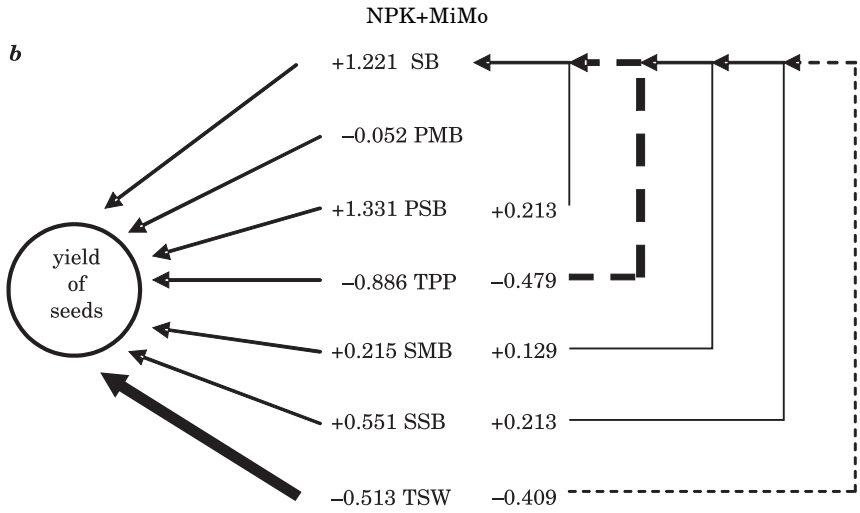

Fig. 2. Path diagram: relationships between yield forming elements and yield of oilseeds rape

The relationship analyzed for the NPK+Mi treatment confirmed the data obtained for the NPK+MiMo treatment. Generally, as implied by the stepwise regression analysis, the number of secondary branches (SB) affected the final yield of seeds with sufficient accuracy, as presented by the following equation:

$$Y = -0.499 + 0.645\ SB \qquad \text{for } R^2 = 84.43\% \qquad (3)$$

This finding has been corroborated by the path analysis (Figure 2c). The results underline the dominating role of the number of secondary branches in yield formation, although the yield is also significantly shaped by the total number of pods per plant.

As demonstrated by the analysis of all the above equations, the yield forming effects of the applied multi-micronutrient fertilizers are related to an increasing number of secondary branches and subsequently occurring events, i.e. a higher number of pods and seeds per pod. Such yield-oriented end-results of the fertilizers sprayed over leaves indirectly suggest significant changes in plant nitrogen economy (Malagoli et al. 2005). It can be concluded that any increase in the number of secondary branches on each oilseed rape plant needs to be balanced by an adequately developed number

of total pods per plant (TPP). Both yield forming characteristics significantly limited the yield increment on the NPK plot due to some excess of secondary branches and shortage of well-developed pods (see Figure 2a). Therefore, any production measure should be oriented towards increasing the number of pods per plant as a prerequisite of an increase in the final yield of seeds, but should also maintain some balance according to the following rule: the higher number of secondary branches, the lower number of total but well-developed pods (see Figures 2b,c).

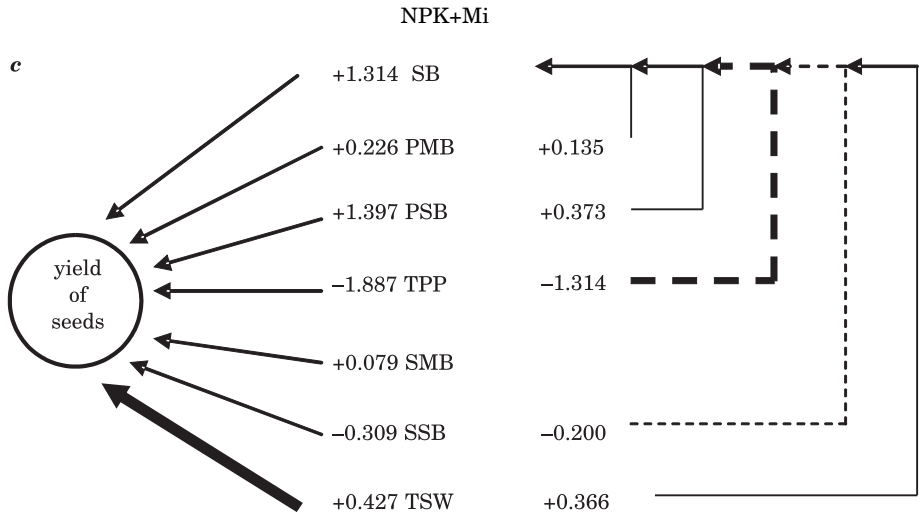

Fig. 2. Path diagram: relationships between yield forming elements and yield of oilseeds rape

Nutritional composition of plants at harvest

The second part of the present paper describes oilseed rape plant nutritional composition at physiological maturity shaped under the influence of the applied multi-micronutrient fertilizers against the background of year-to-year variability. In order to find some interesting rules, the nutrient composition of plant parts is described as shown in Table 2.

In the previous chapter it was explained that the applied multi-micronutrient fertilizers can be mainly affected the number of secondary branches (SB). The content of nutrients in secondary branches (Table 3) tended to be much higher than in a physiologically older main branch (Table 1). The highest differences between both plant parts were observed for calcium and magnesium, second to phosphorus and nitrogen, but excluding potassium. Plants exposed to an external supply of micronutrients significantly increased the content of all macronutrients of the main branch (raceme) and

Table 3

Effect of multi-micronutrient fertilizers on macronutrient content of secondary branches at harvest

Factor	Level of factor	Nutrients (g kg^{-1})				
		N	P	K	Mg	Ca
Fertilizers	NPK	9.283	3.139	17.61	2.222	12.68
	NPK+MiMo	10.05	3.489	17.88	2.383	13.37
	NPK+Mi	10.75	3.389	17.95	2.356	13.45
LSD, $P \leq 0.05$		0.051	0.020	0.021	0.009	0.030
Years	2006	10.04	3.389	17.76	2.283	13.16
	2007	10.03	3.333	17.82	2.300	13.15
	2008	10.01	3.294	17.87	2.379	13.19
LSD, $P \leq 0.05$		-	-	-	0.008	-

secondary branches. However, it was only the magnesium content in secondary branches that responded to both the applied fertilizers and seasons. At the same time, this element showed the highest difference in the concentration between the main and secondary branches, exceeding 80% in the NPK treatment. The MiMo fertilizer resulted in a significant Mg content increase, but caused a slight decrease of the gap between the main and secondary branches (down to 77%), significantly affecting the final yield of seeds:

$$Y_{NPKMiMo} = -2.453\ Mg + 9.841 \text{ for } n = 12,\ R^2 = 0.472 \text{ and } P = 0.01 \quad (4)$$

where:

$Y_{NPKMiMo}$ – yield of seeds, t ha^{-1};
Mg – Mg content in secondary branches, g kg^{-1} DM.

The negative sign of the direction index of the developed linear equation informs us about excess of magnesium in secondary branches. In order to explain this unspecific result, the levels of macronutrients in secondary branches were regressed against the indicative attribute of yield structure, i.e. number of secondary branches. The analysis corroborated the specific yield-forming effect of magnesium (Mg), which significantly affected the number of secondary branches (Y) in two of the three treatments:

1. NPK $Y = -3.457\ Mg + 13.88\ R^2 = 0.607$ for $n = 12$ and $P < 0.001$ (5)

2. NPK+MiMo $Y = -18.03\ Mg^2 + 80.42\ Mg - 82.22\ R^2 = 0.513$ for $n = 12$ (6)

The first equation clearly shows that oilseed rape canopy fertilized with NPK alone had excess magnesium in secondary branches, causing their

number to decrease alongside the increasing content of magnesium, up to 2.100 g kg DM. Foliar application of the MiMo fertilizer changed this relationship, indicating a positive effect of magnesium up to its critical content amounting to 2.230 g kg DM. It is well known that magnesium and molybdenum are an effective controller of nitrates taken up by plants (KAISER et al. 2005). Differences between the main and secondary branches were as follows: NPK – 103%, NPK+MiMo – 108% and NPK+Mi +115%. The last figure is too high, showing some disturbance of the gentle balance between the number of secondary branches and the total number of pods (see and compare Figures 2b and 2c).

The number of pods developed by the main or secondary branches was taken into account as an index of the final yield in the NPK treatment (the main branch, see equation No. 1; Figure 2a) in the NPK+MiMo (minor one, secondary branches, see equation No. 2; Figure 2b). Patterns of nitrogen and phosphorus content in pods followed all the rules found for vegetative branches (Tables 4 and 5). For the other elements, these differences tended

Table 4

Effect of multi-micronutrient fertilizers on macronutrient content of pods of the main branch at harvest

Factor	Level of factor	Nutrients (g kg^{-1})				
		N	P	K	Mg	Ca
Fertilizers	NPK	6.239	2.717	21.71	1.817	22.96
	NPK+MiMo	6.778	2.922	22.06	2.000	23.13
	NPK+Mi	6.717	2.856	22.03	1.956	23.49
LSD, $P \leq 0.05$		0.035	-	0.033	0.012	0.041
Years	2006	6.544	2.939	21.97	1.900	23.40
	2007	6.583	2.983	22.03	1.917	23.27
	2008	6.606	2.572	21.19	1.956	22.91
LSD, $P \leq 0.05$		-	0.027	-	-	0.040

to be negligible. Significant effect of the applied multi-micronutrient fertilizers on macronutrient content was much stronger for the main branch that for secondary branches. In the former case, it was only phosphorus content in pods that did not show any response to the applied fertilizers. Pods of secondary branches responded to the applied fertilizers in terms of the concentrations of N and P, showing significant their increase. The effect of year-to-year variability was generally small, concerning mainly the main branch. The content of calcium was used as a nutritional index for the final

seed yield forecast. Significant relationships were found only for treatments with the multi-micronutrient fertilizers, as shown below:

1. NPK+MiMo Y = 0.597 Ca - 9.796 for R^2 = 0.343 and n = 12, P = 0.05 (7)

2. NPK+Mi Y = 0.551Ca - 8.961 for R^2 = 0.334 and n = 12, P = 0.05 (8)

Seeds are the last yield component to be discussed, although thwey are the target of the oilseed rape crop production. The effect of the applied fertilizers on the macronutrient content in seeds was variable, although seeds are considered to be a conservative plant organ. Seeds developed on the main branch contained much less nitrogen in comparison to those developed on the secondary branches (Tables 6 and 7). The differences can be used to indicate the nutritional status of seeds at harvest. WOJNOWSKA et al. (1995), who demonstrated yields of seeds at a level of 5 t ha^{-1}, showed the

Table 5

Effect of multi-micronutrient fertilizers on macronutrient content of pods of secondary branches at harvest

Factor	Level of factor	Nutrients (g kg^{-1})				
		N	P	K	Mg	Ca
Fertilizers	NPK	8.822	3.144	21.64	1.917	23.14
	NPK+MiMo	9.311	3.339	21.79	2.000	23.52
	NPK+Mi	9.272	3.306	21.77	1.956	23.40
LSD, $P \leq 0.05$		0.022	0.013	-	-	-
Years	2006	9.206	3.322	21.80	1.928	23.56
	2007	9.000	3.250	21.60	1.950	23.47
	2008	9.200	3.217	21.80	1.994	23.04
LSD, $P \leq 0.05$		-	-	-	-	-

total nitrogen content (TNC) comparable to the one obtained here for seeds from the main branch. BARŁÓG et al. (2006) found different values of TNC, showing significant dependence on the level of harvested yield of seeds. For yields above 3.5 t ha^{-1}, the data presented by these authors are within the range found in the present study for the main branch, but for lower yields, the TNC content tended to be much higher. Therefore, the differences between TNC values for seeds of the main and secondary branches can be used as an index of seed immaturity. The effect of the applied multi-micronutrient fertilizers on nitrogen content was generally positive and significant, but only for the main branch. The content of the other nutrients showed a variable response to the applied experimental factor. The most conspicuous effect was found for phosphorus. This nutrient did not show

any significant differences between branches but at the same time significantly responded to the applied fertilizer. In respect of secondary branches, a year-to-year variability in the P content was observed. In comparison to the data presented by BARŁÓG et al. (2006) and Wojnowska et al. (2000), our values for P are much higher but those for calcium and potassium are much lower. However, the nutrients did not show any significant influence on the final yield of seeds.

Table 6

Effect of multi-micronutrient fertilizers on macronutrient content of seeds of the main branch at harvest

Factor	Level of factor	Nutrients (g kg^{-1})				
		N	P	K	Mg	Ca
Fertilizers	NPK	30.89	8.122	8.194	2.433	1.833
	NPK+MiMo	32.58	8.422	8.383	2.556	1.95
	NPK+Mi	32.00	8.461	8.344	2.483	2.056
LSD, $P \leq 0.05$		0.120	0.017	0.014	-	0.017
Years	2006	31.62	8.400	8.344	2.578	1.933
	2007	31.67	8.300	8.267	2.517	1.900
	2008	32.18	8.306	8.311	2.378	2.006
LSD, $P \leq 0.05$		-	-	-	0.013	-

Table 7

Effect of multi-micronutrient fertilizers on macronutrient content of seeds of secondary branches at harvest

Factor	Level of factor	Nutrients (g kg^{-1})				
		N	P	K	Mg	Ca
Fertilizers	NPK	35.20	8.394	8.300	2.411	1.911
	NPK+MiMo	36.31	8.522	8.461	2.500	2.067
	NPK+Mi	36.26	8.556	8.472	2.522	2.044
LSD, $P \leq 0.05$		-	0.014	-	-	0.010
Years	2006	35.44	8.406	8.400	2.494	2.000
	2007	35.90	8.533	8.450	2.500	1.983
	2008	36.42	8.533	8.483	2.439	2.039
LSD, $P \leq 0.05$		-	0.014	-	-	-

CONCLUSIONS

1. The yield gap occurring between the actually harvested and attainable yields of oilsseed rape can be partly covered by foliar application of multi-micronutrient fertilizers.

2. Status of yield structure elements at harvest can be applied as a very sensitive measure of oilseed crop response to the applied multi-micronutrient fertilizers during its pre-anthesis growth.

3. The multi-micronutrient fertilizers, in-season applied, through gentle changes of the nutritional status of oilseed rape plants allow farmers to improve the balance between the number of secondary branches and the total number of pods.

4. In the present experiment, the magnesium content in secondary branches was the most yield-forming nutritional factor.

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AN EVALUATION OF MACRONUTRIENT NUTRITIONAL STATUS OF SUGAR BEETS IN CRITICAL STAGES OF GROWTH IN RESPONSE TO FOLIAR APPLICATION OF MULTI-MICRONUTRIENT FERTILIZERS

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Abstract

The actual yields of sugar beets harvested by farmers in Poland are much below the yielding potential of currently cultivated varieties, even when grown on fertile soils. Deficiency of micronutrients can be considered as a factor which prohibits reaching a state of nutrient balance, especially in nitrogen, by a crop. The aim of the study was to assess the effect of foliar application of multi-microelement fertilizers on a sugar beet nutritional status in early stages of canopy growth and, in turn, on yields of taproots and recoverable sugar. For this purpose, in two growing seasons, 2005 and 2006, eight field trials were set up in the region of Wielkopolska on fertile soils originated from sandy loam. A simple experimental design comprised three treatments: (1) control (a plot fertilized only with NPK), and (2) multi-microelement fertilizer composed of cations (Mi), (3) cations plus boron (MiB). It has been found that the applied micronutrient fertilizers had a significant effect, irrespectively of the field location and seasonal yield variability, on the sugar beet nutritional status, especially in terms of nitrogen. Yields of taproots have increased by 31.6% and 22.1% for the NPK+Mi and NPK+MiB treatments, respectively. Almost the same degree of increase was noted for yields of recoverable sugar. The achieved nitrogen balance at the stage of harvestable part development, as measured at BBCH43, was probably the main reason for high positive response of sugar beet crop to external supply of micronutrients. All the analyzed standards of sugar beet nutritional status, DRIS indices, responded to the investigated experimental factor, i.e., foliar application of multi-microelement fertilizer, but only

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those for nitrogen underwent a change from negative to positive values, thus enabling us to make a reliable yield prognosis.

Key words: sugar beet, macronutrient content, critical stages of growth, DRIS indices, multi-microelement fertilizers.

OCENA STANU ODŻYWIENIA BURAKÓW CUKROWYCH W KRYTYCZNYCH FAZACH ROZWOJU W REAKCJI NA DOLISTNE NAWOŻENIE WIELOSKŁADNIKOWYMI NAWOZAMI MIKROELEMENTOWYMI

Abstrakt

Plony buraków cukrowych zbierane przez rolników w Polsce kształtują się na poziomie dużo niższym, nawet na glebach żyznych, niż plony potencjalne obecnie uprawianych odmian. Niedobór mikroelementów może być rozważany jako jeden z czynników, który uniemożliwia uzyskanie przez rośliny stanu równowagi żywieniowej, zwłaszcza w przypadku azotu. Celem badań była ocena wpływu dolistnie stosowanych wieloskładnikowych nawozów mikroelementowych na stan odżywienia roślin we wczesnych fazach rozwoju buraków, i w konsekwencji na plony korzeni i cukru. Cel zweryfikowano w latach 2005 i 2006, zakładając w Wielkopolsce 8 doświadczeń zlokalizowanych na żyznych glebach, wytworzonych z polodowcowych glin moreny dennej. Prosty schemat doświadczenia zawierał 3 kombinacje: (1) kontrolę (obiekt nawożony NPK) oraz 2 obiekty z dolistnie stosowanymi nawozami mikroelementowymi wieloskładnikowymi zawierające w swym składzie (2) kationy, (Mi), (3) kationy i bor (MiB). Stwierdzono, niezależnie od lokalizacji doświadczenia i zmienności sezonowej plonów, istotne działanie nawozów mikroelementowych na stan odżywienia roślin, zwłaszcza azotu. Plony korzeni zwiększyły się o 31.6% i 22.1%, odpowiednio w przypadku wariantu Mi i MiB. Analogiczną reakcję odnotowano dla plonów cukru. Główną przyczyną dodatniej reakcji buraków cukrowych na dolistną aplikację nawozów mikroelementowych było uzyskanie przez rośliny w fazie BBCH43 zbilansowanego stanu odżywienia roślin azotem. Analizowane normy odżywienia roślin, indeksy DRIS, wykazały reakcję na badany czynnik doświadczalny, czyli nawozy mikroelementowe wieloskładnikowe, lecz tylko w przypadku azotu nastąpiła zmian wartości indeksów z ujemnych na dodatnie, umożliwiając tym samym wiarygodną prognozę plonów.

Słowa kluczowe: buraki cukrowe, makroelementy, krytyczne fazy rozwoju, indeksy DRIS, wieloskładnikowe nawozy mikroelementowe.

INTRODUCTION

Yielding potential of modern sugar beet varieties, i.e. their attainable yields under predominant conditions in Poland, is relatively high. The potential yield of taproots as presented annually in the COBORU reports is at the level of 70 t ha^{-1} (COBORU 2009). The country annual averages however are much lower, ranging in the last decade from 39 (2000) to 51 (2007) t ha^{-1} (GUS, 2001 2009). The annual yield gap, i.e. unharvested part of attainable yield, ranges therefore from 27% to 45%. The main reason for high production risk and concomitant year-to-year yield variability is a low level of soil fertility, in turn increasing sensitivity of this crop to unfavorable meteorological conditions (Grzebisz et al. 2005).

Modern, high yielding sugar beet varieties are highly sensitive to supply of nutrients, including both a high rate of early canopy growth and the final quality of storage roots. In temperate regions of the world, the fast early growth of sugar beet seedlings is crucial for efficient solar energy absorption by developing plant canopy (BOIFFIN et al. 1992). In the course of the growing season, sugar beet crop reaches 85% of the canopy cover, as the target soil surface cover area, when its leaf area index (LAI) is *ca* 3.0 (ANDRIEU et al. 1997). It has been documented that this level of LAI corresponds to 120 kg N ha^{-1} taken up by the expanding sugar beet canopy (MALNOU et al. 2006). The main target of growers is therefore to create growing conditions in a way allowing a crop to take this amount of nitrogen as quickly as possible, as a prerequisite of the highest efficiency of solar energy absorption.

The Diagnosis and Recommendation Integrated System (DRIS) concept is widely applied to evaluate the plant nutritional status of crop plants. The DRIS nutritional concept is based on a comparison of nutrient ratios of currently cultivated crop with optimum ratios developed for a high-yielding group-population (DRIS norms) (WALWORTH, SUMNER 1987). In this study, it has been assumed that by means of the DRIS method it can be possible to identify the most limiting or the most sensitive nutrient to the applied measures.

The objectives of the conducted study were (i) to qualitatively evaluate sugar beet crop canopy nutritional status at early stages of growth (ii) to determine yields of taproots and recoverable sugar in response to foliar application of multi-microelement fertilizers.

MATERIALS AND METHODS

Field experiments were conducted in 2005 and 2006 years on four sugar beet productive fields localized in Central-Western Poland. Soils in the experimental sites are loamy sands lying on sandy loam, classified, according to Polish standards, as a very good rye complex, belonging to the agronomy class IVa. Agrochemical soil characteristics of the experimental plots were determined each year at the beginning of the growing season, i.e. at early stage of sugar beets growth, BBCH16. The main agrochemical soil characteristics are as follows: 1) soil pH: site-to-site variable, predominantly acid to slightly acid; 2) content of available nutrients: phosphorus – low; potassium – high; magnesium – high and very high.

The single-factorial experiment with sugar beet consisted of tree treatments as presented below:

1. NPK (acronym NPK);
2. NPK + multi-microelement fertilizer (NPK+Mi);

3. NPK + multi-microelement fertilizer and boron (NPK+MiB).

All treatments were replicated three times in a simple block design. Each year cereals (winter wheat or spring barley) were a preceding crop for sugar beet. Farmyard manure was applied in three of four sites. In the fourth one white mustard was grown and plowed down in autumn as a green manure. Phosphorus and potassium rates were adjusted to the soil test and applied in the previous autumn (1/XI). Nitrogen application rate was established by means of the N_{min} method in order to reach at the 16^{th} leaf stage N mineral total content of 200 kg ha^{-1} in the soil zone of 0.0-0.9 m. It has been assumed that this level of mineral N is sufficient to harvest 50-55 t ha^{-1} of taproots. Herbicides and all other agro-technologies followed standard practices.

Multi-microelement fertilizers were prepared at concentrations of 70 Cu, 400 Fe, 170 Mn, 20 Mo, 150 Zn mg dm^{-3} in the chelated by EDTA for the Mi formulation and plus 105 g dm^{-3} of B in the form of boron-ethanolamine for the MiB formulation. Both fertilizers were applied on sugar beet foliage at three consecutive stages of plant growth: 16^{th}, 26^{th} and 43^{rd}, according to the BBCH scale at a rate of 3 dm^3 ha^{-1} for each spraying. Yield of taproots was assessed from an area of 10,8 m^2. Yield of recoverable sugar was determined by means of the Venema analytical procedure in the Środa Sugar Beet Factory (Pfeifer & Langen Polska).

For the purpose of the study, 10 plants were sampled at three consecutive stages of wheat growth according to the BBCH scale: 16 (before the first foliar spray), 43 (after the second but before the third foliar spray), 49 (at technological plant maturity). At each sampling date the harvested plant samples were partitioning into subsamples of leaves and taproots and then dried (65°C). Nitrogen content of the plant tissues was determined by using the Kjeldahl method (Kjeltec Auto Distillation). Phosphorus was determined calorimetrically (Analitykjena Specord 40) whereas K, Mg, Mn and Cu by the FAAS method (Flame Atomic Absorption Spectrophotometer, Varian 250 plus). The results are expressed on a dry matter (DM) basis.

The data from experiments were elaborated by using analyses of variance for each year separately and for interaction year and treatments, using computer programs Statistica 7. For F-test showing significant differences, Tukey's test (HSD) at the probability level $\alpha = 0.05$ was additionally performed to compare mean values (LSD). Linear and non-linear regression fittings were determined on mean values (of four replications), by using statistical software Statistica 7. The goodness of fit was evaluated by R^2 values.

Evaluation of the sugar beet nutritional status at growth stages BBCH16 and 43 has been conducted by means of two approaches. The first one relies on comparison of average values for each of the studied nutrient with concomitant sets of standard sufficiency ranges, according to Barłóg (2009). The second method applied during the study was Diagnosis and Recommendation Integrated System (Dris, Walworth, Sumner 1987), which provides

a means of linking leaf nutrient concentrations to the yield of the tested crops. This method requires two sets of data, classically elaborated via dividing the total tested population into two parts, i.e., high and low yielding. Each of these sets of data comprises averaged data on nutrient ratios for each studied pair of nutrients, for example for nitrogen (N) and phosphorus (P) expressed as N/P for the low-yielding population and as n/p for the standard yielding population. In the calculation practice applied in this study the tested set of data was considered as the low population, but the concomitant set for high-yielding norms, i.e., n/p and their coefficient of variation (CV) are as a rule taken from literature (BARŁÓG, 2009, personal communication) – Table 1. In the present study, leaf samples were analyzed for N, P, K, Ca and Mg. In the second step of procedure, for each of the tested pairs specific nutrient ratios f(N/P) were calculated. Based on each function, an index for

Table 1

Diagnosis and Recommendation Integrated System (DRIS) norms from the high yielding subpopulation of sugar beets grown in the central-western part of Poland (Barłóg 2009)

Nutrient ratio	Discriminators			
	yield of taproots, n = 113		yied of recoverable sugar, n = 144	
	average	CV (%)	average	CV (%)
N/P	10.32	27.07	10.38	23.47
N/K	0.933	18.18	0.946	20.39
N/Mg	10.80	49,38	10.86	33.84
N/Ca	6.591	42.52	7.116	50.49
K/P	11.44	32,29	11.40	30.18
K/Ca	7.077	37.47	7.429	38.54
K/Mg	11.70	47.05	11.73	34.05
Ca/P	1.924	65.48	1.846	64.68
Ca/Mg	1.853	59.29	1.811	53.80
Mg/P	1.159	52.80	1.068	43.00

each nutrient was computed separately, accordingly to respective functions and equations. The choice of appropriate functions depends for example on N/P and n/p values, as presented below:

when $N/P > n/p$ then $$f(N/P) = \left[\frac{N/P}{n/p} - 1\right] \cdot \frac{1000}{CV}$$

when $N/P < n/p$ then $$f(N/P) = 1 - \left[\frac{n/p}{N/P}\right] \cdot \frac{1000}{CV}$$

where:

N/P – nutrient ratio of N to P content in the analysed crop,
n/p – nutrient ratio of N to P in the DRIS normative ratio,
CV – coefficient of variation for n/p ratio for the DRIS norm,
1000 – coefficient of recalculation

$$I(N) = \frac{f(N/P) + f(N/K - f(Mg/N - f(Ca/N)}{4}$$

$$I(P) = \frac{f(P/Mg) - f(N/P - f(K/P - f(Ca/P)}{4}$$

$$I(K) = \frac{f(K/P) - f(N/K - f(Ca/K - f(Mg/K)}{4}$$

$$I(Ca) = \frac{f(Ca/N) + f(Ca/P + f(Ca/K - f(Mg/Ca)}{4}$$

$$I(Mg) = \frac{f(Mg/N) + f(Mg/K - f(P/Mg + f(Mg/Ca)}{4}$$

RESULTS AND DISCUSSION

The main objective of the study was to assess the effect of two multi-microelement fertilizers, first containing all basic cations (Mi) and the second one enriched with boron (MiB) on sugar beet crop nutritional status and in turn on yields of taproots and recoverable sugar. The main hypothesis of the conducted study was that any yield increase could be achieved provided that plants were able to reach the point of balanced nutrients supply, but nitrogen-oriented. Taking into account the main target of sugar beet growers, i.e. end-effect of new production measures, yields of taproots (TR) and recoverable sugar (RS) were assessed first. The site-to-site variability of yield of taproots for the NPK treatment, considered as the control, as documented by a coefficient of variation, was low, i.e. less than 10%, and therefore not significant. It was found, however, that the tested multi-microelement fertilizers significantly affected yields of taproots (Figure 1). In comparison to the NPK treatment, yields increased 31.6% and 22.1% in the NPK+Mi and NPK+MiB treatments, respectively. On average, sugar beet crop fertilized with NPK+Mi yielded 70 t ha^{-1}. This volume of harvested yield is equal to the level of attainable yields in Poland during the years of study (COBORU 2009). Plants fertilized with NPK+MiB yielded *ca* 5 t ha^{-1}

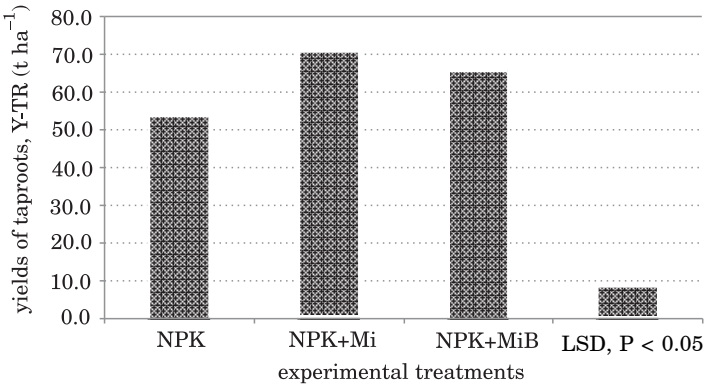

Fig. 1. Yield of sugar beet taproots in response to foliar application of multimicronutrient fertilizers

lower as compared to the NPK+Mi treatment, but significantly higher than in the NPK control. A slightly lower yield increase following additional boron supplementation seems unexpected and can be only explained by means of two hypothesis: i) lower boron uptake by plants due to insoluble borate complexes precipitated in the spraying container, ii) lower uptake of divalent cations such as calcium and magnesium by plants. On the other hand, the yields obtained clearly emphasize the yield-forming role of an external supply of micronutrient factors.

The same pattern of the influence produced by both multi-microelement fertilizers on sugar beet crop appeared in the yield of recoverable sugar (Figure 2). The relative yield increase was 31.3% and 23.7% for the NPK+Mi

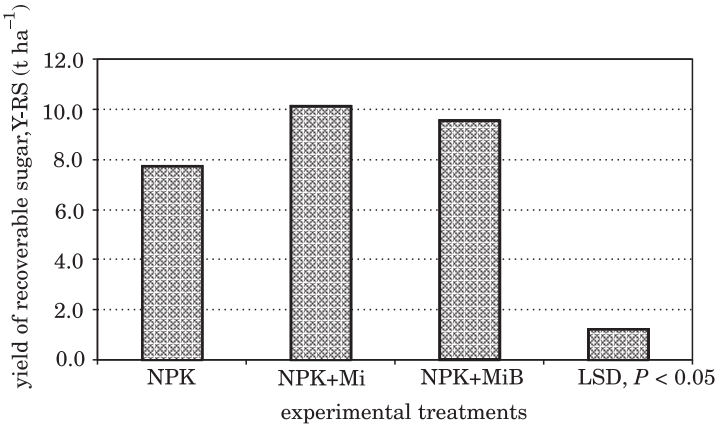

Fig. 2. Yield of recoverable sugar in response to foliar application of multimicronutrient fertilizers

and NPK+MiB fertilizers, respectively. For this end product. the effect of added boron was less limiting that for the yield of taproots. The main reason of a slightly lower yield gap was 0.7% higher content of sugar (data not shown but available from the authors).

The second objective of the study was to explain the yields increments by means of evaluating the sugar beet plants nutritional status over the course of the growing season, with special attention paid to the early stages of sugar beet development. According to DURR et al. (1992), the pattern of individual rate of growth of sugar beet seedlings is established at the stage of 4^{th} leaf, provided sufficient supply of nutrients and water. However, as presented in Table 2, at this sugar beet growth stage favorable growth conditions are rare. In order to verify this hypothesis during the course of sugar beet growing seasons, plants were sampled three times. The first sampling was done when plants reached the stage of 6^{th} leaf (BBCH16). This stage of sugar beet development of canopy should reflect the plant nutritional status just before the first multi-microelement fertilizer application on plant foliage. The sugar beet plant nutritional status evaluation was conducted by BARŁÓG (2009), based on critical ranges worked out for the central-western part of Poland. In 2005, the content of all studied macronutrients in leaves was within their own specific critical ranges. However, the averages for nitrogen, phosphorus and potassium were the lower while those for magnesium and calcium were the medium values of their critical ranges. All values, except for magnesium, were much below the nutrient specific optimum. In 2006, the sameobservations were made, except for nitrogen. Its average content was below the threshold range, but without any nega-

Table 2

Content of macronutrients in sugar beet leaves, stage of growth – BBCH16 (g kg^{-1} DM)

Statistical parameters	Nutrients				
	N	P	K	Ca	Mg
Critical ranges*	36.0-54.5	2.2-8.1	41.0-86.0	5.8-15.5	2.6-7.7
Optimum	45.7	5.2	65.0	10.6	5.1
2005					
Mean	36.58	3.975	43.05	8.500	5.900
SD	±7.702	±0.206	±3.755	±3.160	±0.896
CV, %	21.1	5.1	8.7	31.2	15.9
2006					
Mean	30.60	3.600	42.93	9.200	5.375
SD	±2.15	±0.24	±3.60	±1.0	±0.50
CV, %	7.0	6.8	8.4	10.8	9.5

*Barłóg (2009)

tive impact on the final yield of taproots (Table 2). The assessment of sugar beet plant nutritional status at this stage of sugar beet growth does not give us any knowledge on the current plant nutritional status, but seems to be a very useful indicator of the general growth conditions. At that period of sugar beet growth, the main target of growers is to make the last correction of plant nutritional status in order to reach, as quickly as possible, the targeted 85% canopy cover, required for efficient solar energy absorption, as a prerequisite of high final yield (MALNOU et al. 2006).

Table 3

Content of macronutrients in sugar beet leaves, stage of growth – BBCH43 (g kg^{-1})

Treatment	Level of treatment	Nutrients				
		N	P	K	Ca	Mg
Critical*	ranges optimum	34.7-45.3 40.0	2.7-4.8 3.6	35.7-62.5 49.1	3.1-7.7 5.2	2.3-5.8 3.8
Fertilizers (*F*)	NPK	40.39	3.038	35.13	11.00	5.075
	NPK + Mi	44.18	3.113	36.71	11.98	5.400
	NPK + MiB	43.04	3.113	37.16	11.14	5.188
LSD, $P \leq 0.05$		0.30	-	-	-	-
Year (*Y*)	2005	41.83	3.083	36.18	11.81	5.292
	2006	43.23	3.092	36.49	10.92	5.150
LSD, $P \leq 0.05$		-	-	-	-	-
LSD, $P \leq 0.05$ *F x Y*		-	*x*	-	-	-

*Barłóg (2009);
x – significant interaction

Two of the three foliar sprayings of the multi-microelement fertilizers, conducted during the course of the growing season, took place before the classical stage of sugar beet nutritional status assessment, i.e., development of harvestable part (BBCH43). The average content of the tested elements in fully developed leaves showed some specific differences in nutrients i) in comparison to the previous stage, ii) in response to the applied fertilizers. In comparison to BBCH16 stage, a marketable increase was noted for nitrogen and calcium, while all the other nutirents decreased (Table 3).

Our comparison of the data for the NPK treatment to the nutrient specific threshold optima and ranges clearly indicate a low nutritional status for phosphorus and potassium and excess calcium. The average content of nitrogen in young but fully developed leaves was optimum, but that of potassium was below the lower threshold limit. Having determined such low K values, one should expect it would have a predominant effect on final yields of taproots and/or recoverable sugar. But, as presented in Figures 1

and 2, sugar beet crops did not respond negatively to such temporary K deficiency and or the low P content. Among the analysed nutrients, it was only phosphorus that showed significant response to the year and fertilizer interaction. This interaction was significant due to the differences found in the NPK plot. In the second year, plants fertilized with NPK contained significantly less phosphorus. The other treatments revealed completely opposite albeit not significant relations. The plant growth and yields was unaffected even by the excess of calcium.

Among all the studied nutrients, it was only the nitrogen content that showed a significant response to the multi-microelement fertilizers applied to sugar beet plant foliage. However, some positive trends were also observed for other nutrients, although they were nutrient specific.

Sugar beet growth stage BBCH 43 is considered classical for plant nutritional status evaluation. However, the critical ranges did not enable us to make any ranks of the nutrients with respect to the sugar beet nutritional status. For this purpose, the DRIS concept of plant crop nutritional status can be applied (Barłóg 2009, Walworth, Sumner 1987). The main task of this procedure is to assess plant nutritional imbalance (Table 4). The applied

Table 4

Sugar beet nutritional status at BBCH43 as assessed by means of the DRIS indices

Plant indicator	Experimental treatments	DRIS indices (I)				
		N	P	K	Ca	Mg
Yield of taproots	NPK	-0.623	-8.218	-14.89	16.88	6.856
	NPK + Mi	1.203	-9.888	-16.30	18.00	6.980
	NPK + MiB	0.780	-8,845	-13.82	15.58	6.302
Yield of recoverable sugar	NPK	-1.713	-10.10	-16.22	17.88	10.16
	NPK + Mi	0.218	-12.01	-17.60	19.06	10.33
	NPK + MiB	-0.172	-10,81	-14.99	16.61	9.354

DRIS procedure was conducted taking into account two end products, i.e. yield of taproots (Y-TR) and yield of recoverable sugar (Y-RS), considered as plant specific indicators. For the yield of taproots, irrespective of the experimental treatment, the following order of nutrients was found:

$$K > P > N > Mg > Ca$$

This order clearly demonstrates that potassium was the most limiting nutrient, followed by phosphorus, both presenting negative values of DRIS indices, which simply means that both nutrients were highly unbalanced. It is frequently presented in literature that a DRIS index exceeding an absolute value of ± 15 indicates an acute plant nutritional disorder. In this study, this is the case of potassium, whose absolute values exceeded – 15 in the

NPK+Mi treatment with respect to the yield of taproots and for both treatments with respect to the yield of recoverable sugar. The same but opposite in sign was found for calcium, i.e. > + 15 for both yield indicators. Hence, a regression model of DRIS indices for calcium was developed as a function of the potassium index, as presented below:

$$I(Ca) = -0.9138I(K) + 3.0466 \quad \text{for } R^2 = 0.988 \text{ and } n = 6 \text{ for } P \leq 0.001 \quad (1)$$

However, the variability of both indices due to application of the multi-microelement fertilizers to sugar beet foliage did not significantly affect either the yield of taproots or the yield of recoverable sugar.

Considering the experiment's objectives, nitrogen showed the most interesting response of DRIS indices to the applied fertilizer. The presented indices are of crucial importance for explaining the sugar beet yield response to the applied multi-microelement fertilizers. At first, the nitrogen indices were the lowest, indicating a state of smooth balance. Later, the nitrogen indices underwent a big change in response to the applied fertilizers, from negative for the NPK fertilized crop to positive for treatments receiving the micronutrient fertilizers. The degree of the change as described by DRIS indices for nitrogen, (I)N, enabled us to make a reliable prognosis of the yield of taproots (Y-TR). The regression equation clearly indicates that under a balanced N status the harvested yield of taproots can reach *ca* 60 t ha^{-1}:

$$Y\text{-}TR = 9.110I(N) + 59.33 \quad \text{for } R^2 = 0.994 \text{ and } P \leq 0.001 \quad (2)$$

Quite an opposite change in DRIS indices was observed for phosphorus, I(P). The fertilizers increased the state of imbalance of phosphorus, although a reliable and significant prognosis of taproots yield was still possible:

$$Y\text{-}TR = -9.664I(P) - 23.36 \quad \text{for } R^2 = 0.872 \text{ and } P \leq 0.05 \quad (3)$$

DRIS indices were also developed, but using another set of standards, i.e. for yield of recoverable sugar. In this case, the order of DRIS indices is the same as found for the yield of taproots. Again, the lowest imbalance was calculated for nitrogen, but negative values were noted both for the NPK and NPK+MiB treatments. The fertilizers significantly decreased the DRIS indices for N, but it was only for the NPK +Mi treatment that a change from negative to positive values occurred. The relationship between DRIS indices for nitrogen and phosphorus and yields of recoverable sugar were significant, which made it possible to suggest a reasonable yield prognosis. It is derived from from equation No. 4, where under conditions of N internal balance, the harvested sugar yield can reach *ca* 10 t ha^{-1}:

$$Y\text{-}RS = 1.236I(N) + 9.840 \quad \text{for } R^2 = 0.998 \text{ and } P < 0.001 \quad (4)$$

$$Y\text{-}RS = 1.187(P) - 3.868 \quad \text{for } R^2 = 0.823 \text{ and } P < 0.05 \quad (5)$$

The data on the nutrient content in sugar beet canopy at BBCH43 fully demonstrates the importance of nitrogen balance as a key element for making any sound assessment of canopy nutritional status as a prerequisite for

any reliable prognosis of yield of taproots and recoverable sugar. These data corroborate the thesis that nitrogen in well-balanced sugar beet canopy can be taken as the main plant nutrient responsible for both the rate of dry mater accumulation by growing crop canopy and its division among main organs (leaves and roots) (RUBIO et al. 2003, WEBB et al. 1997).

Table 5

Content of macronutrients in sugar beet leaves, at BBCH49 (g kg^{-1} DM)

Treatment	Level of treatment	Nutrients				
		N	P	K	Ca	Mg
Fertilizers (*F*)	NPK	37.06	3.013	31.70	5.638	4.800
	NPK + Mi	36.44	3.100	35.40	6.338	4.800
	NPK + MiB	37.68	3.188	35.69	5.250	4.512
LSD, $P \leq 0.05$		-	-	-	0.060	-
Year (*Y*)	2005	39.87	3.058	35.53	5.750	4.717
	2006	34.25	3.142	33.00	5.733	4.692
LSD, $P \leq 0.05$		0.33	-	-	-	
LSD, $P \leq 0.05$; *F x Y*		-	-	-	-	*x*

x – significant interaction

The third plant sampling was conducted at sugar beet harvest, related to the stage of BBCH49. Compared to the previous sampling dates, the nutrient content was evaluated both in leaves and in taproots. In comparison to the stage of BBCH43, the content of nutrients in leaves tended to be much lower. It was only the calcium content that showed a significant response to the applied multi-microelement fertilizers. A significant increase was noticed for the NPK+Mi treatment, in contrast to the NPK+MiB treatment, in which the Ca concetration fell. Nitrogen content at this stage of sugar beet growth showed only seasonal variability, being much lower in 2006. Among the five analysed macronutrients, magnesium alone was sensitive to the interaction of fertilizer and season. The main reason for the significant response was a rise in the Mg content in the first year in response to the Mi fertilizer application. This variability can be used to forecast yields. However, it appeared that a statistically accurate prognosis can be made only for recoverable sugar yield:

$$\text{Y-RS} = 37.30\ \text{Mg} - 10.32 \quad r = 0.71 \text{ for } n = 6 \text{ and } P \leq 0.05$$

where:

Y-RS – yield of recoverable sugar, t ha^{-1};

Mg – magnesium content in leaves at BBCH49, g kg^{-1} DM.

Generally, the content of nutrients in taproots is not used to make any assessment of plant nutritional status. Nonetheless, in our experiment, it enabled us to assess the effect of the tested fertilizers on the content of macronutrients (Table 6). With respect to the effect of the multi-microelement fertilizers, significant response was observed only for calcium. The same pattern of its content was found in leaves. Identical but non-siginificant trends were observed for magnesium. Opposite ones appeared for potassium. The potassium content in taproots showed a significant response to

Table 6

Content of macronutrients in sugar beet taproots at BBCH49 (g kg^{-1} DM)

Treatment	Level of treatment	Nutrients				
		N	P	K	Ca	Mg
Fertilizers (*F*)	NPK	10.51	1.339	10.33	0.888	1.300
	NPK + Mi	9.375	1.513	9.900	1.150	1.488
	NPK + MiB	9.325	1.788	1.089	0.838	1.300
LSD, $P \leq 0.05$		-	-	-	0.02	-
Year (*Y*)	2005	1.094	1.558	10.30	0.925	1.383
	2006	8.533	1.533	10.44	0.992	1.342
LSD, $P \leq 0.05$		0.13	-	-	-	-
LSD, $P \leq 0.05$; *F x Y*		*x*	-	*x*	-	-

x – significant interaction

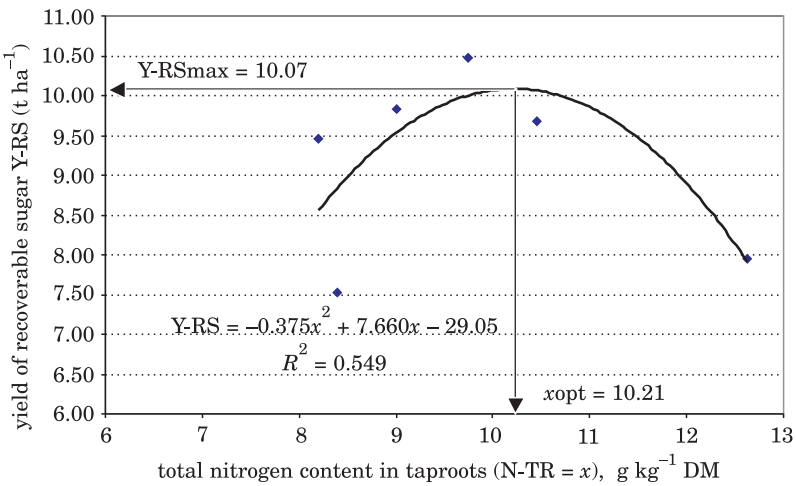

Fig. 3. Yield of recoverable sugar as a function of nitrogen content in taproots at BBCH49

the fertilizer and year interaction, but the results in both years were contrary. In the first year, a significant decrease in the K content in response to the multi-microelement fertilizer application was found. In the second year, the response consisted of a significant increase. The nitrogen content, despite the lack of significant responses, showed a decreasing tendecy, correlatedx to the tested fertilizer and year interaction. The regression of the total N content (TN_{TR}) in taproots against the harvested yield of recoverable sugar (Y-RS) allowed us to calculate an optimum of N content and also to make a reliable prognosis of recoverable sugar yield (Figure 3). In can be concluded that the total nitrogen content in tissues of taproot up to *ca* 10 g kg DM is a prerequisite of a recoverable sugar yield increase.

CONCLUSIONS

1. Sugar beet crops, irrespectively of location and year-to-year variability, showed significant

response to the application of multi-microelement fertilizers, as demonstrated by increased yields of taproots and recoverable sugar.

2. Nitrogen DRIS index can be applied as a very useful measure of sugar beet nutritional status at the beginning of harvestable plant part development.

3. The evaluated sugar beet nutritional status and yield increases indicate an insufficient supply of micronutrients to sugar beet plants during the critical stages of growth.

4. Sugar beet has high tolerance to an internal balance of cations provided that their supply is sufficient to control nitrogen, i.e. to lower nitrogen internal imbalance.

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QUALITY CHARACTERISTICS AND CONTENT OF MINERAL COMPOUNDS IN FRUIT OF SOME CULTIVARS OF SWEET PEPPER (*CAPSICUM ANNUUM* L.)

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Abstract

The study was carried out in 2005–2007. The aim of the experiment was to estimate the quality and mineral compound content in sweet pepper fruits. The experimental material consisted of seven F_1 hybrids grown in the open field: ISI 56511 F_1, ISI 56503 F_1, Axel F_1, Akron F_1, Roei F_1, Elfo F_1 and Polonez F_1 (control cultivar). The fruits were harvested many times. They were collected when green and fully grown. After the harvest, the fruit quality including its mean weight, length, diameter, pericarp weight and pericarp thickness, was evaluated. In 2006-2007, the content of macro– and microelements, such as nitrogen, phosphorus, potassium, sodium, calcium, magnesium, iron, zinc and nitrates was determined in the dry weight of the yield. The results were statistically evaluated by the analysis of variance. Significance of the differences was tested by Tukey's test at $p = 0.05$.

The biometrical traits of fruits were characteristic for each cultivar. The highest content of dry weight was found for cultivar ISI 56511 F_1 (11.2% higher than the mean amount determined for all of the cultivars and 39.8% higher than the lowest dry matter content assessed in fruits of cultivar Roei F_1).

The sweet pepper cultivars tested in the experiment differed significantly in the content of macro- and microelements. The highest content of nitrogen was determined in fruits of cultivar Polonez F_1. Fruits produced by cultivars Elfo F_1, ISI 56503 F_1 and Roei F_1 in comparison with the other cultivars were characterized by a significantly higher content of iron. The highest content of nitrates was assessed in cultivar ISI 56503 F_1 fruit.

Key words: sweet pepper cultivars, quality characteristics of fruit, macro- and microelements content.

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CECHY JAKOŚCIOWE ORAZ ZAWARTOŚĆ SKŁADNIKÓW MINERALNYCH W OWOCACH WYBRANYCH ODMIAN PAPRYKI (*CAPSICUM ANNUUM* L.)

Abstrakt

W latach 2005–2007 badano jakość owoców papryki i zawartość makro- i mikroskładników. Badaniom (uprawa polowa) poddano następujące odmiany papryki: ISI 56511 F_1, ISI 56503 F_1, Axel F_1, Akron F_1, Roei F_1, Elfo F_1 i Polonez F_1 (odmiana kontrolna).

Zbiór owoców papryki wykonywano wielokrotnie, zbierano owoce zielone w pełni wyrośnięte. Po zbiorach owoce poddano ocenie jakościowej, uwzględniając ich masę, długość, średnicę oraz masę i grubość perykarpu. W latach 2006-2007 w suchej masie plonu oznaczono zawartość: makro– i mikroskładników – azotu ogólnego, fosforu, potasu, sodu, wapnia, magnezu, żelaza, cynku oraz azotanów. Wyniki opracowano statystycznie testem Tuckeya, wyliczając półprzedziały ufności na poziomie istotności $\alpha = 0{,}05$.

Badane cechy biometryczne owoców były charakterystyczne dla poszczególnych odmian papryki. Istotnie najwięcej suchej masy zawierały owoce odmiany ISI 56511 F_1 – o 11,2% więcej od średniej zawartości w owocach wszystkich badanych odmian i o 39,8% więcej w porównaniu z najmniejszą jej zawartością, oznaczoną w owocach odmiany Roei F_1.

Badane odmiany papryki różniły się istotnie zawartością makro– i mikroskładników. Najwięcej azotu ogólnego i potasu zawierały owoce odmiany Polonez F_1. W owocach odmian Elfo F_1, ISI 56503 F_1 i Roei F_1 oznaczono – w porównaniu z pozostałymi badanymi odmianami – istotnie większą zawartość żelaza. Istotnie najwięcej azotanów zawierały owoce odmiany ISI 56503 F_1.

Słowa kluczowe: odmiany papryki, cechy jakościowe owoców, zawartość składników mineralnych.

INTRODUCTION

Sweet pepper (*Capsicum annuum* L.) is a vegetable of a very high biological value and specific flavour (KORZEŃ, PERUCKA 1978, GAJC-WOLSKA, SKĄPSKI 2001). Sweet pepper fruits are a rich source of such compounds as vitamins (especially vitamin C), polyphenols, chlorophylls, carotenoids and sugars (AYHAN, FERAMUZ 2007, JADCZAK, GRZESZCZUK 2009). Many studies have proved that those compounds play an important role in protection against many civilization illnesses like cardiopathies, cancer, etc. (HOWARD et al. 2000, PERUCKA et al. 2004). Moreover, sweet pepper fruits are a rich source of mineral compounds, especially magnesium, calcium, potassium, phosphorus and iron (PERUCKA 1995).

Growing interest in field cultivation of sweet pepper has led to the creation of new F_1 hybrids, which are characterized by a short vegetation period, resistance to cold and high quantity and quality of marketable yield (BUCZKOWSKA 1997).

The aim of the experiment was to characterize the quality traits of fruit of some sweet pepper cultivars and to estimate the content of mineral compounds of the fruits.

MATERIAL AND METHODS

The experiment was conducted in the years 2005-2007 in the Department of Vegetable Growing and in the Laboratory of Processing and Storage of Plant Raw Material of Agricultural University in Szczecin. The research material consisted of six Israeli F_1 hybrids: ISI 56511 F_1, ISI 56503 F_1, Axel F_1, Akron F_1, Roei F_1, Elfo F_1. A Polish cultivar, Polonez F_1, was cultivated as a control. The field experiment was set in a randomized block design with four replications. The seedlings of sweet pepper were produced in the greenhouse. Seeds were sown on 23rd March (each year). The seedlings were transplanted into the open field on 31st May in 2005, 2nd June in 2006 and 4th June in 2007. Then, polypropylene non-woven fabric covers were held on plants for a two-week period.

The field was prepared according to agronomic recommendations for sweet pepper cultivation. Mineral fertilization was dosed according to the results of the chemical analysis of the soil. During the growing season, the crop treatment was carried out. It included mainly irrigation, weeding, soil cultivation and plant protection practices.

The fruit harvest was carried out many times. Fruits were collected when they were green and fully grown. After the harvest, the fruit quality including its mean weight, length, diameter, pericarp weight and pericarp thickness was evaluated. In 2006-2007 the content of nitrogen by Kjeldahl's method, phosphorus by the colorimetric method, potassium, sodium and calcium by flame photometry, magnesium, iron and zinc by the method of atomic absorption spectrophotometry (AAS) was determined in the dry weight of the yield. Moreover, the content of nitrates (PN-92/A-75112) in the raw plant was evaluated. The results of nitrate content were counted as mg of NO_3^- per kg of dry matter of fruit.

The results obtained in each year were subjected to analysis of variance. The means of the years were separated by Tukey's test at $p = 0.05$.

RESULTS AND DISCUSSION

The cultivars of sweet pepper tested in the experiment differed significantly in the fruit quality traits of the 1st class marketable yield (Table 1). The highest fruit weight was obtained for the cultivars Axel F_1, Akron F_1 and ISI 56503 F_1. However, fruits of Elfo F_1 and Roei F_1 had a significantly lower fruit weight (respectively 168.5 and 165.9 g) only in comparison with the cultivar Axel F_1. The lowest fruit weight was noted for cv. Polonez F_1. Similar results were obtained for pericarp weight, which was the lowest for cv. Polonez F_1 fruit. The pericarp weight of this cultivar did not differ sig-

Table 1

Quality traits of fruit of tested sweet pepper cultivars (mean for 2005-2007)

Cultivar	Fruit weight (g)	Pericarp weight (g)	Fruit length (cm)	Fruit diameter (cm)	Pericarp thickness (mm)	Dry matter content (%)
Akron F1	195.4	164.4	12.8	8.0	4.7	9.24
Axel F1	210.8	173.9	12.1	8.3	4.8	9.25
Elfo F1	168.5	141.2	12.2	8.1	4.4	9.25
ISI 56511 F1	179.1	148.6	10.2	8.6	4.1	10.29
ISI 56503 F1	194.4	166.6	9.8	9.0	6.7	9.79
Polonez F1	128.1	108.7	9.2	7.7	4.9	9.57
Roei F1	165.9	132.3	8.8	8.0	5.6	7.36
$LSD_{\alpha = 0.05}$	35.21	34.81	1.92	1.13	0.77	0.496

nificantly from the pericarp weight of cultivars Elfo F_1 and Roei F_1. Buczkowska (1997) claims F_1 hybrids differ significantly with specified cultivars on the quality traits. The mean fruit weight of F_1 hybrids was 75.3-89.2 g, while the mean weight of the fruit produced by cv. Kujawianka was 56.2 g. In the research by Gajc-Wolska and Skąpski (2001) the mean weight of F_1 hybrids fruit of sweet pepper was also higher in comparison with the fruit of the cultivars tested in our study, and varied between 82 and 130 g. Our analysis of the fruit length proved that cultivars Akron F_1, Axel F_1 and Elfo F_1 formed significantly longer fruits in comparison with the other cultivars tested in the experiment. Moreover, a significantly larger diameter was noticed for cultivar ISI 56503 F_1, but only in comparison with cultivar Polonez F_1. The largest pericarp thickness was determined for cultivar ISI 56503 F_1 fruits, while the smallest one was recorded for cultivars Axel F_1, Akron F_1, Elfo F_1 and ISI 56511 F_1. Gajc-Wolska and Skąpski (2001) state that the mean pericarp thickness of sweet pepper is 5–6 mm while Buczkowska (2004) suggests it ranges from 5.5 to 5.8 mm.

The dry matter content of sweet pepper fruits differed significantly between the cultivars. Most dry matter was noted for cultivar ISI 56511 F_1 (10.29%) and the least for cultivar Roei F_1 (7.36%). Gajc-Wolska and Skąpski (2001) determined lower amounts of dry matter of sweet pepper, i.e. from 4.6 to 8.4%. Olszewska and Nowaczyk (2004) assessed on average 8.81% of dry matter in fruits of sweet pepper, while Buczkowska et al. (2001) determined that sweet pepper fruit contained 10.1%.

The sweet pepper cultivars tested in the experiment were characterized by a high content of mineral compounds (Table 2). Significantly higher content of total nitrogen (24.70 g kg^{-1} d.m.) and potassium (23.22 g kg^{-1} d.m.) was assessed in fruits of cultivar Polonez F_1. The least nitrogen was in

Table 2

Content of mineral compounds in the yield of tested cultivars of sweet pepper (mean for 2006-2007)

Cultivar	(g kg^{-1} d.m.)						(mg kg^{-1} d.m.)		
	N-total	P	K	Ca	Na	Mg	Fe	Zn	NO_3^-
Akron F1	19.88	3.60	20.06	2.62	0.23	1.01	365.0	10.0	50
Axel F1	19.51	3.25	21.14	2.59	0.25	1.11	370.0	11.2	27
Elfo F1	20.09	3.22	19.24	2.62	0.23	1.02	600.0	12.3	25
ISI 56511 F1	18.48	3.02	20.32	2.33	0.17	1.25	350.0	12.0	50
ISI 56503 F1	20.49	3.22	20.52	2.62	0.22	1.10	600.0	10.2	115
Polonez F1	24.70	3.60	23.22	2.62	0.15	0.75	350.0	15.1	24
Roei F1	20.64	3.05	21.34	2.33	0.17	0.87	600.0	15.0	24
Mean	20.54	3.28	20.83	2.53	0.20	1.02	462.1	12.3	45
$LSD_{\alpha = 0.05}$	0.946	n.s.	0.523	0.121	n.s.	n.s.	46.421	3.486	3.1

n.s. – non-significant differences

fruits of cultivar ISI 56511 F_1 (18.48 g kg^{-1} d.m.), while the lowest concentration of potassium was found in fruits of cultivar Elfo F_1 (19.24 g kg^{-1} d.m.). Among the cultivars examined in the experiment, Roei F_1 and ISI 56511 F_1 were characterized by a higher content of calcium than the other cultivars. There were no significant differences found between the tested sweet pepper cultivars in the content of phosphorus, sodium and magnesium. Comparable amounts of magnesium in hot pepper fruit were obtained by Perucka et al. (2004). Osunde and Musa Makama (2007) determined 20.4 mg of calcium and 693.0 mg of potassium in 100 g of dry weight of sweet pepper.

A significantly higher content of iron was assessed in fruits of cultivars Elfo F_1, ISI 56503 F_1 and Roei F_1 in comparison with the other cultivars and by 29.8% in comparison with the mean content of iron of all the tested sweet pepper cultivars. A significantly higher content of zinc was determined in fruits of cultivars Polonez F_1 and Roei F_1 (15.1 and 15.0 g kg^{-1} d.m., respectively) but only in comparison with cultivars Axel F_1, ISI 56503 F_1 and Akron F_1. Fruits of cultivar ISI 56503 F_1 were characterized by the highest content of nitrates (115 g kg^{-1} d.m.). The content of nitrates in fruits of cultivars Akron F_1 and ISI 56511 F_1 was 50 g kg^{-1} d.m. A lower content of nitrates, from 24 to 27 g kg^{-1} d.m., was determined in fruits of the other tested cultivars of sweet pepper.

CONCLUSIONS

1. The cultivars of sweet pepper tested in the experiment differed significantly in the quality traits of fruits.

2. The highest dry matter content was found in fruits of cultivar ISI 56511 F_1 while the lowest one – in fruits of cultivar Roei F_1, in comparison with the control.

3. There were significant differences found in the macro– and microelements content between the tested cultivars of sweet pepper. The highest amounts of nitrogen and potassium were noted in fruits of cultivar Polonez F_1. Cultivars Elfo F_1, ISI 56503 F_1 and Roei F_1 were characterized by a significantly higher content of iron in comparison with the other cultivars.

4. Among the cultivars tested in the experiment, the highest content of nitrates was determined in fruits of cultivar ISI 56503 F_1.

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ENVIRONMENTAL FACTORS PREDISPOSING TO PAIN SYNDROMES AMONG ADOLESCENT GIRLS WITH DIAGNOSED IDIOPATHIC SCOLIOSIS*

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Abstract

Idiopathic scoliosis (IS), despite multidirectional therapy, involves a significant impairment of the quality of life of the patients. It is caused by pain syndromes frequently accompanying IS, the etiology of which has not been entirely discovered. The present research on environmental factors predisposing to pain syndromes in girls with IS involved analysis of 54 girls under ambulatory care, aged 14-17 (on average 15.3-years-old ±0.99). The patients' natural environment was analysed, including time spent in a sedentary position during the day, sleeping time, time spent on physical activity during the week, regularity of meals consumed, as well as the quantitative composition of the diet. The research also concerned the location and characteristics of ailment reported, as well as pain intensity on the Numerical Rating Scale (NRS).

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It was found that the most frequent locations of back pain were: lumbar section – in 28 persons (51.9% of the examined population), and thoracic section of the spine – in 21 persons (38.9% of the examined population). Pain occurring at the same time in the cervical, thoracic and lumbar sections of the spine was reported for 4 persons (7.4% of the examined population). Fifty girls (92.6%) were aware of controlling their body posture, and 23 persons (42.6%) were able to correct it. The group of girls under examination suffering from pain in the lumbar section of the spine was characterized by a longer period of retaining a sedentary position, less time dedicated to any therapeutic rehabilitation programme, higher intensity of pain, and a higher average age than the group with pain in the thoracic section of the spine.

The research proved that patients with pain syndrome located in the lumbar part of the spine were characterized by more time spent in a sedentary position, less time spent on a therapeutic rehabilitation programme, higher intensity of pain and a higher average age than the group suffering from pain in the thoracic section of the spine. The lifestyles of the patients under examination and their lack of awareness of the need to control the body posture can affect the type and intensity of pain.

Key words: idiopathic scoliosis, pain, adolescent girls, environmental factors.

CZYNNIKI ŚRODOWISKOWE PREDYSPONUJĄCE DO ZESPOŁÓW BÓLOWYCH KRĘGOSŁUPA W POPULACJI DZIEWCZĄT ZE SKOLIOZĄ IDIOPATYCZNĄ

Abstrakt

Idiopatyczna skolioza kręgosłupa (ISK), pomimo wielokierunkowej terapii, wiąże się z istotnym obniżeniem jakości życia chorych. Przyczyną tego są często towarzyszące IKS zespoły bólowe, których etiologia nie jest jeszcze całkowicie poznana. Realizując badania nad wskazaniem czynników środowiskowych predysponujących do zespołów bólowych kręgosłupa u dziewcząt z ISK, poddano analizie 54 objęte opieką ambulatoryjną dziewczęta w wieku od 14 do 17 lat (średnio 15,3 lat ±0,99). Dokonano analizy środowiska naturalnego pacjentek, m.in.: czasu przebywania w pozycji siedzącej w ciągu doby, czasu trwania snu, ilości czasu przeznaczonego na aktywność ruchową w ciągu tygodnia oraz regularności spożywanych posiłków, a także składu ilościowego diety. Przedmiotem badań była też lokalizacja i charakterystyka zgłaszanych dolegliwości oraz ocena intensywności bólu według skali numerycznej NRS (Numerical Rating Scale).

Stwierdzono, że najczęstszymi lokalizacjami bólu pleców były odcinki: lędźwiowy u 28 osób (51,9% populacji badanej) oraz piersiowy kręgosłupa u 21 osób (38,9% populacji badanej). Ból obejmujący jednocześnie odcinki szyjny, piersiowy oraz lędźwiowy kręgosłupa występował u 4 osób (7,4% populacji badanej). Świadomość kontroli postawy ciała miało 50 badanych dziewcząt (92,6%), a skorygować ją potrafiły 23 osoby (42,6%). Grupa badanych dziewcząt z bólem odcinka lędźwiowego kręgosłupa dłuższy czas przebywała w pozycji siedzącej, mniej czasu poświęcała na program usprawniania terapeutycznego, skarżyła się na większą intensywność bólu i miała wyższą średnią wieku niż grupa z bólem odcinka piersiowego kręgosłupa.

W badaniach wykazano, że pacjenci z zespołem bólowym zlokalizowanym w części lędźwiowej kręgosłupa dłuższy czas przebywali w pozycji siedzącej, mniej czasu poświęcali na program usprawniania terapeutycznego oraz zgłaszali większą intensywność bólu i mieli wyższą średnią wieku w porównaniu z grupą z bólem odcinka piersiowego kręgosłupa. Tryb życia chorych objętych badaniem i brak świadomości kontroli postawy może mieć wpływ na rodzaj i intensywność dolegliwości bólowych.

Słowa kluczowe: skolioza idiopatyczna, ból, dorastające dziewczęta, czynniki środowiskowe.

INTRODUCTION

Research and analyses concerning the quality of life in relation to environmental factors of patients with IS remain a valid area of study. Prevention of the development of spinal curvatures is the main aim of preventive treatment (Wiśniewska et al. 2006). Paradoxically, research projects on the effectiveness of preventive treatment are more difficult when children and teenagers receive much medical and social care (Grivas et al. 2006). Currently, it seems impossible to compare such patients to a group of untreated patients, deprived of even natural physical activity and unintentional prophylactic activities, such as physical education lessons, amateur sport or recreational swimming (Goldberg et al. 1994, Weinstein et al. 2003).

IS is a problem concerning a large group of adolescents, particularly girls (Ponseti, Freidman 1950, Weinstein et al. 2003). In spite of multidirectional, preventive and (increasingly more often) invasive therapy, this condition involves a significant impairment in the life quality of the IS patients caused by pain syndromes, which often accompany the illness (Ponseti, Freidman 1950).

MATERIAL AND METHODS

The aim of the research has been to indicate which environmental factors predispose girls with idiopathic scoliosis to spinal pain syndromes.

The research involved examination of 54 girls aged 14-17 (on average 15.3 years ±0.99), including 28 girls with pain in the lumbar section (average age 15.39 years ±0.99), 22 girls with pain in the thoracic section (average age 14.73 years ±0.98) and 4 girls with pain in cervical, thoracic and lumbar sections (average age 15.75 years ±1.25). Further analysis concerned only the girls with pain in lumbar and thoracic sections of the spine. All persons examined were under ambulatory care because of IS. In the whole group, first grade IS was diagnosed in 44 persons and second grade IS in the remaining 10 persons. The data obtained underwent bidirectional analysis. The first part of the research was to analyse the natural environment of patients, including the time spent in a sedentary position during the day, sleeping time, time spent on physical activity during the week and regularity of meals consumed, as well as the quantity composition of the diet. The second epart included location and characteristics of the reported ailment as well as the intensity of pain on the Numerical Rating Scale (NRS), in which self-evaluation of pain is specified using a scale of 0-10 points. On this scale, zero point means no pain, while 10 points describe the pain of the highest intensity. Attention was also paid to the programme of therapeutic rehabilitation applied, as well as to the patients' awareness of the body posture control.

The data for our analyses were obtained with a purpose-designed survey, which included nine points:

1) age of the patient,
2) time spent in a sedentary position during the day:
   a) at school,
   b) at home,
   c) during extracurricular classes;
3) sleeping time per 24 hours,
4) regular meal times,
5) eating habits,
6) duration of physical activity in 1 week:
   a) lessons of physical education at school,
   b) additional extracurricular classes,
   c) physical rehabilitation programme,
   d) other;
7) numerical rating scale of the pain,
8) characteristics and location of the back pain:
   a) cervical section,
   b) thoracic section,
   c) lumbar section,
9) body posture awareness.

Whenever atypical deformations of the spine are suspected, the cause and character of the ailment should be thoroughly searched for and treated as an interdisciplinary problem. In such cases, specialist diagnostic tests was performed apart from an X-ray examination, for example computer tomography (TK and TK 3 D) and magnetic resonance (MRI), which make it possible to precisely describe the deformation, its location and possible changes to the vertebral canal, intervertebral foramens as well as locations of intervertebral discs.

The results obtained during our study were subjected to statistical analysis in order to calculate the means and standard deviation.

RESULTS

The girls suffering from pain in the thoracic section spent on average 11.02 hours in a sedentary position, while girls suffering from pain of the lumbar section – 12.14 hours. The time spent in a sedentary position at home was, 3.41 hours among girls with pain located in the thoracic section and 4.43 hours among girls suffering for pain in the lumbar location. A prolonged sitting position prevailed as a posture assumed during a daily routine of the persons examined. Sleeping time in the examined group of persons with pain in the thoracic section was on average 7.27 hours and in the group

Table 1

Analysis of sitting position and sleep during the day and motor activity during a week for a thoracic section of the spine

	Sitting position (h)			Sleep time	Physical activity (h)							Pain (numerical scale 0-10)	Age
	school	home	extracurricular classes		physical education	additional classes					therapeutic rehabilitation		
						swimmng pool	hippo-therapy	volley-ball	no classes	dancing			
Thoracic section	8	5	2	7	3	1	0	0	0	0	2	5	17
	7	2	0	8	0	1	0	0	0	0	2	5	14
	7	7	2	5	3	1	0	0	0	0	0	5	17
	7	3	0	9	3	0	1	0	0	0	1	4	15
	7	3	3	9	4	0	0	1	0	0	7	5	14
	7	3	2	6	3	0	0	0	0	1	2	2	14
	6	5	0	8	4	0	0	0	0	1	4	5	14
	6	5	1	9	0	0	0	0	0	1	3	6	16
	8	5	0	10	0	0	0	0	0	1	1	2	14
	8	5	0	6	4	0	0	0	0	1	4	3	15
	5	4	0	6	3	0	0	0	1	0	2	5	14
	6	5	0	7	3	0	0	0	1	0	2	4	14
	6	3	0	7	3	0	0	0	1	0	2	4	15
	7	3	0	7	0	0	0	0	1	0	1	4	15
	7	3	0	7	0	0	0	0	1	0	1	4	14
	7	2	0	7	0	0	0	0	1	0	2	4	14
	7	2	0	7	0	0	0	0	1	0	2	4	14
	7	2	0	7	3	0	0	0	1	0	1	4	16
	7	2	0	7	3	0	0	0	1	0	1	4	14
	7	2	0	7	3	0	0	0	1	0	0	4	15
	7	2	0	7	3	0	0	0	1	0	0	5	15
	7	2	0	7	3	0	0	0	1	0	0	5	14
Total	151	75	10	160	48	3	1	1	12	5	40	93	324
Mean	6.86	3.41	0.45	7.27	2.18	0.14	0.05	0.05	0.55	0.23	1.82	4.23	14.73
Standard deviation	0.710	1.469	0.912	1.162	1.563	0.351	0.213	0.213	0.510	0.429	1.622	0.973	0.985

Table 2

Analysis of sitting position and sleep during the day and motor activity during a week for a lumbar sections of the spine

Lumbar section	Sitting position (h)			Sleep time (h)	Physical activity (h)						Pain (numerical scale 0-10)	Age
	school	home	extracurricular activities		physical education	additional classes				therapeutical rehabilitation		
						swimming pool	hippotherapy	volleyball	no classes			
	7	7	0	6	3	1	0	0	0	2	7	16
	6	1	2	7	3	1	0	0	0	1	3	17
	7	4	2	7	3	1	0	0	0	2	1	17
	7	4	2	6	2	1	0	0	0	2	6	15
	6	5	2	7	0	0	1	0	0	2	6	16
	8	4	1	6	3	0	0	1	0	2	10	16
	7	6	2	8	2	0	0	1	0	1	8	15
	7	6	2	7	3	0	0	1	0	0	4	15
	7	3	0	8	3	0	0	0	1	0	4	17
	7	3	0	7	0	0	0	0	1	0	3	16
	8	3	0	7	0	0	0	0	1	0	4	14
	8	6	2	8	4	0	0	0	1	1	6	15
	7	5	0	7	4	0	0	0	1	1	3	15
	7	5	0	8	4	0	0	0	1	0	6	15
	6	5	1	8	3	0	0	0	1	2	2	15
	7	8	0	5	0	0	0	0	1	1	5	15
	5	2	1	6	3	0	0	0	1	0	6	14
	8	3	3	8	0	0	0	0	1	1	6	17
	6	3	0	6	4	0	0	0	1	2	3	16
	7	3	0	7	0	0	0	0	1	1	5	15
	7	5	0	8	3	0	0	0	1	0	5	15
	7	5	3	7	3	0	0	0	1	2	5	14

cont. Table 2

	8	6	0	6	4	0	0	0	1	2	6	14
	6	5	0	6	3	0	0	0	1	2	6	17
	6	5	0	10	3	0	0	0	1	1	6	14
	7	4	0	7	3	0	0	0	1	1	6	16
	7	5	0	7	3	0	0	0	1	1	7	15
	7	3	0	7	3	0	0	0	1	0	7	15
Total	193	124	23	197	69	4	1	3	20	30	146	431
Mean	6.89	4.43	0.82	7.04	2.46	0.14	0.04	0.11	0.71	1.07	5.21	15.39
SD	0.737	1.550	1.056	0.999	1.401	0.356	0.189	0.315	0.460	0.813	1.912	0.994

Table 3

Analysis of sitting position and sleep during the day and motor activity during a week for cervical, thoracic and lumbar sections of the spine

Cervical + thoracic + + lumbar section	Sitting position (h)			Sleep time (h)	Physical activity (h)				Pain (numerical scale 0-10)	Age
	school	home	extracurricular activities		physical education	additional classes		therapeutical rehabilitation		
						no classes	dancing			
	7	2	6	7	0	0	1	1	3	17
	7	2	3	5	2	1	0	2	7	16
	7	5	2	8	3	1	0	1	2	14
	8	2	0	6	3	1	0	0	6	16
Total	29	11	11	26	8	3	1	4	18	63
Mean	7.25	2.75	2.75	6.50	2.00	0.75	0.25	1.00	4.50	15.75
SD	0.500	1.500	2.500	1.291	1.414	0.500	0.500	0.816	2.380	1.258

Table 4

Qualitative composition of the diet in the examined population – part 1

	Liquid (dm^3)	Number of persons	Average consumption of liquids (dm^3)
Type and amount of liquids consumed during the day	milk, cocoa 0.00	26	0.17
	0.20	15	
	0.50	13	
	mineral water 0.50	10	1.05
	1.00	25	
	2.00	7	
	Juice 0.20	6	0.69
	0.50	12	
	1.00	17	
	fizzy drinks 0.25	9	0.43
	0.50	2	
	1.00	2	
	0.50 (sometimes)	6	
	cola drinks 0.25	7	0.42
	0.50	3	
	1.00	1	
	0.50 (sometimes)	5	
	other 0.25	5	0.51
	0.50	9	
	1.00	3	

with pain in the lumbar section it was 7.04 hours. The average weekly time spent at physical education classes at school was 2.18 hours for persons with pain in the thoracic section and 2.46 hours in the group of girls with pain in the lumbar section (Tables 1, 2). Regular breakfast time was observed by 41 persons, and lunchtime – by only 13 persons. Dinner was eaten at regular time by 32 persons and supper – by 23. The examined girls most often drank mineral water and juice (Table 4). Dairy products, vegetables, fruit and sweets were the main constituents of their diet (Table 5).

Table 5

Qualitative composition of the diet in the examined population – part 2

	Product	Number of persons	Average consumption of products
Frequency of consuming individual products in the diet	dairy products, yoghurts 7 times a week	34	5.22 times a week
	4 times a week	8	
	once a week	1	
	once a month	11	
	fish twice a week	4	0.96 times a week
	once a week	25	
	once a month	19	
	never	6	
	meat 7 times a week	26	4.90 times a week
	4 times a week	11	
	3 times a week	11	
	1 month	6	
	vegetables, fruit 7 times a week	34	5.38 times a week
	4 times a week	10	
	twice a week	7	
	once a month	4	
	flour products 7 times a week	20	5.22 times a week
	4 times a week	5	
	once a week	1	
	once a month	6	
	sweets 7 times a week	28	5.08 times a week
	4 times a week	8	
	3 times a week	1	
	twice a week	2	
	once a month	9	

The most frequent location of back pain was the lumbar section (28 persons; 51.9% of the examined population) and the thoracic section of the spine (21 persons; 38.9% of the examined population). Simultaneous pain covering in the cervical, thoracic and lumbar sections occurred in 4 persons (7.4 % of the examined population). Double scoliosis was diagnosed in 52 girls, and cases of single and triple scoliosis were diagnosed for single patients. In 28 persons (51.9%), it was found that the pain was reduced to the lumbar area and in 7 (13%) cases, it additionally radiated to lower limbs. MRI tests performed in the later group made it possible to diagnose discopathy of this section of the spine. The examined patients with pain syndrome in the lumbar section described the intensity of pain at the level of 5 and 6 points, which corresponds to strong pain. On the other hand, girls with pain syndrome in the thoracic section described the intensity of pain at the level of 4 and 5 points, which corresponds to pain of moderate intensity (Figure 1). A prolonged sedentary position during the day was indicated as causing back pain in 20 cases (37%). Additional activities conducted as part

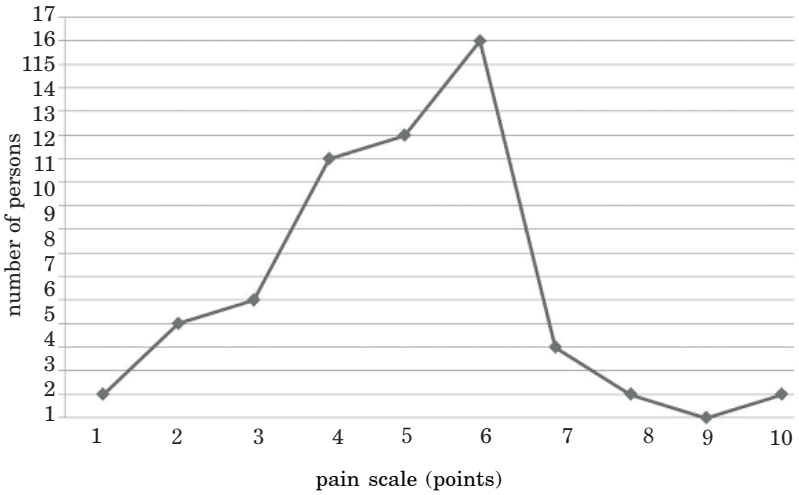

Fig. 1. Numerical rating scale of pain

of a therapeutic program were attended regularly by 36 persons (66.7%). The average time spent on such physical activity was 1.82 hours a week for persons with pain in the thoracic section and 1.07 hours a week for persons with pain in the lumbar section. Fifty of the examined girls (92.6%) claimed they were aware of the need to control the body posture. The correct body posture could be maintained deliberately by 23 persons (42.6%).

The highest progress in the unbalanced nature was observed in the thoracic part of the spine, while the highest intensity of pain and changes of a neurological character were observed in the lumbar section. The results of this study indicate high intensity of pain experienced by patients who,

despite their young age, revealed significant disorders in spinal statics. The quality and frequency of meals eaten were not related to the intensity and location of pain syndromes of the spine among the examined girls.

DISCUSSION

IS occurring during adolescence is the most frequent type of this condition, constituting at least 80% of cases diagnosed clinically (RISEBOROUGH et al. 1973). The etiology of the disease is in most cases unknown, and its symptoms often appear in previously healthy children (LONSTEIN et al. 1984). While looking for causes of the disease, researchers most often consider genetic predispositions and environmental factors, such as latitude (mostly northern) and related ambient temperature, air humidity or insolation, which, through specific mediators, affect cells of the human body (GRIVAS et al. 2006, MILLER 2000). The role of the hypothalamic neuroendocrine system, increasingly more often emphasized, should also be mentioned, as well as dysfunctions in the secretion of such substances as melatonin and leptin, since such irregularities can be accountable for more frequent occurrence of IS among girls in the adolescence period (BURWELL et al. 2008). Asymmetries found in infancy such as spinal deformations, which are revealed in the period of dynamic changes in body height during the pubertal body growth spurt, can play an important role in the development of IS (VOJTA 1988).

Essential aims of treating IS include prevention of further development of spinal curvatures, improvement of functions of the respiratory system and pain relief (WEISS et al. 2006).

The examined population of girls with diagnosed, non-invasively treated IS is characterized by a frequent occurrence of back pain and low physical activity. It is consistent with observations by WEINSTEIN et al. (2003), who explicitly confirmed significantly higher occurrence of spinal pain syndromes among persons suffering from IS in relation to the control group of healthy persons. It should be noted that the majority of cases in the examined group were diagnosed as first degree IS, but, as observed by PONSETI and FREIDMAN (1950), pain intensity in scoliosis does not depend on the angle of curvature but on its morphology and location. Curvatures located in the thoracic or lumbar sections more frequently involve the feeling of pain. This fact was also observed in the current study, in which the most frequent locations of pain included the sections of the spine described above.

The population of adolescent girls with low grade IS is a group of patients who, in survey examinations conducted by other authors, was characterized by a low level of self-esteem, frequent reporting of various types of ailments and, in consequence, depressed quality of life (GOLDBERG et al.

1994). What should be noticed is that persons suffering from IS should receive comprehensive care, covering a full scope of non-invasive treatment (including psychotherapeutic procedures) and surgical treatment whenever appropriate (KOTWICKI et al. 2008).

Damage to the central nervous system (CNS), causing subsequent developmental disturbances such as spinal deformation, can be caused by toxic elements commonly found in the natural environment. KOZIELEC et al. (2000) claim that an excessive amount of toxic metals, e.g. lead, cadmium, mercury, arsenic and aluminium, in the human body can disturb proper functions of the nervous and osseous systems. When accompanied by a concurrent shortage of basic bionutrients, such as calcium, magnesium, zinc, copper, iron, selenium, sulphur, cobalt, manganese and iodine, the concentration of toxic metals in the body increases. Such significant deviations from rational nutrition models, which lead to disturbances in the supply of proteins, vitamins, macro- and micronutrients (particularly in the phase of rapid growth in the developmental age) can inhibit psychomotor growth, lower the quality of bone tissue and lead to osteopenia and spinal deformations (KOZIELEC et al. 2000).

Processes of environmental and health stimulation in the form of corrective kinesitherapy of spinal deformations can bring about positive effects, known as the so-called long term synaptic potentiation (LTP). This fact explains the ability to maintain the growing effectiveness of synaptic conduction for a period of some hours or days (JOHNSSON et al. 2003), which has a positive influence on the process of remembering learnt patterns of proper body posture. Such results can also be expected after subsequent, repeated series of physiotherapeutic procedures, regarding as a specific type of environmental stimulation (KOWALSKI et al. 2007). Perhaps, excessive stimuli from the natural environment in the form of overloading kinesiological stimulation (as overtraining) leads to a reverse process, i.e. long-term depression of synaptic transmission (LTD) (JOHNSSON et al. 2003). A prolonged sedentary position can be also regarded as a form of overtraining, which is linked with disorders related to inhibition and stimulation of muscles by the CNS. Such stimulation results in disorders of muscular tension equilibrium and disorders of motor stereotypes. An excessive amount of information from the external environment, as well as a large number of improper stimuli lead to overloads in effectors, i.e. muscles, which is frequently manifested by pain. Motor stereotypes developed by everyday improper motor models cause disorders in the physiological posture, and disorders of posture sense; consequently, they can initiate many pathologies related to spinal deformations (JOHNSSON et al. 2003).

Backpain, often observed in patients with IS, can be therefore regarded as a specific indicator of improper arrangement of individual skeleton parts against one another. New, proper motor patterns introduced and coded in the CNS, along with regular, everyday training of aware and positively mo-

tivated IS patients carried out by therapists is a prerequisite of successful therapy (WIŚNIEWSKA et al. 2006). It should be also emphasized that only a radical change of everyday habits, mainly correction of improper body posture, control of the proper angle of the pelvis position, and increased time spent doing properly selected physical activity can lead to successful treatment and consequently, to improved quality of life for IS patients (WIŚNIEWSKA et al. 2006, KIEBZAK et al. 2009).

CONCLUSIONS

1. Patients with pain syndrome located in the lumbar part of the spine are characterized by a longer time spent in a sedentary position, less time devoted to a programme of therapeutic rehabilitation, higher intensity of pain and higher average age as compared to the group suffering from pain in the thoracic section of the spine.

2. The lifestyle of the examined patients and lack of awareness of the need to control of the body posture can affect the type and intensity of pain.

3. Qualitative composition of the examined persons' diet had no influence on the location or the intensity of pain suffered.

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EFFECT OF FOLIAR APPLICATION OF ANTHRACENE AND PYRENE (PAH) ON YIELDS AND CHEMICAL COMPOSITION OF BUTTERHEAD LETTUCE (*LACTUCA SATIVA* L.) GROWN UNDER VARIED ABUNDANCE OF SUBSTRATE IN NUTRIENTS

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Abstract

The purpose of this study has been to evaluate the effect of foliar application of PAH organic chemical compounds (anthracene, pyrene) on yield, chemical composition and uptake of nutrients by cv. Vilmorin butterhead lettuce (*Lactuca sativa* L.). Lettuce was grown under minimum and three-fold higher abundance of substrate in nutrients, as determined according to threshold amounts. A pot trial was established in four replicates and performed twice, in the spring of 2008 and 2009, in a greenhouse at the University of Warmia and Mazury in Olsztyn. Lettuce seedlings were planted in pots containing 10 dm^3 of mineral substrate. Fertilization (N, P, K, Mg, Na and Cl) was carried out prior to the planting of lettuce. Under the minimum nutrient abundance of the substrate, all the dose of nitrogen was supplied as a pre-sowing treatment, whereas when the abundance in nutrients was raised three-fold, the nitrogen dose was divided (2/3 pre-sowing and 1/3 10 days after planting). Contamination of lettuce plants with anthracene (ANT) or pyrene (PYR) and their mixture started 10 days after planting lettuce. Foliar application of either of the PAHs continued for 25 days until the vegetative growth of lettuce terminated. Determination of the concentration of macronutrients (N, P, K, Mg, Ca and Na) was performed using standard methods on the mineralised (H_2SO_4+H_2O_2), previously dried at 60şC lettuce plant material. The determinations were completed by referring to certified material (CTA-VTL-2). The amount of fresh mass of butterhead lettuce depended primarily on the abundance of the substrate in nutrients. The three-fold increase in the substrate's abundance in N, P, K,

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Mg, Na and Cl caused an increment in the yield of lettuce head mass by 13.3%. Foliar application of ANT and PYR caused an increase in the yield of lettuce head mass. The concentration of N, K, Na, Mg, Ca and Mg in lettuce was modified first of all by the abundance of the substrate and, to a lesser degree, by the applied PAHs.

Key words: *Lactuca sativa* L., macronutrients, anthracene, pyrene, fertilizer rates.

WPŁYW DOLISTNEJ APLIKACJI ANTRACENU I PIRENU (WWA) NA PLONOWANIE I SKŁAD CHEMICZNY SAŁATY MASŁOWEJ (*LACTUCA SATIVA* L.) UPRAWIANEJ W WARUNKACH ZRÓŻNICOWANEJ ZASOBNOŚCI PODŁOŻA W SKŁADNIKI POKARMOWE

Abstrakt

Celem badań była ocena oddziaływania wpływu dolistnej aplikacji organicznych związków chemicznych z grupy WWA (antracen oraz piren) na plon, skład chemiczny oraz pobranie składników pokarmowych przez sałatę masłową (*Lactuca sativa* L.*)* odmiany Vilmorin. Sałatę uprawiano w warunkach minimalnej i 3-krotnie zwiększonej zasobności podłoża w składniki pokarmowe, którą ustalono wg liczb granicznych. Doświadczenie wazonowe w 4 powtórzeniach prowadzono 2-krotnie wiosną w latach 2007-2008, w hali wegetacyjnej UWM w Olsztynie. Rozsadę sałaty wysadzono do wazonów o pojem. 10 dm^3 podłoża mineralnego. Nawożenie (N, P, K, Mg, Na i Cl) zastosowano przed sadzeniem sałaty. W warunkach minimalnej zasobności podłoża azot również podano w całości przedsiewnie a w warunkach 3-krotnie zwiększonej zasobności podzielono na 2 części (2/3 przedsiewnie i 1/3 po 10 dniach od posadzenia). Skażanie roślin antracenem (ANT) oraz pirenem (PYR) i ich mieszaniną rozpoczęto po 10 dniach od posadzenia sałaty. Aplikacja dolistna wybranych WWA trwała przez 25 dni do końca okresu wegetacji. Oznaczenia zawartości makroskładników (N, P, K, Mg, Ca, Na) dokonano standardowymi metodami po mineralizacji (H_2SO_4+H_2O_2) wysuszonego w 60ºC materialu roślinnego. Oznaczenia wykonano wobec materiału certyfikowanego (CTA-VTL-2). Ilość świeżej masy sałaty masłowej zależała przede wszystkim od zasobności podłoża w składniki pokarmowe. Trzykrotne zwiększenie zasobności podłoża w N, P, K, Mg, Na i Cl spowodowało przyrost plonu masy główek o 13,3%. Dolistnie aplikowane związki ANT i PYR powodowały przyrost masy główek sałaty masłowej. Zawartość N, K, Na, Mg, Ca i Mg w sałacie była modyfikowana przede wszystkim przez zasobność podłoża i w mniejszym zakresie przez aplikowane wybrane WWA.

Słowa kluczowe: *Lactuca sativa* L., makroelementy, antracen, piren, dawki nawozów.

INTRODUCTION

Intensive growth of particular branches of both industrial and agrcultural production, coupled by an increase in the human population and its expansiveness, means that growing amounts of carcinogenic and mutagenic substances, including polycyclic aromatic hydrocarbon compounds (PAHs), are emitted into the environment. Eventually, about 90% of such compounds end in soil (by sedimenting on plants) and are practically present in all elements of the natural environment in which we live (Wcisło 1998, Oleszczuk 2002, Kluska 2003). PAHs are mainly created during processes of incomplete combustion of organic matter, but also as a product of its mineral-

isation. The fact that there are few reports dealing with the effect of PAHs on the chemical composition of crops (WIECZOREK et al. 2006), especially when nutrients are deficient or excessive in substrate, stimulated our study.

The aim has been to assess the effect of foliar sedimentation of the PAH organic chemical compounds (anthracene and pyrene) on the yield, chemical composition and uptake of nutrients by cv. Vilmorin butterhead lettuce (*Lactuca sativa* L.), which was grown under the minimum and three-fold enhanced abundance of substrate in nutrients.

MATERIAL AND METHODS

A two-factor pot experiment, in four replicates, was set up on cv. Vilmorin butterhead lettuce (*Lactuca sativa* L.) grown in a greenhouse at the University of Warmia and Mazury in Olsztyn. Six-week seedlings of lettuce were purchased and planted on 25 April 2007 and 7 April 2008 in pots containing 10 dm^3 of substrate. The substrate used for the trials had the following chemical properties: 4.1 mg $N\text{-}NO_3$, 5.5 mg $N\text{-}NH_4$, 44.2 mg P, 173.3 mg K, 60.9 mg Mg, 921.9 mg Ca, 8.3 mg Na, 13.4 mg Cl^-, 71. mg $S\text{-}SO_4$ in dm^3, pH – 6.5, EC – 0.11 mS cm^{-1}. The following rates of nutrients were added per 1 dm^3 of the substrate: 60, 180 mg N; 50, 150 mg P; 50, 150 mg K; 40, 120 mg Mg; 20, 60 mg Na and 30.8 oraz 92.4 mg Cl^- (1^{st} factor). Before planting the lettuce seedlings, the soil surface was sprayed with propyzamide in the amount of 0.65 mg dm^{-3} of substrate. One gram of anthracene (ANT) or pyrene (PYR) was dissolved in 10 cm^3 of acetonitrile (ACN), filled up to 100 cm^3 with deionised water. Afterwards, 10 cm^3 of this solution was trasferred to 1000 cm^3 flasks and filled up to 1 dm^3 with deionised water. Control plants were sprayed with a solution of ACN of the same concentration or with deionised water. The first spraying treatment was performed 10 days after the lettuce had been planted and conducted twice daily for the next 25 days, supplying 1.8 cm^3 day^{-1} of ACN, ANT, PYR or ANT+PYR solutions of the concentration of 100 mg dm^{-3} (2^{nd} factor). The lettuce plants were harvested six weeks after planting the seedlings. During the harvest, lettuce heads were weighed. The chemical analysis involved only aerial parts of each lettuce plant (the treatments were not aggregated into combinations). The determinations of the concentration of N_{org} (Kjeldahl method), P (the vanadium-molybdene method), K, Ca, Na (the flame atomic emission spectrometric method, ESA), and Mg (the atomic absorption spectrometric method, AAS), having first wet mineralised the material in H_2SO_4. The determinations were completed with reference to cetrified material (CTA VTL-2), at the following error: P – 4.5%, K – 2%, Ca – 2.8%, Mg – 1.5%, Na – 7%.

The results were processed statistically using analysis of variance for a two-factor experiment in a completely random design, with an aid of the software package Statistica v. 7.0. The results are given as means from 8 replications for two years.

RESULTS AND DISCUSSION

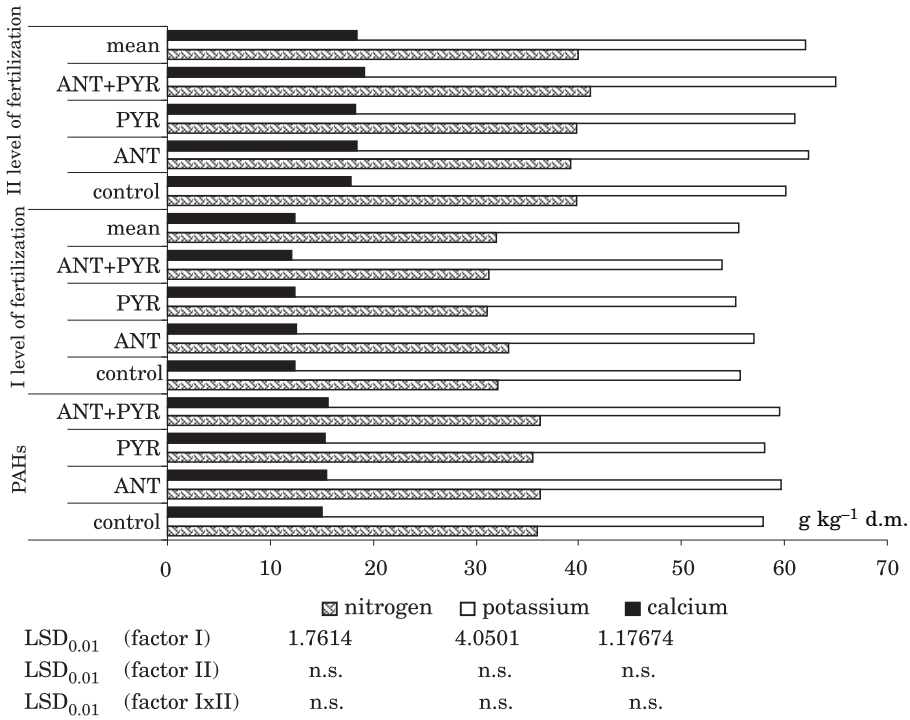
The increase in fresh matter of aerial parts of butterhead lettuce was significantly dependent mainly on the concentration of nutrients in the substrate (Table 1). The three-fold increase in the abundance of soil in nutrients (N, P, K, Mg, Na, Cl) caused an over 1.13-fold increase in the weight of lettuce heads. Foliar application of PAHs (ANT, PYR) raised an increase in the lettuce biomass. ANT demonstrated a stronger effect on the produced fresh mass of butterhead lettuce grown on soil less abundant in nutrients, while PYR – on substrate three-fold richer in nutrients. In a study completed by WIECZOREK et al. (2003), the increase in the aerial part of lettuce obtained after foliar application of anthracene was smaller. Such a result was observed in the present experiment only when substrate was more abundant in nutrients (on average, a 2.3 g per pot increase in weight).

Table 1

Effect of fertilization and foliar application of PAHs on the average mass of butterhead lettuce (*Lactuca sativa* L.) heads (g)

Fertilization level	Control	ANT	PYR	ANT + PYR	X ± SD
I	174.7	182.7	180.0	174.9	178.1 ± 22.47
II	196.2	193.9	202.8	214.0	201.7 ± 18.68
X ± SD	185.5 ± 21.2	188.3 ± 17.9	191.4 ± 23.6	194.5 ± 31.4	-
$LSD_{0.01}$ (factor I) – 13.84		$LSD_{0.01}$ (factor II) – n.s.		$LSD_{0.01}$ (factor I´II) – n.s.	

The concentration of nitrogen, potassium and calcium in leaves of butterhead lettuce was dependent mainly on the availability of nutrients in substrate (Figure 1). The substrate three-fold richer in nutrients resulted in an average increase in the concentration of nitrogen by 28.9% and potassium by 11.9% in leaves of the tested lettuce. Although calcium was not supplied with the fertilizers, its concentration in plants changed largely depending on the abundance of substrate in nutrients. The three-fold increase in quantities of available nutrients in substrate caused a 49.2% increased Ca concentration in plant tissues. In an experiment conducted by other researchers, such as MICHAŁOJĆ (2000), JAROSZ, DZIDA (2006), the concentration of nitrogen in leaves also increased following nitrogen fertilization, while the

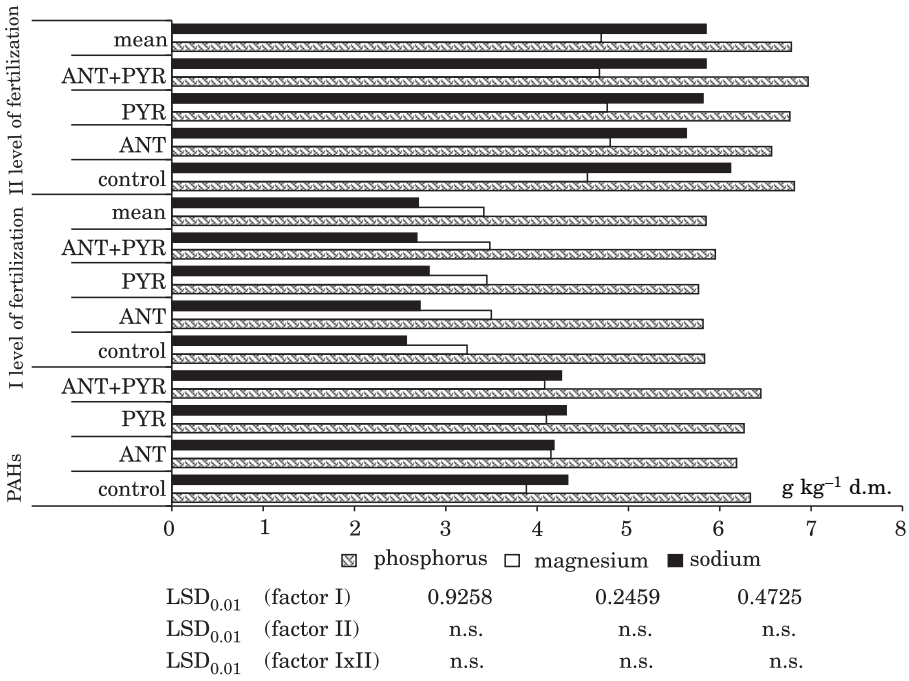


$LSD_{0.01}$	(factor I)	1.7614	4.0501	1.17674
$LSD_{0.01}$	(factor II)	n.s.	n.s.	n.s.
$LSD_{0.01}$	(factor IxII)	n.s.	n.s.	n.s.

Fig. 1. Effect of fertilization and foliar application of PAHs on the concentration of nitrogen, potassium and calcium in dry matter of butterhead lettuce heads (g kg^{-1} d.m.)

concentration of Ca did not change. This, however, did not lead to its increased content in substrate. PAHs reach plants predominantly via atmospheric precipitations or airborne dust.

Plants can also be contaminated with soil dust from substrate on which vegetables are grown (Maliszewska-Kordybach 1986, Bell, Failey 1991, Lodovici et al. 1994). There are very few reports on the influence of these compounds on the chemical composition of plants. In our study, the concentration of nitrogen in lettuce leaves affacted by the PAHs was less changed when lettuce grew on a substrate richer in nutrients than on a poorer one. The examined hydrocarbons raised the concentration of potassium in lettuce plants growing on a substrate three-fold richer in nutrients. When PAHs were sprayed over leaves of lettuce growing on a substrate poor in nutrients, the concentration of potassium in plant tissues was nearly unchanged or even decreased when ANT+PYR was applied. A reverse tendency was observed when lettuce was cultivated under improved availability of nutrients. Based on this observation, it can be concluded that the influence of PAHs on the concentration of Ca in lettuce was predominantly conditioned by the abundance of substrate in nutrients. In a study completed by



$LSD_{0.01}$	(factor I)	0.9258	0.2459	0.4725
$LSD_{0.01}$	(factor II)	n.s.	n.s.	n.s.
$LSD_{0.01}$	(factor IxII)	n.s.	n.s.	n.s.

Fig. 2. Effect of fertilization and foliar application of PAHs on the concentration of calcium and phosphorus in dry matter of butterhead lettuce heads (g kg^{-1} d.m.)

WIECZOREK et al. (2006), foliar application of anthracene raised the concentration of magnesium and calcium in leaves, potassium and sodium in stems and caclium and sodium in seeds of lupine.

The concentration of phosphorus, magnesium and sodium in lettuce was also significantly dependent on fertilization (Figure 2). Increasing amounts of P, Mg and Na in substrate led to an increase in their concentration in butterhead lettuce leaves by 16.1% for phosphorus, 37.4% for magnesium and 116% for sodium. No significant effect of ANT and PYR on the concentration of the analysed nutrients has been demonstrated. However, an increasing concentration of magnesium after foliar application of anthracene, pyrene as well as their mixture was observable, irrespective of the abundance of substrate. In turn, anthracene when sprayed over lettuce plants caused a decline in the concentration of phosphorus and sodium in leaves of butterhead leaves, especially when the substrate was highly abundant in nutrients.

The accumulation of macronutrients (N, P, K, Ca, Mg and Na) in aerial parts of burtterhead lettuce was significantly dependent on the availability of nutrients in soil (Table 2). The three-fold increase in the amounts of available nutrients in soil led to their enhanced uptake. The uptake of sodi-

Table 2

Accumulation of macronutrients in aerial parts of butterhead lettuce depending on fertilization and foliar application of PAHs

Treatments			N	P	K	Ca	Mg	Na
			mg pot^{-1}					
Factor I	fertilization 1st level	control	383.3	68.93	658.4	147.8	38.52	31,10
		ANT	382.0	68.30	663.2	144.4	41.00	31,90
		PYR	372.2	68.23	660.4	148.0	41.10	33,52
		ANT+PYR	366.5	69.70	640.0	142.7	41.50	31,57
		mean	376.0	68.8	655.5	145.7	40.50	32.03
	fertilization 2nd level	control	483.1	80.80	730.7	222.9	56.48	74.90
		ANT	474.7	76.20	761.9	230.2	58.23	66.35
		PYR	485.4	81.90	751.4	227.6	57.38	68.80
		ANT+PYR	511.9	82.70	809.3	243.7	59.02	72.70
		mean	488.8	80.40	763.3	231.1	57.8	70.69
Factor II PAHs		control	433.2	74.87	694.6	185.3	47.50	53.00
		ANT	428.4	72.23	712.6	187.3	49.62	49.13
		PYR	428.8	75.07	705.9	187.8	49.24	51.15
		ANT+PYR	439.2	76.18	724.7	193.2	50.16	52.13
$LSD_{0.01}$ (factor I)			20.74	6.823	25.72	23.72	3.292	4.978
$LSD_{0.01}$ (factor II)			n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
$LSD_{0.01}$ (factor I II)			n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

um was raised the most evidently, followed by that of calcium (even though the substrate was not fertilised with this element), magnesium, nitrogen and – finally – phosphorus and potassium, whose uptake increased to a comparable degree. The effect of the other factor was not significant, although it could be noticed that under the effect of anthracene, the uptake of nitrogen, phosphorus and sodium declined while the accumulation of potassium, calcium and magnesium increased. The same PAH applied in conjunction with pyrene caused an increased uptake of all the macronutrients except for sodium.

CONCLUSIONS

1. The yield of butterhead lettuce heads was mainly affected by the fertilization and, to a lesser extent, by the contamination of the plants with anthracene, pyrene or their mixture.

2. The concentration of macronutrients in lettuce was predominantly modified by the amounts of nutrients available to lettuce plants from substrate.

3. Anthracene and pyrene as well as their mixture, when applied as foliar sprays, increased significantly the mass of butterhead lettuce heads.

4. Butterhead lettuce accumulated significantly more nutrients in aerial parts when growing on a substrate three-fold more abundant in N, P, K, Mg, Na and Cl.

5. No significant effect of foliar application of anthracene and pyrene on the accumulation of N, P, K, Ca, Mg and Na in aerial parts of butterhead lettuce has been observed.

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INFLUENCE OF THE ADMINISTRATION OF SELENIUM COMPOUNDS ON TISSUE MAGNESIUM CONCENTRATION IN RATS

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Abstract

Magnesium and selenium belong to important bioelements. Magnesium is the second most abundant intracellular macroelement, which takes part in the metabolism of carbohydrates, nucleic acids, protein and lipids. Selenium is an essential microelement, whose deficit has been stated in many different pathological states. Much research on safe and effective selenium supplementation has been performed for the last fifty years but the results still remain unsatisfactory.

The aim of our study was to investigate the influence of inorganic sodium selenite Na_2SeO_3 and two selenoorganic compounds synthetized at our chair on magnesium concentration in tissues of adolescent male Wistar rats. Inorganic selenite was administered as a water solution, whereas organic compounds: 4-(o-tolilo)-selenosemikarbazyd of 2-chlorobenzoic acid of a chain structure (ORG-C) and 3-(o-chlorobenzoylamino)-2-(o-tolylimino)-4--methyl-4-selenazoline of a ring structure (ORG-R) were suspended in emulsion (oil, arabic gum and water). Selenium compounds were given to rats at a dose of $5 \cdot 10^{-4}$ mg Se g^{-1} b.w. once a day for a period of 10 days. The control group was treated with saline. The administration was performed with use of a stomach tube.

In comparison to the control group, selenium supplementation caused decrease in magnesium concentration in kidney and lung tissues, but did not cause any changes in the brain and heart muscle. In the liver and spleen it was only ring selenazoline that affected magnesium concentration, increasing it in the liver and decreasing in the spleen. In the femoral muscle it was only the selenosemicarbazide chain that exerted the significant effect causing a decrease in Mg concentration vs the control group.

Selenium supplementation influences the tissue magnesium concentrations depending on tissue and structure of the supplement. Irrespective of the administered compound, it lowered magnesium in kidneys and lungs but caused no changes in the brain and heart muscle. In the liver, spleen and femoral muscle, alterations in the magnesium concentration were dependent on the provided supplement.

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Key words: male rats, organoselenium compounds, supplementation, magnesium.

WPŁYW PODAWANIA ZWIĄZKÓW SELENU NA TKANKOWE STĘŻENIE MAGNEZU U SZCZURÓW

Abstrakt

Magnez i selen należą do biopierwiastków bardzo ważnych dla prawidłowego funkcjonowania organizmu. Magnez jest drugim co do ilości makropierwiastkiem wewnątrzkomórkowym, który odgrywa istotną rolę w metabolizmie węglowodanów, kwasów nukleinowych białek i lipidów. Selen jest niezbędnym mikroelementem, którego deficyt został stwierdzony w różnych stanach patologicznych. Przez ostatnie 50 lat prowadzono rozległe badania nad skuteczną i bezpieczną suplementacją tego pierwiastka, ale uzyskane wyniki nie są do końca satysfakcjonujące.

Celem pracy było zbadanie wpływu nieorganicznego selenianu(IV) sodu Na_2SeO_3 i dwóch organicznych związków selenu o różnej budowie na stężenie magnezu w tkankach młodych samców szczurów rasy Wistar. Nieorganiczny selenian(IV) sodu podawano w postaci wodnego roztworu, natomiast organiczne związki selenu: 4-(o-tolilo)-selenosemikarbazyd kwasu 2-chlorobenzesowego (ORG-C, budowa łańcuchowa) i 3-(2-chlorobenzoiloamino-)-2-(o-toliloimino-)-4-metylo-4-selenazolina (ORG-R, budowa pierścieniowa) w formie emulsji złożonej z oleju, gumy arabskiej i wody. Grupa kontrolna otrzymywała sól fizjologiczną. Związki podawano sondą dożołądkowo w dawce $5 \cdot 10^{-4}$ mg Se g^{-1} m.c. 1 raz dziennie przez okres 10 dni.

W porównaniu z grupą kontrolną nieotrzymującą selenu, suplementacja związkami Se wpłynęła na statystyczny spadek stężenia magnezu w tkankach nerki i płuca, natomiast nie spowodowała żadnych zmian w tkance mózgu i mięśnia serca. W tkance wątroby i śledziony jedynie cykliczna selenazolina wpłynęła na stężenie magnezu – w wątrobie zaobserwowano wzrost, a w śledzionie spadek. W tkance mięśnia uda jedynie łańcuchowy selenosemikarbazyd wywarł istotny wpływ, powodując obniżenie stężenia Mg w stosunku do grupy kontrolnej.

Suplementacja selenu wpływa na tkankowe stężenie magnezu w sposób zależny od rodzaju tkanki i struktury zastosowanego suplementu. Niezależnie od budowy podawanego związku, zaobserwowano obniżenie stężenia magnezu w tkance nerki i płuca, natomiast nie zauważono żadnych zmian w mózgu i mięśniu serca. W tkankach wątroby, śledziony i mięśnia uda zmiany stężenia magnezu były zależne od rodzaju podawanego związku.

Słowa kluczowe: szczury samce, organiczne związki selenu, suplementacja, magnez.

INTRODUCTION

Magnesium is the second most abundant intracellular macroelement (Telci et al. 2002). It takes part in the metabolism of carbohydrates, nucleic acids, protein and lipids (Barbosa et al. 2010). Many disorders of functions in a human body can be connected with magnesium deficiency e.g.: disturbance of the cardiovascular system and homeostasis of other bioelements, muscle weakness as well as decreased parathyroid hormone secretion (Shoback 2008, Assadi 2010).

Selenium belongs to essential trace bioelements and its deficiency in an organism may result in numerous severe diseases, for example a low selenium level has been found in cases of alimentary tract illnesses (Skelton et al. 2006), dermatic and nephrological disorders (Ingen-housz-oro et al. 2004, Zachara et al. 2004) and AIDS development after HIV-infection (Rayman 2000). The question of selenium supplementation is rather complicated because of the narrow range between therapeutic and toxic doses (Hawkes et al. 2008). Moreover, its bioavailability depends on the structure of a used supplement (Burk et al. 2006). For the last fifty years extensive research on supplementation of selenium has been carried out (Combs 2005, Řezanka, Sigler 2008, Selamoglu Talas et al. 2009), including both inorganic (sodium selenite or selenate) (Ivancic, Weiss 2001, Uezono et al. 2006) and organic compounds (selenomethionine, selenocyanates, selenic acids of a chain structure as well as compounds of a cyclic form e.g. ebselen which has a benzisoselenazolone structure) (Xia et al. 2004, Burket et al. 2006, Cui et al. 2008). However, the problem of safe and efficient Se-supplementation still remains unsolved.

Two selenium organic compounds synthesized at our chair: 3-(2-chlorobenzoylamino)-2-(o-tolylimino)-4-methyl-selenazoline (Musik et al. 2009) possessing an ebselen-like, ring structure and 4-(o-tolyl)-selenosemicarbazide of 2-chlorobenzoic acid (Musik et al. 2002b) of a chain structure were studied in regard of the possibility of their application as a selenium supplement. Taking into account the importance of magnesium for proper functions of a human organism, we investigated the effect of the oral administration of the above compounds on the Mg tissue concentrations in rats.

MATERIALS AND METHODS

The experiment was carried out on four groups of adolescent male Wistar rats (ten animals each):

Group I – control treated with saline (n=10);
Group II – treated with water solution of sodium selenite (Na_2SeO_3), n=10;
Group III – treated with 4-(o-tolyl)-selenosemicarbazide of 2-chlorobenzoic acid – compound ORG-C (n=10);
Group IV – treated with 3-(2-chlorobenzoylamino)-2-(o-tolylimino)-4-methyl-4--selenazoline – compound ORG-R (n=10).

The weight of the animals at the beginning of the study was within the range of 110-150 g. Organic compounds given to groups III and IV were suspended in emulsion composed of oil, arabic gum and water in the following proportions 2:1:1.5. Selenium compounds were given to rats at a dose of $5 \cdot 10^{-4}$ mg Se g^{-1} b.w. once a day for a period of 10 days. The administration was performed with use of a stomach tube. The body weight of animals was measured every day before Se administration. Rats had free access to standard feed LSM and drinking water.

After the experiment, the animals were sacrificed under pentothal narcosis and the tissues of their kidneys, liver, brain, spleen, femoral muscle, heart muscle and lungs were collected. Ten per cent (w/v) tissue homogenates were prepared in 0.1 mol dm^{-3} Tris – HCl buffer, pH = 7.4. Supernatants were obtained by centrifugation at 5000 x g for 30 min. The prepared material was stored at -18°C.

Magnesium concentration in the supernatants was measured by the reaction with xylidyl blue (diagnostic set Liquick Cor-MG 60), using the colorimetric method. The wavelength was 520 nm. The assays were carried out with the help of a SPECORD M40 (Zeiss Jena) spectrophotometer.

Comparisons between the control and tested groups as well as between the selenium supplemented groups were made using c-Cochran-Cox test. The values were considered significant at $p < 0.05$.

The study was performed according to the statutory bioethical standards and approved by the Local Ethics Commission of the Medical University of Lublin, approval 65/AM/2004.

RESULTS AND DISCUSSION

The results obtained in the present experiment provided evidence to support interaction between magnesium and selenium. This effect was mainly observed in kidney and lung tissues. In comparison with the control group (without Se), selenium supplementation, regardless of its form, caused a decrease in the magnesium tissue concentration. In contrast, none of the Se supplement caused any changes in the brain and heart muscle. In the liver and spleen, it was only ring selenazoline that affected the magnesium concentration, casuing its increase in the liver and a decrease in the spleen. In the femoral muscle it was only chain selenosemicarbazide that exerted a significant effect vs the control group, causing a decrease in the Mg concentration.

Our comparison between the Se-treated groups showed that in some tissues the influence of selenium on Mg tissue concentration was dependent on its form. In the liver, ring selenazoline (group IV) increased Mg in comparison with the other groups receiving selenocompounds, significantly vs group III (given chain selenosemicarbazide). In the spleen, organic compounds diminished the Mg concentration in comparison with inorganic selenite and in group IV this effect was significant vs group III. In the femoral muscle, a distinct difference between organic compounds was displayed, namely chain selenosemicarbazide decreased Mg whereas ring selenazoline enhanced the level of this element, an effect which was evident vs group II (sodium selenite) and group III (selenosemicarbazide).

All the results of determinations are presented in Table 1.

Table 1

Magnesium tissue concentration in rats receiving different selenium supplements

Group	Magnesium tissue concentration (mmol kg^{-1} of wet tissue)						
	kidney $\overline{x}$ ± SD	liver $\overline{x}$ ± SD	brain $\overline{x}$ ± SD	spleen $\overline{x}$ ± SD	femoral muscle $\overline{x}$ ± SD	heart muscle $\overline{x}$ ± SD	lung $\overline{x}$ ± SD
Group I Control n=10	9.61 ± 0.37	5.30 ± 1.59	4.20 ± 0.44	9.75 ± 1.73	9.53 ± 1.39	5.85 ± 1.46	.71 ± 1.13
Group II Treated with Na_2SeO_3 n=10	5.15 ± 0.90 ***	5.79 ± 1.12	3.11 ± 0.26	9.47 ± 2.18	8.05 ± 1.08	5.89 ± 0.55	3.76 ± 0.98 ***
Group III Treated with ORG-C n=10	4.73 ± 1.45 **	5.74 ± 0.46	4.10 ± 0.43	7.62 ± 1.52	6.73 ± 0.72 **	6.00 ± 0.70	3.34 ± 0.35 ***
Group IV Treated with ORG-R n=10	5.09 ± 0.50 ***	7.08 ± 0.39 $^{*, Y}$	4.50 ± 0.58	5.04 ± 1.27 $^{**, \#}$	11.21 ± 1.62 $^{\#, Z}$	6.65 ± 0.50	2.79 ± 0.50 ***

Values are mean ± standard deviation
* $p < 0.05$
$^{**}p < 0.01$
*** $p < 0.001$ vs. group I
$^{\#}$ $p < 0.01$ vs. group II
^{Y} $p < 0.01$; ^{Z} $p < 0.001$ vs. group III
n – number of animals in the group

In our previous experiment, we studied the effect of similar selenoorganic compounds on magnesium concentration in mice. The obtained results were the same in the brain, where no changes were observed. In the kidney and liver, inorganic selenite exerted the same effect causing significant decrease in the kidney and no alterations in the liver. A slight modification of the structure of an organic chain supplement (4-(o-tolyl)-selenosemicarbazide of 4-chlorobenzoic acid instead of 4-(o-tolyl)-selenosemicarbazide of 2-chlorobenzoic acid) resulted in distinct differences in its influence – in the kidney magnesium did not change significantly, whereas in the liver a decrease was observed. When the ring-structured supplement was applied, a more appreciable difference in the structure (3-(4-chlorobenzoylamino)-2--(o-tolylimino)-4-phenyl-4-selenazoline instead of 3-(2-chlorobenzoylamino)-2--(o-tolylimino)-4-methyl-4-selenazoline) altered its influence in the kidney, where no changes were noticed, and in the liver, where significant Mg depletion was determined. In the brain, the above modification of the supplement's structure did not change the effect of Se-supplementation (Musik et al. 2002a).

Relationships between Mg and Se have already been reported (Osada et al. 2002, Erdal et al. 2008). Both elements play an important role in the diabetes therapy (Matej-butrym, Schabowski 2008) and were reported to be effective agents for mercury toxicity (Shukla et al. 2007). Decrease in the serum magnesium and selenium was found in diabetic patients (Kamal et al. 2009). In epileptic patients treated with valproic acid, increased serum selenium was observed but the magnesium concentration in serum was unaltered (Hamed et al. 2004).

Investigating changes in the magnesium level in tissue of animals subjected to selenium supplementation seems to be advisable because such determinations are possible only using an animal model. In horses administered a selenium-containing diet, no alterations of the plasma Mg were observed, irrespective of both Se-source (selenite or Se-yeast) and Se-dose (Calamari et al. 2010). In rats receiving sodium selenite, enhanced fractional reabsorption of magnesium was displayed (Sakly et al. 2003). Inorganic selenium caused some increase in the Mg^{2+} accumulation in K562 cells (Jun--ying, Cun-Shuan 2009).

The effect of selenium can depend on other substances administered to experimental animals. In our study, Se did not alter Mg in the heart muscle of healthy rats. In rabbits administered daunorubicin, a significant decrease in the myocardial magnesium was accompanied by a slight increase in selenium (Šimunek et al. 2005). Rats treated with dietary cadmium showed depressed Se and Mg in the liver (Noël et al. 2004), although we did not observe any effect of the selenium treatment (sodium selenite or chain selenosemicarbazide groups) or an increase in the liver magnesium (ring selenazoline). Other factors can also influence the effect of Se-administration. In horses given selenium with vitamin E after exercise, a significant increase in serum Mg was observed, whereas before exercise no changes were

obtained (YUR et al. 2008). Sodium selenite caused only a slight enhancement of magnesium in lenses of rats exposed to cigarette smoke (DILSIZ et al. 1999). Relationships between magnesium and selenium in an organism are also dependent on time. In rats fed a magnesium-deficient diet the kidney selenium was increased after 7 days, whereas in the heart such an effect was not observed until day 70 (JIMENEZ et al. 1997). Similarly, in the present study, short-term administration of selenium compounds did not change the heart muscle magnesium.

CONCLUSIONS

1. Selenium supplementation influenced tissue magnesium concentrations depending on tissue and structure of the supplement.

2. Selenium supplementation, irrespective of an administered compound, caused magnesium depression in the kidney and lungs and no changes in the brain and heart muscle.

3. In the liver, spleen and femoral muscle, alterations of magnesium concentrations were was dependent on the provided supplement.

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EFFECT OF SOIL CONTAMINATION WITH ARSENIC AND APPLICATION OF DIFFERENT SUBSTANCES ON THE MANGANESE CONTENT IN PLANTS

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Abstract

The aim of the study has been to determine the effect of some substances such as dolomite, loam, compost, pinewood bark, peat, lime, charcoal, natural and synthetic zeolite on reducing the impact of soil contamination with arsenic on the content of manganese in some plant species. The content of manganese in the test plants depended on the degree of soil contamination with arsenic, application of different substances as well as on the plant species and organ. Soil contamination with arsenic caused either an increase or a decrease in the content of manganese in plants depending on a plant species and organ. In the series without soil amending substances, in the arsenic contaminated objects the manganese content decreased in above-ground parts of cocksfoot and swede but increased in above-ground parts and roots of maize and yellow lupine, in roots of cocksfoot and swede and in straw and roots of spring barley. On the other hand, the highest rates of arsenic depressed the content of manganese in roots of cocksfoot, swede and spring barley. Addition of any of the aforementioned substances to contaminated soil changed the content of manganese in the plants. The most unambiguous effect of the different substances was determined in the case of above-ground parts of maize as well as above-ground parts and roots of cocksfoot, in which the manganese content fell down, and in roots of yellow lupine, grain and straw of spring barley, in which the content of manganese rose. Charcoal and loam caused the largest and synthetic zeolite led to the smallest changes in the content of manganese in plants.

Key words: arsenic contamination, substances application, plants, manganese content.

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WPŁYW ZANIECZYSZCZENIA GLEBY ARSENEM I APLIKACJI RÓŻNYCH SUBSTANCJI NA ZAWARTOŚĆ MANGANU W ROŚLINACH

Abstrakt

Celem badań było określenie oddziaływania dodatku do gleby wybranych substancji: dolomitu, iłu, kompostu, kory sosnowej, torfu, wapna, węgla drzewnego, zeolitu naturalnego i zeolitu syntetycznego na ograniczenie wpływu zanieczyszczenia gleby arsenem i zawartość manganu w wybranych roślinach. Zawartość manganu w badanych roślinach zależała od poziomu zanieczyszczenia gleby arsenem, aplikacji substancji oraz od gatunku i organu roślin. Zanieczyszczenie gleby arsenem powodowało zwiększenie lub zmniejszenie zawartości manganu w roślinach, w zależności od ich gatunku i organu. W serii bez dodatków, w obiektach zanieczyszczonych arsenem, odnotowano zmniejszenie zawartości manganu w częściach nadziemnych kupkówki i brukwi oraz zwiększenie jego zawartości w częściach nadziemnych oraz korzeniach kukurydzy i łubinu żółtego, w korzeniach kupkówki pospolitej i brukwi pastewnej, a także w słomie i korzeniach jęczmienia jarego. Jednakże najwyższe dawki arsenu wywołały zmniejszenie zawartości manganu także w korzeniach kupkówki, brukwi i jęczmienia jarego. Dodatek do gleby różnych substancji spowodował zmiany w zawartości manganu w badanych roślinach. Ich najbardziej jednoznaczny wpływ stwierdzono w częściach nadziemnych kukurydzy oraz w częściach nadziemnych i korzeniach kupkówki, w których następowało, na ogół, zmniejszenie, oraz w korzeniach łubinu żółtego, ziarnie i słomie jęczmienia jarego, gdzie wykazano zwiększenie zawartości manganu. Węgiel drzewny i ił powodowały największe zmiany w zawartości manganu w roślinach, a zeolit syntetyczny najmniejsze.

Słowa kluczowe: zanieczyszczenie arsenem, aplikacja substancji, rośliny, zawartość manganu.

INTRODUCTION

Air emission is one of the major sources of arsenic in soil, water and plants. Arsenic accumulation occurs via dry and wet deposition, i.e. with the participation of atmospheric precipitations and gravitational forces (HŁAWICZKA 1998). Poland is not seriously threatened by environmental pollution with arsenic. Moreover, the emission of noxious elements to the atmosphere, including arsenic, has been decreasing in the last years. However, there are millions of people worldwide who are at risk of contracting illnesses caused by the toxic effect of arsenic (HAN et al. 2003). The response of a human organism to arsenic depends on the amount of the element, type of contact, duration of the exposure, sources and chemical form of arsenic. At the third oxidation level, this element is 60-fold more toxic than at its fifth oxidation level. In turn, mineral forms of arsenic are up to 100-fold more harmful than organic ones. Also, the way and duration of the contact of arsenic with a human body have a large influence on its impact (ATSDR 2000, JAIN, ALI 2000, CAUSSY 2003). According to HAN et al. (2003), in 2000 a potential load of arsenic introduced to arable soils by man's activity was 283 kg As km^{-2}, which was 31-fold more than in 1990. The same authors estimate that one

kg of the surface layer of soil (10 cm) potentially receives an average 2.18 mg As every year. Arsenic is highly vulnerable to redox conditions. Its presence and mobility in the environment largely depend on interactions between several other biogeochemical conditions, such as pH, microbial activity, ionic relations as well as the presence of loamy minerals or organic substance (LIPIŃSKI 2000, WARNER 2003, KARCZEWSKA et. al. 2005). Such factors, which can be modified by natural processes as well as human activity, can largely affect the processes of absorption and desorption or dissolution and precipitation, which in turn modify the mobility of arsenic in the environment (WARNER 2003) and the effect arsenic produces on plants, including their ability to uptake macro- and microelements. Thus, effective methods are being searched for to reduce the effect of this metal on soil properties and plants.

The present study has been conducted to determine the effect of adding to soil certain substances, such as dolomite, loam, compost, pinewood bark, peat, lime, charcoal, natural zeolite and synthetic zeolite, on reducing the influence of soil pollution with arsenic on the content of manganese in several plant species.

MATERIAL AND METHODS

The study consisted of a pot experiment conducted in a greenhouse of the University of Warmia and Mazury in Olsztyn. The pot trials were established on three soils similar in their physicochemical properties (during 4 years), all collected from the humus layer of typical Eutric Cambisols soil, characterised by the grain-size distribution of loamy sand. They were acidic or slightly acidic in reaction, moderately abundant in available phosphorus and potassium, moderately or poorly abundant in available magnesium. The content of arsenic and other trace elements was low and did not exceed the threshold levels set for lands used for agricultural purposes (*Ordinance of the Ministry for Environment* 2002). Out tests on the effect of soil contamination with arsenic added to soil in the doses of 10, 20, 30 and 40 mg As kg^{-1} of soil were carried out on cv. Juno yellow lupine (*Lupinus luteus* L.), and these which included 25, 50, 75 and 100 mg As kg^{-1} of soil involved cv. Scandia maize (*Zea mays* L.) cv. Nawra cocksfoot (*Dactylis glomerata* L.), cv. Ortega spring barley (*Horendum vulgare* L.) and cv. Sara swede (*Brassica napus* var. *napobrassica*). In order to reduce the influence of arsenic on the plants, the soils were enriched with the following substances: lime, natural zeolite, charcoal, loam and compost in tests on maize; lime, natural zeolite, charcoal, loam, compost and synthetic zeolite in tests on cocksfoot and yellow lupine; peat, pinewood bark, loam, dolomite and synthetic zeolite in tests on spring barley and swede. Both zeolites were relatively rich in manganese; also loam contained higher amounts of this ele-

ment. Peat, pinewood bark, dolomite and calcium oxide had little manganese. The substances were added to soil in the amounts equal 3% in relation to the soil mass in a pot (9 kg) whereas lime and dolomite were introduced in the quantities corresponding to 1 hydrolytic acidity (Hh). In addition, a control series of tests was run (without any extra substances added to soil). The test soils were enriched with NPK fertilizers added in rates adjusted to fertilization requirements of particular crops. Arsenic was added to soil as sodium arsenate, nitrogen was introduced as urea, phosphorus as triple superphosphate and potassium as potassium salt, with all these compounds being applied as aqueous solutions. Having prepared the soils, the plants were sown in pots. After their emergence, the following stands were left per pot: 10 maize plants, 8 cocksfoot plants, 9 yellow lupine plants, 15 spring barley plants and 3 swede plants. The vegetative pot experiments were performed in 3 replications. During the vegetative growth of the plants, soil moisture content was maintained at 60% field water capacity. The plants were harvested at the technological maturity phase, after which the plant material was sampled for laboratory analysis.

The plant material was fragmented, dried at 60ºC and ground. The content of manganese was determined by the atomic absorption spectrophotometry method (OSTROWSKA et al. 1991). The results underwent statistical processing (two-factorial analysis of ANOVA variance) using the software package Statistica (STATSOFT, INC. 2007). Additionally, Pearson's simple correlation coefficients were computed between the rates of arsenic and the content of manganese in the plant organs.

RESULTS AND DISCUSSION

The content of manganese in the plants depended on the degree of soil contamination with arsenic, application of the additional substances as well as the species and organs of plants (Table 1). Soil contamination with arsenic resulted in an increase or decrease in the concentration of manganese in plants, depending on a plant species and organ (Table 1). In the series without any substances added to soil, the content of manganese decreased in above-ground parts of cocksfoot and swede but increased in above-ground parts and roots of maize and yellow lupine, in roots of cocksfoot and swede and in straw and roots of spring barley in all the objects polluted with arsenic. The decrease in the concentration of manganese in above-ground organs of cocksfoot and swede was on a similar level and equalled, respectively, 23% (r=-0.847) and 21% (r=-0.947). Soil pollution with arsenic raised the content of manganese the highest in spring barley straw. The stimulating effect was the weakest in above-ground parts of maize and yellow lupine. The increase in the manganese content in roots was higher than in above-

Table 1

Effect of arsenic on manganese content (Mn) in plants in a series without additions (mg kg^{-1} d.m.)

Arsenic doses	Plant					
	yellow lupine (*Lupinus luteus* L.)	maize (*Zea mays* L.)	cocksfoot (*Dactylis glomerata* L.)	swede (*Brassica napus* L. var. *napobrassica* L. Rchb.)	spring barley (*Hordeum vulgare* L.)	
Above-ground parts					grain	straw
0	65.56	79.80	336.50	156.40	48.96	110.25
I	67.11	84.50	275.60	138.25	48.91	158.95
II	67.67	84.20	266.00	135.70	48.76	171.75
III	71.11	83.20	263.70	131.00	48.06	174.75
IV	72.45	83.40	254.40	123.05	47.11	176.60
Average	68.78	83.02	279.24	136.88	48.36	158.46
r	0.975**	0.496	-0.847**	-0.947**	-0.915**	0.844**
LSD	3.76**	3.62**	13.72**	8.93**	n.s.	11.82**
Roots						
0	171.12	70.50	185.00	57.75	160.48	
I	172.29	86.25	197.55	62.35	162.26	
II	188.12	85.40	221.15	64.65	192.71	
III	212.21	91.50	248.35	71.10	189.32	
IV	n.a.	98.75	198.25	60.25	159.71	
Average	185.94	86.48	210.06	63.22	172.90	
r	0.937**	0.939**	0.488	0.427	0.243	
LSD	5.03**	4.51**	12.34**	3.58**	10.22**	

Arsenic doses:
yellow lupine: I – 10; II – 20: III – 30 and IV– 40 mg As kg^{-1} of soil;
other plants: I – 25; II – 50: III – 75 and IV – 100 mg As kg^{-1} of soil;
Significant for: * p=0.05, ** p=0.01; n.s. – differences non-significant;
r – correlation coefficient;
n.a. – not analysed because of an insufficient amount of plant material

ground parts of maize and yellow lupine. Under the effect of arsenic contamination, the content of manganese in maize roots increased by 40% (r=0.939) and in yellow lupine roots it rose by 24% (r=0.937). The highest increase in the accumulation of manganese in the arsenic contaminated treatments occurred in spring barley straw where it reached 60% (r=0.844).

Increased levels of manganese were also found in roots of cocksfoot and swede up to the contamination rate of 50 mg As kg^{-1} of soil and in spring barley roots but only up to the dose of 75 mg As kg^{-1} of soil. The highest rates of arsenic (100 mg As kg^{-1} of soil) caused depressed concentrations of manganese in the above organs of these plants.

The relevant literature contains very few publications which deal with the effect of arsenic contamination of soil on the content of manganese in plants. According to PÄIVÖKE and SIMOLA (2001), under the effect of arsenic the content of manganese in plants declines, which has been partly confirmed in the authors' own research. SHAIBUR et al. (2008) found lower levels of manganese in shoots and roots of barley growing on a substratum contaminated with arsenic. In our research, the effect of arsenic was strongly dependent on the species and organ of a plant, although the dominant correlation was that between an increasing content of manganese in plants growing on increasingly contaminated objects.

Introduction of different substances to soil caused changes in the content of manganese in the analysed plants (Table 2). The least ambiguous effect of these substances occurred in above-ground parts of maize and in above-ground parts and roots of cocksfoot, where manganese tended to decline, as well as in roots of yellow lupine or grain and straw of spring barley, where more manganese was determined. The negative impact of the different substances was stronger in above-ground parts than in roots of maize. As for cocksfoot, a reverse tendency was revealed – modifications in the manganese content were larger in roots than in above-ground organs. The concentration of manganese in maize above-ground organs was depressed on average from 33-36% (lime, natural zeolite, compost) to 49-50% (loam, charcoal). In roots of maize, the negative effect on manganese occurred only when charcoal (34%) and natural zeolite (23%) had been applied. An analogous negative effect of compost and lime on the content of manganese in cocksfoot roots (46% less manganese) was larger that that produced by charcoal (41%), loam (33%) and natural zeolite (16%). In the above-ground parts of this plant, such an effect was evidently weaker. Loam and compost produced the strongest effect on roots of yellow lupine, although the other substances also favoured the accumulation of manganese in roots of this plant. In contrast, above-ground parts of yellow lupine, like roots of swede, were only slightly affected by the additional substances, except for natural zeolite, which depressed the content of manganese in above-ground organs of yellow lupine. Synthetic zeolite, dolomite and loam in particular depressed the content of manganese in above-ground parts of swede, unlike peat and pinewood bark. All the substances increased the content of manganese in grain and straw of barley. Peat and synthetic zeolite, in addition to the above, also raised the levels of this element in spring barley roots. There were only two substances – pinewood bark and dolomite – which reduced, albeit very slightly, the content of manganese in roots of this plant. The

Table 2

Effect of different substances on manganese content (Mn) in plants –average from series (mg kg^{-1} d.m.)

Substances	Plant					
	yellow lupine (*Lupinus luteus* L.)	maize (*Zea mays* L.)	cocksfoot (*Dactylis glomerata* L.)	swede (*Brassica napus* L. var. *napobrassica* L. Rchb.)	spring barley (*Hordeum vulgare* L.)	
Aerial parts					grain	straw
Without additions	68.78	83.02	279.24	136.88	48.36	158.46
Charcoal	66.29	40.90	348.60	-	-	-
Natural zeolite	56.50	55.88	235.75	-	-	-
Synthetic zeolite	69.11	-	270.56	121.09	53.58	191.13
Loam	73.56	41.98	250.86	78.70	67.95	188.51
Compost	68.74	53.43	251.12	-	-	-
Lime	75.58	55.73	262.80	-	-	-
Peat	-	-	-	151.11	57.67	172.44
Bark	-	-	-	154.00	60.73	208.39
Dolomite	-	-	-	107.59	79.05	185.19
Average	68.37	55.16	271.40	124.90	61.22	184.02
LSD	3.24**	4.17**	20.05**	9.67**	n.s.	n.s.
Roots						
Without additions	185.94	86.48	210.06	63.22	172.90	
Charcoal	219.85	57.26	124.23	-	-	
Natural zeolite	197.71	66.30	175.52	-	-	
Synthetic zeolite	225.83	-	227.56	62.53	201.43	
Loam	238.23	86.66	141.29	59.21	180.69	
Compost	253.28	86.65	113.88	-	-	
Lime	188.95	94.24	114.03	-	-	
Peat	-	-	-	69.76	212.13	
Bark	-	-	-	60.55	150.73	
Dolomite	-	-	-	59.46	164.12	
Average	215.68	79.60	146.50	62.46	180.33	
LSD	6.37**	6.18**	38.80**	4.05**	13.77**	

Significant for: *p = 0.05, **p = 0.01; n.s. – differences non-significant

strongest and positive influence on the content of manganese was produced by loam (41%) and dolomite (63%) in grain as well as pinewood bark (32%) in straw of spring barley.

The content of manganese in plants depends on its availability in soil, which in turn is affected by some properties of soil, such as sorptive characteristics, conditioned by the presence of organic matter in soil and soil acidity. These soil properties can be modified by adding to soil organic substance and lime, which affects the availability of manganese and other nutrients taken up by plants. MONGIA et al. (1998) found out that the liming of acidic soil caused lower levels of manganese in rice grain and straw. Analogous effects in other crops were reported by ANDERSSON and SIMAN (1991). The results provided by GUO et al. (2007) implied that a change in soil pH led to a depressed content of manganese in barley roots and leaves. Analogous relationships were determined in roots of plants by HAHN and MARSCHNER (1998) after applying dolomite lime to soil. Thus, the uptake of manganese by plants depends on both soil acidity and its sorptive capacity. A study carried out by CUMMINGS and XIE (1995) shows that higher soil reaction causes a decline in the soil's content of bioavailable forms of manganese and, consequently, leads to less Mn in crops. These authors did not find out any positive effect produced by dolomite lime on the content of water soluble forms of manganese in soil, although enrichment of soil with bird manure was positively correlated with their content. According to UYANOZ et al. (2006), introduction of organic substance to soil leads to higher concentrations of manganese in plants, with the actual effect being dependent on the type of organic matter used, which can be given in the following decreasing order: municipal sewage > bird manure > farmyard manure > compost. Our results partly confirm the above reports.

CONCLUSIONS

1. The content of manganese in the test plants depended on the degree of soil contamination with arsenic, application of different substances as well as the species and organs of plants. Soil contamination with arsenic caused either an increase or a decrease in the content of manganese in crops, depending on their species and organs.

2. In the series without any additional substances, in the arsenic contaminated treatments, a decreased content of manganese was found in above-ground parts of cocksfoot and swede, whereas the concentration of this element in above-ground parts and roots of maize and yellow lupine, in roots of cocksfoot and swede and in straw and roots of spring barley increased. Nevertheless, the highest arsenic rates caused a decrease in the content of manganese in roots of cocksfoot, swede and spring barley.

3. Soil pollution with arsenic caused the largest increase in the content

of manganese in straw of spring barley; the weakest effect of this factor occurred in above-ground parts of maize and yellow lupine.

4. Enrichment of soil with different substances caused changes in the content of manganese in the test plants. The least ambiguous effect of the various substances added to soil was determined for above-ground parts of maize and above-ground parts and roots of cocksfoot, where the manganese content tended to decline, as well as in roots of yellow lupine, grain and straw of spring barley, where more manganese was determined.

5. Charcoal and loam caused the largest and synthetic zeolite the smallest changes in the content of manganese in plants.

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EFFECTS OF GLUTAMATE AND ZINC IONS ON THE CONTRACTILITY OF VASCULAR SMOOTH MUSCLE PREPARATIONS

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Abstract

It was shown in this study that isolated porcine coronary arteries (PCA) contracted by depolarization with high K_o or by histamine are dose-dependently relaxed by glutamic acid, aspartic acid, N-methyl-asparate (NMDA) and γ-aminobutyric acid (GABA). Zn^{2+} was also shown to relax dose-dependently PCA contractions induced by 50 mM KCl with an ED_{50} value of about 1.5 mM and to inhibit dose-dependently histamine-induced contractions, shifting ED_{50} values from 6μM to 40 μM, not affecting however corresponding cumulative concentration-response (CCR) curves established for acetylcholine-induced contractions. Furthermore, since Zn^{2+} ions are co-localized in many glutamatergic synapses of the central nervous system, it has been postulated in analogy to glutamate neurotoxicity that perturbations of the synaptic zinc concentrations might be a triggering factor in several cerebral diseases, such as ischemic strokes and sustained seizures. Unfortunately, little is known so far about effects of glutamate and zinc ions on the vascular tone. Although the nature of the glutamatergic receptors occurring in the blood vessels investigated in this study remains unclear, the results suggest that glutamate and Zn^{2+} ions interact with voltage-gated as well with ligand-operated Ca-channels. An interesting aspect might be the putative role of glutamate and zinc as long-term toxic agents in the early steps of the pathomechanisms leading to degenerative vascular lesions.

Key words: monosodium glutamate, aspartate, N-methyl-D-aspartate (NMDA), GABA, zinc ions, blood vessels.

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INTRODUCTION

Glutamate receptors, exhaustively investigated in the central nervous system (CNS), are classified according to the nature of the signal transduction mechanism, with ionotropic receptors acting as ligand-gated cation channels and metabotropic receptors operating via second messengers (Dingledine et. al. 1999, Danysz, Parson 1998). Ionotropic receptors gated by L-glutamate are distinguished on the basis of pharmacological criteria into three main subtypes: N-methyl-D-aspartate (NMDA), á-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) and kainate receptors. NMDA receptors are characterized by high Ca permeability and a voltage-dependent Mg^{2+} block. The gating of the associated ion channels is modulated by glycine (acting as a co-agonist to glutamate) and by Zn^{2+} ions. Zn^{2+} ions are also present in synaptic glutamatergic vesicles and co-released with glutamate. NMDA receptors have been shown to play an important role in several cerebral functions endowed with high synaptic plasticity, such as learning and memory; however, they have also been implicated in neuronal injury and death caused by massive release of L-glutamate, which might take place in several types of traumatic lesions, such as a head injury, stroke, epilepsy and chronic neurodegenerative diseases (Benveniste et. al. 1984, Faden et al. 1989, Barnes 1988, Olney 1990). Quite similar neurotoxicity has been postulated for zinc ions (Choi, Koh 1998). Notwithstanding the ambiguous role played by glutamate and zinc in the cerebral neurotransmission, both substances are widely present in daily life, with a reputation of being "virtually nontoxic" (Choi, Koh 1998, Valee, Falchuk 1993). The aims of the present study were: (a) to investigate the effects of glutamate and other related substances with transmitter function and of Zn^{2+} ions on the contractility of arterial preparations activated by different stimuli and (b) to detect a putative deleterious effect of excess glutamate on the vascular cells similar to the excitotoxicity observed in neurons, whereby a possible implication as triggering factors in pathological lesions of the vascular wall might also be discussed.

MATERIAL AND METHODS

Large branches of epicardial coronary arteries (right, anterior descending and circumflex arteries; outside diameter 2.5 mm-0.9 mm) were prepared from pig hearts freshly obtained from a nearby slaughterhouse. The vessel preparations were cut into helical strips (approx. 10-15 mm in length and 2 mm in width), pierced by two hooks and mounted vertically in 6 parallel setups of organ baths each filled with 25 ml of physiological salt solution (PSS) maintained at 37˚C and aerated continuously with 95% O_2 and 5% CO_2. The PSS had the following composition (mM): NaCl 137; KCl 2.7;

$CaCl_2$ 1.4; $MgCl_2$ 0.5; $NaHCO_3$ 11.9; NaH_2PO_4 0.4; Glucose 8. The changes in force or length developed by the preparations were measured isometrically (Statham Instruments transducers) or isotonically (HF-Modem-Hugo Sachs Elektronik K.G.) and registered on conventional chart recorders. The maximal wall stress (force/cross sectional area; mN mm^{-2}) was approximated by multiplying the force by the tissue length and dividing by the wet weight. The following chemicals were used: aspartic acid, α-aminobutyric acid (GABA), 3.4-diaminopyridine (3.4-AP), glutamic acid, glycine, histamine dihydrochloride, NaF, tetraethylammonium (TEA), $ZnCl_2$. All the drugs were purchased from Sigma. The data were expressed as the mean ± S.E.M. and the number of strip preparations excised from the same coronary artery. For statistical analysis, Student's t-test for paired and unpaired values was used. P values less than 0.05 were considered significant.

RESULTS

1. Relaxations induced by glutamate, GABA, NMDA and aspartate in PCA depolarized by high K or stimulated by histamine

Whereas the basal tone of unstimulated PCA preparations was not affected by application of glutamate or related agonists (GABA, NMDA, aspartate), preparations contracted by depolarization with high K_o or after stimulation with 10 mM histamine were strongly relaxed by glutamate (0.1-5 mM). However, the relaxing efficacy differed according to the type of activation. The amplitude of the relaxation induced by glutamate (5 mM) in preparations depolarized by high external K^+ and contracted via Ca influx through L-type Ca channels, was much smaller than that recorded in histamine--stimulated preparations (Table 1).

Table 1

Comparison of the magnitude of relaxations induced by glutamate, aspartate, NMDA and GABA on PCA preparations contracted by KCl (50 mM) and by histamine (10 μM). The values are expressed as percentage of the maximal height of contraction obtained by depolarization with 50 mM KCl and of the tonic component of the biphasic contraction induced by 10 μM histamine, respectively. 100 % correspond to the values in mN mm^{-2} given in the parentheses; n = 6 for each agonist

	KCl 50 mM	Histamine 10 μM
Glutamate 5 mM	18.1 ± 4.3% (14.9 ± 1.3 mN mm^{-2})	92.5 ± 3.1% (9.1 ± 1.3 mN mm^{-2})
Aspartate 5 mM	-	78.8 ± 8.8 % (1.5 ± 0.5 mN mm^{-2})
NMDA 1.5 mM	10.2 ± 1.6% (13.8 ± 1.5 mN mm^{-2})	34.0 ± 6.8% (3.9 ± 0.6 mN mm^{-2})
GABA 5 mM	19.8 ± 3.2% (14.9 ± 1.8 mN mm^{-2})	48.3 ± 4.9% (2.9 ± 0.5 mN mm^{-2})

Moreover, in the first case the relaxations were transient, whereas in histamine-stimulated preparations the relaxations were sustained. Contrasting with the general observation that histamine characteristically exerts a predominantly dilator effect on vascular smooth muscle, very powerful contractions were recorded when histamine was applied on PCA preparations at all concentrations (Figures 1 and 2); these contractions are most probably linked to the activation of ligand-operated receptor channels. Figure 1 summarizes the relaxing effect of several agonists of glutamatergic receptors on PCA preparations stimulated by 50 mM KCl or by 10 μM histamine. The largest relaxations were those induced by aspartate, NMDA and GABA on preparations stimulated by histamine.

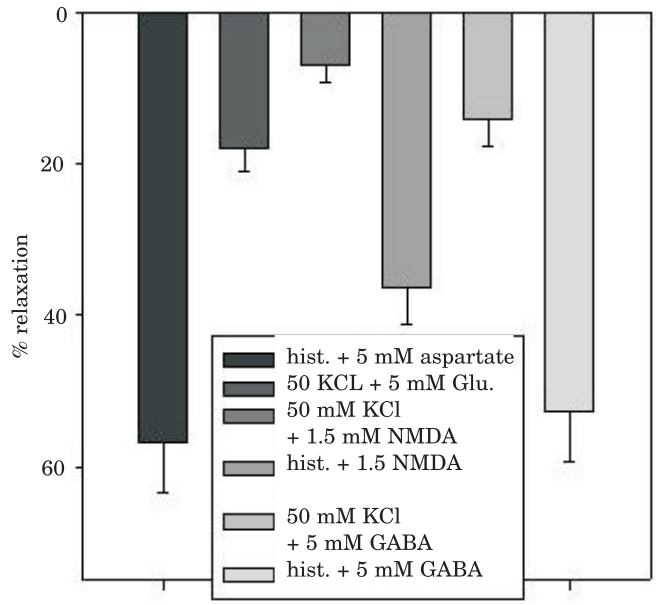

Fig. 1. Effects of NMDA-agonists on PCA stimulated by 50 mM KCl or by 10 μM histamine. The error bars represent means values ± SEM and n = 6 for each column. See text for further details

At first sight, the finding that glutamate as well as the other investigated agonists relax contractions induced by histamine or by depolarization with high K^+ is unexpected, since it is well-known that in the CNS glutamate acts mainly as an excitatory neurotransmitter. If gating of the channels associated with glutamatergic receptors results in Ca entry, then contractions would be expected to occur when glutamate is added to stimulated PCA preparations. The results presented in Figure 1 show that the contrary is observed and that without any exception only relaxations are recorded. Although no experimental evidence is presented here to support the following

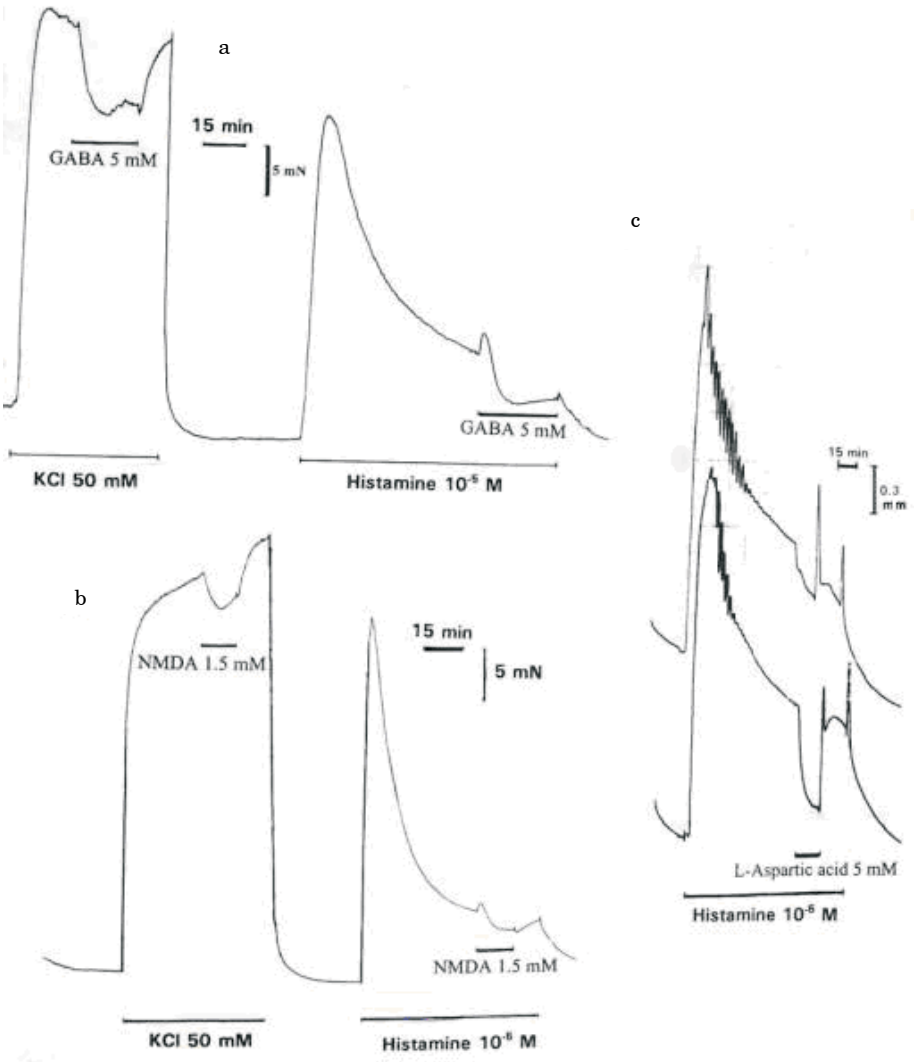

Fig. 2. Representative mechanograms for the relaxations induced by GABA (a), for NMDA (b) and for aspartic acid (c)

assumption, a rational explanation for the contradiction encountered in this study may be that the stimulation of glutamatergic receptors in the vascular smooth muscle, which has not been investigated until now, is associated with an increasing potassium conductance. This point will be further explained in details in the discussion. Figure 2 shows representative experiments obtained for GABA (a), NMDA (b) and aspartate (c).

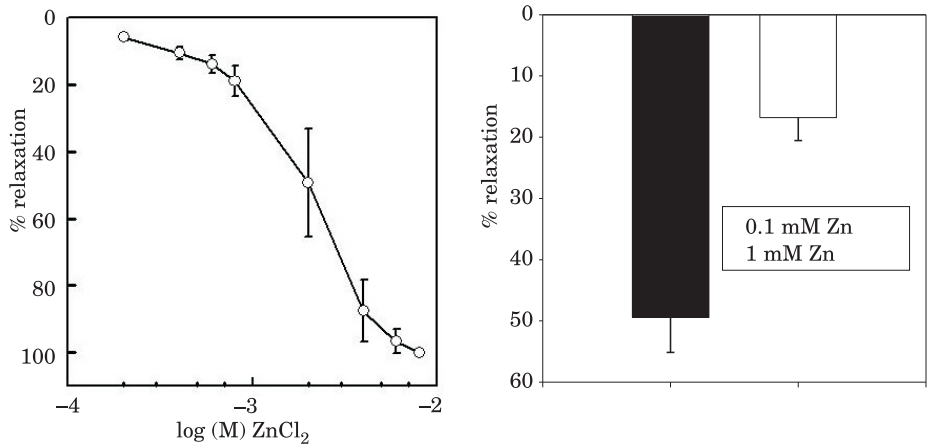

Fig. 3. The diagram on the left shows the relaxations induced by cumulative addition of zinc ions on PCA depolarized by high K^+. 100 % correspond to the maximal contractions induced by 50 mM KCl: 17 mN mm^{-2}; $n = 6$. The diagram on the right shows the effects of two zinc ions concentrations (0.1 and 1 mM) on the relaxations induced by 5 mM glutamate on PCA preparations contracted by 10 μM histamine. The error bars represent means values ± SEM

2. Inhibiting effects of Zn^{2+} ions on depolarization-induced PCA-contractions and on relaxations induced by glutamate in histamine-stimulated preparations

On the left side of Figure 3 it is shown that Zn^{2+} ions relax dose-dependently PCA contractions induced by 50 mM external K^+ with an ED_{50} value of approx. 1.5 mM. This finding is easily interpreted, if it is assumed that the depolarization induced by high external K^+ opens a Ca-channel of L-type, leading to the contraction of the vascular smooth cells. Thus Zn^{2+} ions seem able to inhibit Ca-entry by binding to a specific site of the channel pore.

On the right side of Figure 3 it can be seen that addition of Zn^{2+} ions reduces the amplitude of the relaxations induced by 5 mM glutamate in histamine-stimulated PCA preparations. They amount to resp. 49.38 ± 5.77% with 0.1 mM Zn^{2+} and to 16.82 ± 3.72% with 1 mM Zn^{2+}; $n = 12$; $p < 0.05$. The diagram on the left side of the same figure shows relaxations induced by cumulative addition of zinc ions on PCA depolarized by high K^+. 100% correspond to the maximal contractions induced by 50 mM K^+: 17 mN mm^{-2}; $n = 6$. The results presented in Figure 3 are consistent with the ability of Zn^{2+} ions to act as a modulator of both voltage-sensitive and ligand-gated ion channels. Thus, taken together the results seem to suggest that Zn^{2+}

ions modify the kinetics of ion channels by binding to sites located on Ca or K channels.

3. Effects of Zinc ions on PCA contractions induced by histamine and by acetylcholine

Figure 4 shows the effects of $ZnCl_2$ (0.5 mM) on contractions induced by histamine and by ACh. Whereas CCR-curves for histamine-induced contractions were significantly shifted to the right (ED50 values increased from 0.9 µM to 4 µM), indicating a blockade of Ca entry through ligand-gated channels by Zn^{2+} ions, the same treatment did not affect the corresponding effects induced by ACh.

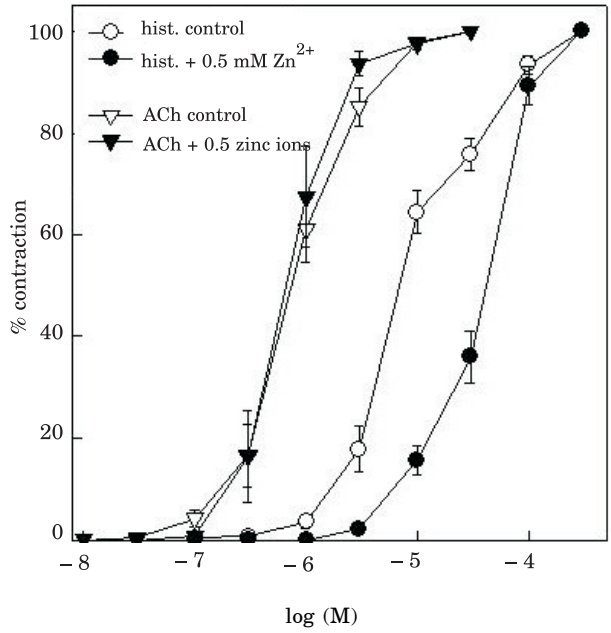

Fig. 4. Effects of $ZnCl_2$ (0.5 mM) on contractions induced by histamine and ACh (n = 12). 100 % =9.98 ± 1.2 for histamine and 8.1 ± 1.5 mN mm^{-2} for ACh., n = 12. The error bars represent mean values ± SEM

4. Effects of K channel blockers on glutamate-induced relaxations of PCA stimulated by histamine

In order to test the possibility that glutamate-induced relaxations are caused by an increased potassium conductance, PCA preparations relaxed by glutamate during a stimulation with histamine were treated with K-channel blockers (3, 4-diaminopyridine and tetraethylammonium). As can be seen in Figure 5 (third and fourth column from the left) application of 1 mM 3, 4-diaminopyridine reversed completely the relaxations induced by glutamate in the histamine-stimulated preparations. A similar treatment of the preparations with 5 mM TEA was not able to significantly affect the glutamate-induced relaxations. The amplitude of contractions induced by 50 mM K^+ and 10 μM histamine are shown in the first and second column of Figure 5 in order to allow a comparison with the glutamate-induced effects.

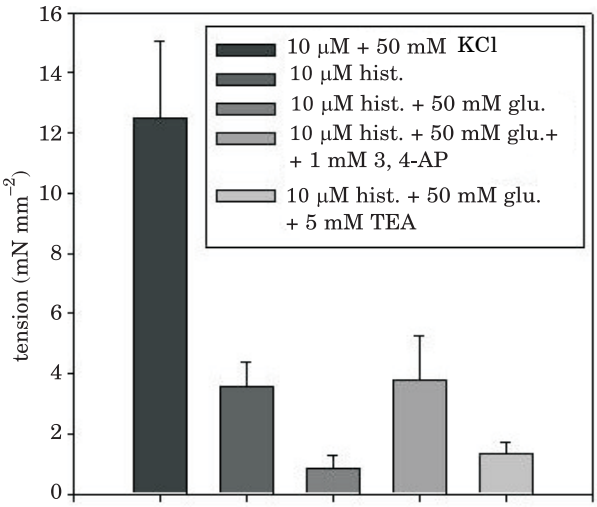

Fig. 5. Effects of K channel blockers on glutamate-induced relaxations of PCA stimulated by 10 μM histamine. The two columns at the left obtained from parallel experiments which show the height of the contractions induced by high K^+ and by histamine are included for the purpose of comparison. The error bars represent means values ± SEM and n = 6 for each column

The representative mechanogram shown in Figure 6 shows the reversal of glutamate-induced relaxations in contractions after a pre-treatment with 3,4-diaminopyrimidine. By itself 3,4-diaminopyridine elicited a strong phasic and unsustained contraction.

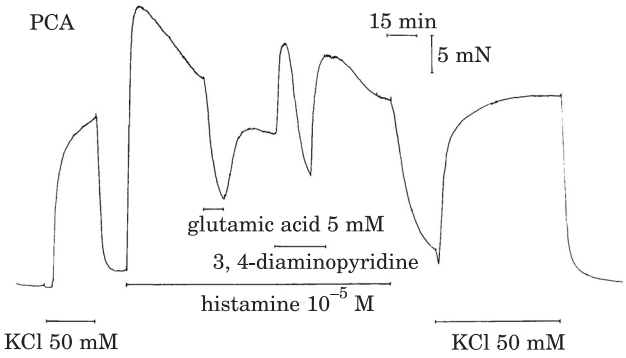

Fig. 6. Relaxing effect of glutamate (5 mM) on PCA preparations stimulated by histamine (10 μM) and its reversal to contraction after a pre-treatment with 3, 4-diaminopyrimidine (5 mM).
The preparations were contracted at the beginning and at the end of the experiment in order to test their reactivity.

5. Effects of glutamate on PCA preparations contracted by histamine in presence and in absence of glycine

It has been reported that glycine, which represents an essential coagonist, is indispensable for the physiological activation of NMDA receptors (Danysz, Parson 1998). In agreement with this requirement, the ED_{50} values of CCR curves obtained for the relaxation induced by L-glutamate in histamine-stimulated PCA preparations were shifted leftward from 0.8 mM to 0.25 μM in presence of 1 mM glycine (Figure 7).

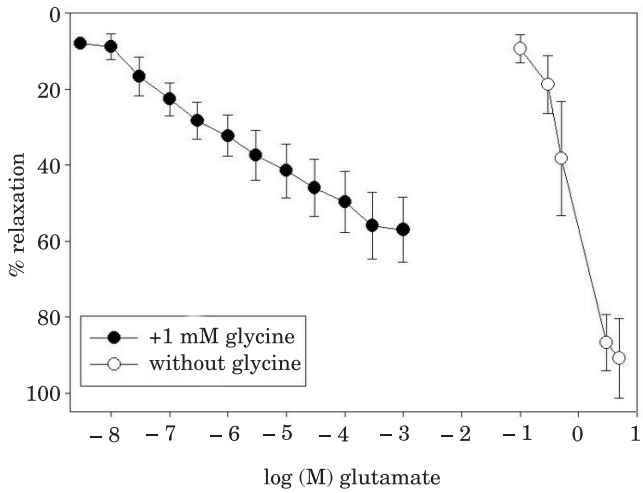

Fig. 7. Effects of glutamate on PCA preparations contracted by histamine in presence and in absence of glycine. The error bars represent means values ± SEM; n = 12.
See text for further details

6. Effects of glutamate on F^--induced PCA contractions and complete loss of contractility elicited by administration of L-glutamate on PCA preparations overactivated by treatment with F^- -ions

Depending of the level at which it is applied in the signal transduction chain, glutamate might exert quite different effects. CCR curves established for contractions induced by depolarization with K_o (10-100 mM) were slightly and significantly shifted to the right in presence of 5 mM glutamate (Figure 8, the left diagram), suggesting that glutamate-induced relaxations partly rely on a reduction of Ca entry. By contrast, the effects elicited by glutamate on F^--induced contractions were more complex (Figure 8, the right diagram). It has been shown in a previous study (NGUYEN-DUONG 1994) that fluoride ions, which form spontaneously $[AlF_4]^-$ (fluoroaluminate) are able to stimulate the signal transduction chain downstream of the receptor site by

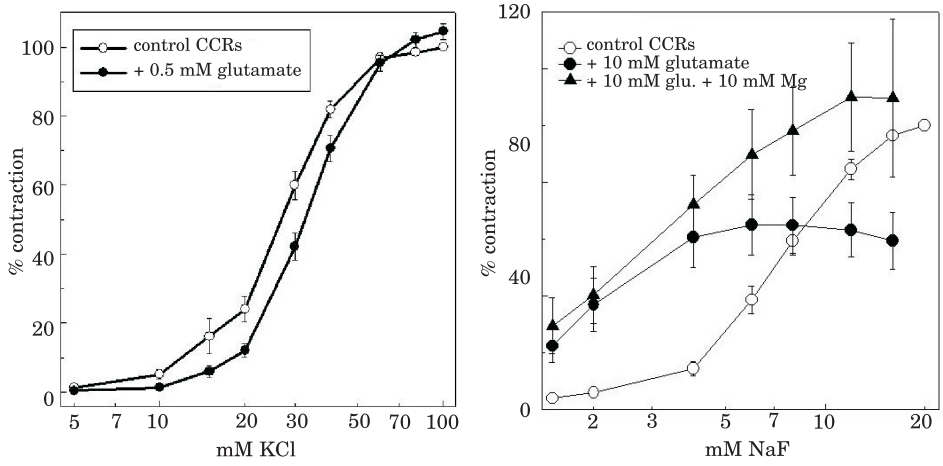

Fig. 8. Comparison of glutamate effects on contractions induced by cumulative increasing concentrations of external KCl (right) and of NaF.
Left diagram: 100 % = 24.56 ±1.73 mN mm^{-2}, n = 12. Right diagram: 100 % = 14.24 ± 1.52 mN mm^{-2}; n = 12. The errors bars represent means values 24± S.E.M. See text for further details

acting at the level of inositide signalling a pathway as a phosphate analogue with G proteins. In keeping with the assumed mechanism, the CCR curves obtained for contractions induced by NaF (1.5-12 mM) were significantly shifted to the left (ED_{50} from 6.3 to 3.1 mM), indicating that the sensitivity of contractions triggered by direct activation of G-proteins was increased in the presence of glutamate. Parallel to the leftward shift of the CCR curves, a depression of the maximum effect (reflecting a reduced intrinsic efficacy) was also observed at higher F^- concentrations (Figure 8, the right diagram). This observation may be explained as follows: since NMDA ion channels are subject to voltage-dependent Mg^{2+} blockade, a moderate depolarization elic-

ited by F^- would displace the channel-bound Mg^{2+} ions, leading in turn to an opening of channels associated with glutamatergic receptors and most probably to increased K^+ efflux. The ensuing hyperpolarization would lead to a reduced Ca entry and to inhibition of the vascular tone. Consistent with the proposed mechanism, this depression could be reversed by increasing the external Mg^{2+} to 5 mM (Figure 8 the right diagram).

When L-glutamate (10 mM) was added to F^--stimulated PCA preparations, within 1 to 2 hours a complete and irreversible loss of contractility and reactivity toward depolarization by 50 mM K^+ or applied NaF was regularly observed (not shown); control experiments performed in absence of L-glutamate demonstrated that contractility and reactivity of the PCA preparations remained completely intact within the same lapse of time (not shown).

DISCUSSION

The results presented in this study suggest that substances known for their ability to activate glutamatergic receptors in the CNS have pronounced relaxing effects on preparations excised from PCA. Although only some sporadic information exists on pharmacological effects of amino acids on the vascular smooth muscle, to my knowledge there is as yet no published report on receptors stimulated by binding of NMDA, GABA or kainate in PCA. Hence it is not possible at the moment to interpret the present results on the basis of available biochemical, molecularbiological or electrophysiological data. The first conspicuous discrepancy encountered in the pharmacological behaviour of the PCA preparations is the relaxation in response to the application of agonists of glutamatergic receptors, which has been recorded throughout and without exception. This finding is in contrast with the fact that in the CNS amino acids are excitatory by nature and that stimulation of NMDA receptors regularly leads to Ca entry in the postsynaptic cells. A logical inference is that the glutamatergic receptors involved in PCA are associated with K^+ channels. This assumption is consistent with the reversal of glutamate-induced PCA-relaxations observed after addition of a specific K^+ blocker (Figures 5 and 6). Glutamate seems also to unspecifically interfere with Ca entry through voltage-gated channels; however, the effects were transient, whereas the corresponding effects recorded after stimulation of ligand-operated receptors were sustained and of a larger amplitude (Table 1 and Figure 1). An additional hint for the hypothetical occurrence in the vascular wall of glutamatergic receptors of unidentified subtype is the extreme sensibilization of the glutamate-induced effects by glycine, a co-agonist of NMDA-receptors in the CNS (Figure 7) – NGUYEN-DUONG 2001. It has been postulated that Zn^{2+}, a co-factor and structural component of many

enzymes, which also plays a role as an intercellular signalling messenger, may be partly responsible for the neuronal death associated with transient global ischemia, with sustained seizures and with some neurological diseases (BENVENISTE et al. 1984). The different effects of Zn^{2+} described in this study seem to be consistent with its function as an endogenous modulator of ligand- and voltage-gated ion channels (Figures 3 and 4). A very strong argument for a modulator role of Zn^{2+} ions in ligand-gated receptors is their ability to inhibit histamine-stimulated PCA preparations, whereas the corresponding effects induced by acetylcholine were unaffected (Figure 4). Inside channel proteins Zn^{2+} ions coordinate to histidine, cysteine, aspartate and glutamate residues; since histamine is a derivative of histidine, the finding described in Figure 4 might not be wholly casual.

Because of the vital role played by zinc ions in the metabolism of proteins, carbohydrates, lipids, as well as in gene transcription, immune response, and many other fundamental biological processes, this essential trace element is strongly controlled by various homeostatic mechanisms, regulating their absorption, their cellular uptake and their distribution among intracellular compartments. On account of the extreme efficacy of the underlying homeostatic mechanisms, the nontoxicity of even excessive zinc ingestion has been generally taken for granted (VALLEE, FALCHUK 1993). However, recent research demonstrating that dysregulation of zinc and copper homeostasis in the brain might play an extremely critical role in Alzheimer disease (MELONI et al. 2007) emphasize the necessity of re-evaluating this far too simplistic assumption made on the innocuousness of excessive zinc (VALLEE, FALCHUK 1993) and support precautions to be taken with regard to zinc as an environmental toxicant and as a cerebral neurotoxin. In terms of integrated systems with regulatory functions, however, the pharmacological and toxicological significance in the organism of copper and zinc ions is only understood when these ions are considered by pairs, as reciprocal antagonists, in the same way as it is done with calcium and magnesium ions.

The relaxing effect of glutamate described in the present study may be linked to the well-known so-called Chinese restaurant syndrome, which manifests itself as headaches and flushing (OLNEY 1990). The ED_{50}, i.e. the dose producing a half-maximum relaxation in PCA preparations amounted to about 0.9 mM. This value is of the same order of magnitude that reported in plasma of humans after an experimental ingestion of monosodium glutamate, in which the concentration peaked until a value of about 0.5 mM (GRAHAM et al. 2000).

A „cytotoxic" effect of glutamic acid has been also demonstrated in an isolated rat lung (SAID 1999), which seems to correspond to that observed in neurones and glial cells and can be related to the neurotoxic effect described in the CNS. The observation made in the present study that a high concentration of L-glutamate (10 mM) when added to F^--stimulated PCA preparations led to a complete and irreversible loss of contractility and reactivity

might be explained as follows: stimulation with fluoride ions takes place downstream of the receptor site and represents a rather unspecific intervention that targets several classes of G proteins, impinging upon several effector systems; together with a fluoride-induced intracellular Ca overload, this would in turn inevitably lead to a rather indiscriminate stimulation of proteases, protein kinases and phospholipases. An activation of phospholipase A_2 and of cyclooxygenase would for example generate free-radical species overwhelming the endogenous scavenging mechanisms and producing lipid peroxidation and membrane damage.

In conclusion, the data presented in this study, which give a preliminary account of the sensitivity of peripheral blood vessels to glutamate, to zinc ions and to related substances, need undoubtedly further electrophysiological investigations before therapeutic implications could be deduced. Nevertheless, the total loss of contractility of hyperstimulated blood vessels after the administration of glutamate reported in the present study let one speculate on initial stages in the pathogenesis of atherosclerotic lesions. It is conceivable that similar mechanisms might underlie permeability changes of the cell membrane, leading to local oedema of the blood vessels, favouring in turn the accumulation of lipids, the activation of macrophages and provoking eventually an irreversible production of foam cells.

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CONTENT OF CHEMICAL ELEMENTS IN MUSCULAR TISSUE AND LIVER OF MALE KIDS AND RAM LAMBS IN CENTRAL-EASTERN POLAND

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Abstract

The aim of the study was to estimate the content of Pb, Cd, Zn, Mn, Cu, Fe, Ca in meat and liver of male kids and ram lambs fed mixtures containing 10% of flax seeds. Castrated male kids ($n = 7$) of White Upgraded breed and castrated ram lambs ($n = 7$) of Polish Lowland sheep fattened up to about 35 kg of body weight were used as experimental material. The animals were fed mixture CJ *ad libitum*, supplemented with 10% of flax seeds and meadow hay as a structural supplement. Contents of the chemical elements were analysed in samples of *longissimus dorsi* muscle.

The content of Cd (0.01 mg kg^{-1}) in male kid meat was lower than in ram lamb meat ($p \leq 0.05$). Similarly, Pb content was lower ($p \leq 0.05$) in male kids (0.04±0.003 mg kg^{-1}) than in ram lambs (0.07±0.002 mg kg^{-1}). Slightly lower content of Pb (by 0.01 mg kg^{-1}) and Cd (by 0.01 mg kg^{-1}) in male kid liver was determined, and the differences were statistically insignificant. Significantly larger ($p \leq 0.01$) Cu content (1.14±0.07 mg kg^{-1}) in muscular tissue of ram lambs was also stated. Male kid meat, however, was richer in Mn, Fe, Zn and Ca, with the differences tested at $p \leq 0.05$ and $p \leq 0.01$. The liver was an organ which accumulated not only Pb and Cd but also Cu, Mn and Zn both in ram lambs and male kids. Especially high level of Cu and Mn in liver was found, which could be the result of a high content of the chemical elements in mixtures. Moreover, significantly larger content of Ca (15.24±1.68 mg kg^{-1}) in ram lamb liver compared to male kid liver was stated. On the other hand, muscular tissue of male kids contained more Ca (21.94±1.74 mg kg^{-1}) than that of ram lambs.

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The content of chemical elements (especially heavy metals) in muscular tissue and liver was lower than the norms established by the Minister for Health and the European Committee, which prove that the tested food products were fully safe for consumption.

Key words: goat kids, ram lambs, flax seeds, muscular tissue, liver, chemical elements, heavy metals.

POZIOM METALI W TKANCE MIĘŚNIOWEJ I WĄTROBIE KOZIOŁKÓW I TRYCZKÓW Z REGIONU ŚRODKOWO-WSCHODNIEJ POLSKI

Abstrakt

Celem badań było określenie zawartości Pb, Cd, Zn, Mn, Cu, Fe, Ca w mięsie i wątrobie koziołków i tryczków żywionych mieszanką pełnoporcjową z 10% udziałem nasion lnu. Materiał doświadczalny stanowiły wykastrowane koziołki ($n = 7$) rasy białej uszlachetnionej i wykastrowane tryczki ($n = 7$) polskiej owcy nizinnej, tuczone do masy ciała ok. 35 kg. Zwierzęta doświadczalne otrzymywały do woli przemysłową mieszankę treściwą CJ, dodatek 10% nasion lnu oraz siano łąkowe jako dodatek strukturalny. Próby do analizy pobrano z mięśnia najdłuższego grzbietu (*m. longissimus dorsi*), w którym oznaczono zawartość pierwiastków.

W mięsie koziołków stwierdzono niższą zawartość Cd (0.01 mg kg^{-1}) niż u tryczków – $p \leq 0.05$. Podobnie poziom Pb był niższy ($p \leq 0.05$) w grupie koźląt (0.04±0.003 mg kg^{-1}) w porównaniu z tryczkami (0.07±0.002 mg kg^{-1}). W wątrobie koźląt stwierdzono nieznacznie niższy poziom Pb oraz Cd (0.01 mg kg^{-1}) – różnice nieistotne statystycznie. Istotnie wyższą ($p \leq 0.01$) zawartość Cu (1.14±0.07 mg kg^{-1}) w tkance mięśniowej stwierdzono u tryczków. Natomiast mięso koziołków było bogatsze w Mn, Fe, Zn i Ca – różnice $p \leq 0.05$ i $p \leq 0.01$. Wątroba była narządem koncentracji nie tylko Pb i Cd, ale Cu, Mn i Zn zarówno u tryczków, jak i koziołków. Zaobserwowano szczególnie wysoki poziom Cu i Mn w wątrobie, czego przyczyną mógł być wysoki poziom tych pierwiastków w paszy. Ponadto w wątrobie tryczków stwierdzono istotnie ($p \leq 0.01$) wyższy poziom Ca (15.24±1.68 mg kg^{-1}) niż u koziołków. Natomiast tkanka mięśniowa koziołków zawierała więcej Ca – 21.94±1.74 mg ≤ kg^{-1}.

Stężenie pierwiastków, szczególnie metali ciężkich, w tkance mięśniowej i wątrobie było poniżej dopuszczalnych norm podanych przez Ministra Zdrowia i WE, co kwalifikuje je jako surowce żywnościowe w pełni bezpieczne do spożycia.

Słowa kluczowe: koziołki, tryczki, nasiona lnu, tkanka mięśniowa, wątroba, pierwiastki, metale ciężkie.

INTRODUCTION

Environmental pollution has worsened the health quality of food, which is one of the main sources of exposure of people to harmful trace minerals. Young kid meat is praised for its excellent quality and delicate taste. It is also a source of phosphorus, sulphur, copper, iron and calcium. This meat competes with veal and lamb in nutritive value (HUMANN-ZIEHANK et al. 2008, KRUPA, KOGUT 2000, LIDWIN-KA•MIERKIWICZ et al. 2006, NIEDZIÓŁKA et al. 2008). Addition of oil plant seeds to mixtures influenced the fatty acid content,

which is of great importance in cholesterol regulation, and also affected heavy metal content (Krełowska-Kułas 1998, Morawiec 1991, Niedziółka et al. 2007, Pieniak-Lendzion 2006). Studies that have been conducted recently showed an altered content of chemical elements in tissues and edible organs, from trace values to levels which exceeded physiological values.

The aim of this study was to test the content of Pb, Cd, Zn, Mn, Cu, Fe, Ca in meat and liver of male kids and ram lambs fed mixtures containing 10% of flax seeds.

MATERIAL AND METHODS

Castrated male kids (n = 7) of White Upgraded breed and castrated ram lambs (n = 7) of Polish Lowland sheep fattened up to 35 kg of body weight were used as experimental material. Kids and rams were kept together with their dams until the 60th day of life. At the age of 1 month all kids and ram lambs were castrated using a surgical method. The experimental animals were fed *ad libitum* on mixture CJ supplemented with 10% of flax seeds and meadow hay as a structural supplement. The mixtures were produced from components originating from Podlasie. The slaughter was conducted according to methodology presented by the Zootechnic Institute. Samples were taken from *longissimus dorsi* muscles. The content of chemical elements such as Zn, Cu, Mn, Fe was tested in an atomic absorption spectrophotometer AAS. The content of Pb and Cd was analysed using the extraction method. An Atomic absorption spectrophotometer AAS-30 manufactured Carl Zeiss Jena was used to conduct the analyses. Each time, 10 g of the material was dried at 150°C for 24 hours. Then, each sample was combusted in a muffle stove (temp. 420°C). The ash was moistened with nitric acid and, after distilling nitrogen oxide, the ash was placed in a muffle stove at 420°C for 30 minutes. The white rest was dissolved in muriatic acid (1 mol dm^{-3}) and was analysed directly from an aqueous solution – aspirating to flame of the atomic absorption spectrophotometer. The content of heavy metals in meat was tested in a laboratory of the Institute of Chemistry at the University of Podlasie.

The results were statistically presented in tables, showing mean values (x) and standard deviation (SD) for each tested value. Significance of differences between means for both species was analyzed by Tukey test. Statistical calculations were done using Statistica 6.0PL.

RESULTS AND DISCUSSION

The content of chemical elements in muscular tissue was low and did not exceed the norms accepted by the Minister for Health and the European Committee, which indicated that kid and lamb meat are healthy products suitable for consumption (*Minister for Health* 2003, EC 2006). Lead and cadmium (Table 1) are toxic elements and their accumulation in ram lamb tissues was larger than in kid tissues by 0.03 and 0.01 mg kg^{-1}, respectively

Table 1

Content of lead and cadmium in meat and liver (mg kg^{-1} fresh tissue)

Chemical elements	Analyzed tissue	Statistical parameters	Species	
			goat kids	ram lambs
Pb	meat	$\overline{x}$	0.04*	0.07*
		SD	0.003	0.00
	liver		0.25	0.26
		SD	0.03	0.03
Cd	meat	$\overline{x}$	0.02*	0.03*
		SD	0.003	0.008
	liver	$\overline{x}$	0.03	0.04
		SD	0.006	0.01

*value significant at $p \leq 0.05$. **value significant at $p \leq 0.01$

– $p = 0.05$. Two-fold more Cd was found in liver, from 0.03 mg kg^{-1} (kids) to 0.04 mg kg^{-1} (ram lambs) and a four-fold higher level of Pb (0.25 and 0.26 mg kg^{-1}) was also demonstrated. The addition of flax seeds to mixtures lowered the Pb and Cd content, which was confirmed by a larger content of Pb and similar content of Cd in muscular tissue and liver found in our earlier studies on lambs and kids (KRUPA, KOGUT 2000, NIEDZIÓŁKA et al. 2007). The aim of the study performed by LIDWIN-KA•MIERKIEWICZ et al. (2006) was to examine cadmium, lead, copper, mercury and zinc in raw beef, beef boiled in water without NaCl and in water with NaCl. The lowest concentration of Pb was in brisket boiled in water without salt and the highest in raw entrecote. The Cd content in raw beef as well as in beef boiled in water without salt was low.

The source of lead and, possibly cadmium, for animals is mainly green forage, silage, whole plants from roots and, to a lesser degree, cereal grain. Consequently, the risk of contamination with Pb and Cd is the highest for sheep, cattle and wild boar game. Skilful indoor nourishment with the cereals and oilplant parts combined with beneficial influence of spicy plants can

limit the content of heavy metals in animal bodies (Bayçu et al. 2003, Krupa, Kogut 2000, Pieniak-Lendzion et al. 2008)

Significantly larger ($p = 0.01$) content of Cu (1.14 mg kg^{-1}) in muscular tissue of ram lambs was found (Table 2). Noteworthy is the fact that both kid and ram lamb livers contained high level of copper. However, the level of copper was 14.37 mg kg^{-1} higher in ram lamb liver. The content of copper in male kid muscular tissue (0.88-0.96 mg kg^{-1}) was similar, while that in kid liver (73.78-92.58 mg kg^{-1}) was larger than in a study by Pieniak-Lendzion et al. (2008). The content of copper depended on the gender and male kids accumulated less Cu in muscular tissue but more Cu in liver. In other studies (Johnson et al. 1995), the average content of Ca in muscular

Table 2

Content of manganese iron, zinc, copper and the calcium in meat and the liver (mg kg^{-1} fresh tissue)

Chemical elements	Analyzed tissue	Statistical parameters	Species	
			goat kids	ram lambs
Mn	meat	$\overline{x}$	0.46**	0.32**
		SD	0.05	0.03
	liver	$\overline{x}$	5.00*	5.77*
		SD	0.33	0.72
Fe	meat	$\overline{x}$	28.27*	21.70*
		SD	5.58	1.34
	liver	$\overline{x}$	53.11	48.55
		SD	4.13	7.41
Zn	meat	$\overline{x}$	59.90**	52.99**
		SD	1.47	4.14
	liver	$\overline{x}$	69.42	69.04
		SD	3.96	6.72
Cu	meat	$\overline{x}$	0.97**	1.14**
		SD	0.08	0.07
	liver		109.58	123.95
		SD	17.02	21.48
Ca	meat	$\overline{x}$	21.94**	18.32**
		SD	1.74	0.75
	liver		6.66**	15.24**
		SD	0.38	1.68

*value significant at $p \leq 0.05$. **value significant at $p \leq 0.01$

tissue of young kids was 28.1 mg 100 g^{-1}, the content of Zn – 92.3 mg 100 g^{-1} and Cu – 4.4 mg 100 g^{-1}. Male kid meat was richer in Mn, Fe, Zn and Ca, with the significant differences appearing at $p = 0.05$ and $p = 0.01$. The iron content in muscular tissue of male kids was larger by about 6.57 mg kg^{-1} than that of ram lambs ($p = 0.05$). Hoffman et al. (2003) demonstrated a lower content of iron (16.29-18.83 mg kg^{-1}) in muscular tissue of lambs slaughtered at 40 kg of body weight. The aim of a study completed by Pięta, Patkowski (2009) was to determine the content of elements in the *longissimus dorsi* muscle of lambs, dependent on the system of maintenance**.** It was noted that in lambs kept with their mothers in an outdoor system it was higher compared to lambs kept indoors (16.29 vs 10.47 mmol kg^{-1}). The content of iron was 447.3 µmol kg^{-1} (indoor) and 486.6 µmol kg^{-1} (outdoor), being similar to the levels determined in our own investigations. The liver was an organ which not only accumulated Pb and Cd, but also Cu, Mn and Zn both in ram lambs and male kids. It was proved that the Cu content in the liver exceeded 100 mg kg^{-1}, and the Mn content was over 5 mg kg^{-1}, which resulted from a higher level of the chemical elements in the mixtures. However, a statistically significantly larger Ca content in muscular tissue than that in the liver both in ram lambs and male kids was found. Elevated amounts of calcium in muscular tissue of male kids (by 3.62 mg kg^{-1}, $p \leq 0.01$) and in the liver of ram lambs (by 8.58 mg kg^{-1}, $p \leq 0.01$) were stated. The correlations regarding increased levels of iron, manganese, copper and calcium in the liver than in muscular tissue were confirmed by other researchers (Humann-Ziehank et al. 2008, Morawiec 1991, Niedziółka et al. 2008, Pieniak-Lendzion et al. 2007).

CONCLUSIONS

1. It was found that the species differentiated the content of chemical elements in meat and the liver of male kids and ram lambs fed mixtures supplemented with flax seeds, but the levels of the elements did not exceed the accepted norms.

2. The content of Cd and Pb was significantly lower in muscular tissue and slightly lower in the liver of male kids than those of ram lambs.

3. The liver was an organ which accumulated not only Pb and Cd, but also Cu, Mn and Zn both in ram lambs and male kids. The effect of a species on the accumulation of Cu and Ca was proved.

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CONTENT OF ORGANIC C AND pH OF BOG AND POST-BOG SOILS VERSUS THE PRESENCE OF GROUND BEETLES *CARABIDAE* IN STARY DWÓR NEAR OLSZTYN

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Abstract

The present study consisted of an evaluation of assemblages of epigeic carabid beetles (*Col. Carabidae*) colonizing hydrogenic soils (bog and post-bog ones), different in the soil development degree. The observations were conducted on a drained, low bog area called Stary Dwór, which today is used as a cut meadow. This is an oblong depression, filled in with (partly mucky) rush peats and situated in the sandur landscape. It lies in the mesoregion called Pojezierze Olsztyńskie (Olsztyn Lake District) near Olsztyn (UTM DE 65), about 3 km of the southern borders of the town. The field observations for determination of the soil type were conducted using soil catenas. A transect was established, which cut across different types and sub-types of bog and post-bog soils. In this paper, the authors have attempted to answer the question whether the sequence of hydrogenic soils and some parameters chosen to describe them have any influence on assemblages of epigeic carabid beetles dwelling in such habitats. Based on the results, it has been concluded that the soils present in the analyzed peat bog were characterized by the following sequence: muckous soils → peat-muck soils → peat soils. Their properties depended on the position in the soil relief, advancement of muck formation and content of organic carbon. It has been found out that the highest soil ash content in the surface horizons was in muckous soil (90.39%), and the lowest – in profile 3 of peat-muck soil (18.77%). The reaction of the analyzed soils ranged from slightly acidic to neutral and tended to decrease towards the centre of the depression, reaching the lowest value in peat soil. During the two years of our observations, a total of 673 individuals of *Carabidae* belonging to 29 species were captured. It has

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been determined that the type of soil as a factor significantly affected the number of captured carabid beetles, but did not influence the species abundance. The decreasing pH gradient as well as an increasing content of organic C were associated with a decreasing number of the species of carabid beeetles tolerant to moisture conditions (mesophilous species), which were being replaced by hygrophilous individuals. As the acidic reaction of soil increased and the soil content of organic matter rose, so did the abundance of mixophagous species at the expense of predatory individuals.

Key words: organic C, soil pH, *Carabidae*, bog soils, post-bog soils.

ZAWARTOŚĆ C ORGANICZNEGO I pH GLEB BAGIENNYCH I POBAGIENNYCH A WYSTĘPOWANIE NAZIEMNYCH *CARABIDAE* W OBIEKCIE STARY DWÓR k. OLSZTYNA

Abstrakt

W badaniach poddano ocenie zgrupowania epigeicznych biegaczowatych (*Col.*, *Carabidae*) zasiedlających gleby hydrogeniczne (bagienne i pobagienne) o różnym stopniu rozwoju. Terenem badań było odwodnione torfowisko niskie Stary Dwór, użytkowane jako łąka kośna. Obiekt stanowi podłużne zagłębienie wypełnione torfami szuwarowymi (częściowo zmurszałymi) w krajobrazie sandrowym. Jest on zlokalizowany w mezoregionie Pojezierza Olsztyńskiego, w pobliżu Olsztyna (UTM DE 65), w odległości ok. 3 km od południowych granic miasta. Prace terenowe, w których określano typ gleby, prowadzono metodą katen glebowych. Wyznaczono transekt przebiegający przez różne typy i podtypy gleb bagiennych i pobagiennych. W pracy próbowano uzyskać odpowiedź na pytanie, czy sekwencja gleb hydrogenicznych oraz wybrane parametry opisujące te gleby mają wpływ na zgrupowania zasiedlających je epigeicznych biegaczowatych. Stwierdzono, że opisane na badanym torfowisku gleby charakteryzowały się następującą sekwencją: gleby murszaste → gleby torfowo-murszowe → gleby torfowe. Ich właściwości były uzależnione od usytuowania w reliefie, zaawansowania procesu murszenia oraz zawartości C organicznego. Największą popielność stwierdzono w poziomach powierzchniowych gleby murszastej (90,39%), natomiast najniższą w profilu 3 (18,77%) gleby torfowo-murszowej. Odczyn badanych gleb kształtował się od lekko kwaśnego do obojętnego i malał w kierunku centrum obniżenia, osiągając najniższe wartości w glebie torfowej. W czasie dwuletnich obserwacji na badanym obiekcie odłowiono łącznie 673 osobniki *Carabidae* należące do 29 gatunków. Stwierdzono, że typ gleby okazał się czynnikiem istotnie wpływającym na liczbę odłowionych osobników *Carabidae*, natomiast nie wpływał na ich bogactwo gatunkowe. Malejący gradient pH oraz wzrastająca zawartość C organicznego wiązały się ze spadkiem liczebności osobników badanej grupy chrząszczy, mało wrażliwych na zmieniające się warunki wilgotnościowe (mezofile), i zastępowaniem ich przez osobniki wilgociolubne. Wraz ze zwiększającą się kwasowością gleby i zawartością materii organicznej wzrastała również liczebność miksofagów, kosztem zmniejszania się grupy osobników drapieżnych.

Słowa kluczowe: C organiczny, pH gleby, *Carabidae*, gleby bagienne, gleby pobagienne.

INTRODUCTION

Soil properties are of an immense importance for the shaping of components of agrocenoses, and especially populations of arthropods dwelling in soil habitats. Among arthropods, carabid beetles are a very important and valuable group of animals, very strongly connected with the soil environment. These beetles are mostly predators, hence they can limit gradation of phytophagous animals; besides, they are commonly used for zoological indication (RAINIO, NIEMELA 2003). Soil properties are characterized by a number of physical and chemical parameters, which also affect *Carabidae*. Some of the more important chemical properties of soil are soil reaction (pH), soil salinity and soil content of calcium carbonate. Other important properties of soil are the particle size distribution and soil content of organic matter. These factors have a large influence on soil microclimate, including soil moisture and temperature. The vertical gradient of changes in these parameters affects migration of soil invertebrates and modifications in quantitative ratios between trophic groups of these organisms (BEZKOROVAINAYA, YASHIKHIN 2003). SĄDEJ et al. (2008) demonstrated a significant, positive effect of the content of organic C and positive effect of total nitrogen in lessive soil on the density of soil macrofauna.

This article deals with an assessment of epigeic carabid beetles dwelling on hydrogenic soils (bog and post-bog soils), different in the development degree. The properties of these soils in the surface horizon, for example specific and bulk density, total porosity and pH, differed as well. The authors hoped to answer the question whether the sequence of hydrogenic soils and the analyzed soil parameters had some influence on epigeic carabid beetles which colonized these soils.

THE RESEARCH AREA AND METHODS

The study was carried out on a drained, low bog area called Stary Dwór, now used as a cut meadow. This is an oblong depression filled in with (partly mucky) rush peats in the sandur landscape. It is situated in the mesoregion called Pojezierze Olsztyńskie (Olsztyn Lake District) near Olsztyn (UTM DE 65), about 3 km south of the town's borders. The field tests were carried out with the soil catena method. A transect was established that ran across different types and sub-types of bog and post-bog soils. The sequence of soils within the analyzed areas was as follows (from the edges towards the centre):

– muckous soils GM,
– peat-muck soils GMT1 and GMT2,
– peat soils GT.

The soil samples underwent the following determinations: ash content, bulk density, reaction and content of organic matter (C_{org}). The determinations were performed with the methods used in soil sciences for organic and mineral formations (SAPEK, SAPEK 1997). Longwise the established transect, four modified Barber traps were set up, one on each of the analyzed types of soil. They were filled with a fixing liquid and exposed from May to October in 2006 and 2007. The collected material was analyzed in terms of its species composition, dominance structure and zoogeography. In addition, the ecological characterization of the captured speciemens of *Carabidae* was drawn (THIELE 1977). The structure (classes) of dominance of the carabids was presented according to the following pattern: superdominants (>30%), eudominants (30-10%), dominants (10-5.1%), subdominants (5-2.1%), recedents (2-1.1%) and subrecedents (≥1%) (GÓRNY, GRÜM 1981). The siginifance of differences between the number of species and individuals captured on the analyzed types of soil was evaluated with the ANOVA analysis of variance, using the software package Statistica 8.0 PL. In order to describe changeability of *Carabidae* assemblages dwelling on the analyzed types of soil, different in soil parameters, ordinance techniques were used with an aid of the software package Canoco v. 4.5 (TER BRAAK 1986). The statistical significance of canonical axes was established with Monte Carlo tests.

RESULTS AND DISCUSSION

The soils present in the analyzed peatland belong to the division of hydrogenic soils (division IV), to the order of bog (order IVa) and post-bog soils (order IVb). The latter soil was represented by the type of muck soils, subtype of peat-muck (GMT1 and GMT2) soils and by mucky soils belonging to the subtype of muckous soils. Bog soils are the peat soils of lowland bogs, characterized by active accumulation of organic sedmients (e.g. peat), which can accumulate to over 30 cm in thickness. The peat soil found in the centre of the analyzed area was developed from lowland, rush peat, moderately (R2) and strongly (R3) decomposed. Post-bog soils, in turn, are formed from bog soils, which have been drained. In such soils, the muck formation process occurs due to the penetration of air into soil pores in the upper soil horizones. The peat-muck soils found in Stary Dwór were formed from mucky rush peats. The thickness of the peat muck varied from 21 to 40 cm. Muckous soils (GM) are a further stage in the development of post-bog soils in the decession phase. They contain between 3-10% of organic matter (peat and muck-peat soils have over 20% organic matter). The organic matter has the characteristics of peat muck and does not form complex bonds with the mineral components of soil. In the analyzed soil, the muckous formation lay on a loose sand substrate 30 cm deep into the soil profile. The properties of the analyzed soils depended on their position of the relief, advancement of

the muck formation process and content of organic matter (Table 1). The highest soil ash content in the surface horizons was in the muckous soil (90.39%), and the lowest – in profile 3 of the peat-muck soil (18.77%). In the muckous soil, the ash content was rising with the depth, in contrast to the peat-muck and peat soils. The specific density of the peat-muck and peat soils rises with the depth, and ranges within 1.46-2.47 Mg m^{-3}. The specific density in the muckous soil varies from 2.45 do 2.68 Mg m^{-3}. The bulk

Table 1

Some properties of surface horizons of the soils in Stary Dwór peatland

Analyzed soil	pH (H_2O)	Ash content	Organic carbon (g kg^{-1})	Bulk density (Mg m^{-3})
	pH	Pop	M org	Vo
Muckous soil GM	7.2	903.9	5.57	1.4
Peat-muck soil GMT1	6.55	255.9	43.16	0.47
Peat-muck soil GMT1	6.6	187.7	47.12	0.26
Peat soils GT	6.23	312.9	39.86	0.44

density of the peat-muck and muck soils decreases with the depth. It ranges between 0.13 and 0.26 Mg m^{-3} in the surface layers. In the muckous soils, the bulk density attains the highest values (1.40-1.55 Mg m^{-3}). The total porosity was the lowest in the muckous soils, decreasing in deeper layers of the soil profile (42.86-36.74%). In the surface horizon of the peat-muck and peat soils, the total porosity was 67.81-88.89%. It rose in deeper layers of the soil, reaching the maximum value of 94.74% in the peat-muck soil at the depth of 21.42 cm. The reaction of the analyzed soils varied from slightly acidic to neutral. The lowest values of pH in H_2O and KCl were determined in the muckous soil. The reaction was decreasing towards the centre of the land depressing, reaching the lowest values in the peat soil. The soils of Stary Dwór bog area are characterized by a varied content of organic substance. The lowest concentration of organic matter was found in the muckous soil (9.60%). As the land inclined, the content of organic matter in soil rose. In mucks of the peat-muck soils, it was 74.41 to 81.23%, increasing in the layers of rush peat to 84.56-92.78%. In the peat soil, however, the content of organic matter rose in the deeper layers of the profile, ranging between 68.71% and 89.90%.

During the two-year observations carried out in Stary Dwór, a total of 673 specimens of *Carabidae* beetles representing 29 species were captured (Table 2). The analysis of variance demonstrated that the type of soil was a factor that significantly affected the number of captured carabid beetles (p=0.0222), but did not produce a significant effect (p=0.0818) on the qualita-

Table 2

Species composition and dominance (%) of the carabid beetles on the analyzed types of soil

Species		Muckous soil	Peat--muck soil GMT1	Peat-muck GMT2	Peat soils
Latin name	abbrev.				
Amara aenea (De Geer.1774)	A_aene	0.93	1.20	1.48	2.68
Amara communis (Panzer.1797)	A_comm	0.00	0.40	1.97	0.00
Amara convexior Stephens.1828	A_conv	0.00	0.40	1.48	0.89
Amara eurynota (Panzer.1797)	A_eryi	0.93	1.60	1.97	5.36
Amara familiaris (Duftschmid.1812)	A_famil	0.93	0.00	0.00	0.00
Amara ovata (Fabricius.1792)	A_ova	0.93	0.40	0.00	0.00
Amara similata (Gyllenhal.1810)	A_simi	0.00	1.60	1.48	1.79
Amara spreta Dejean.1831	A_spre	0.93	0.00	0.00	0.00
Amara tibialis (Paykull.1798)	A_tibi	0.00	0.00	0.00	1.79
Anisodactylus binotatus (Fabricius.1787)	An_bin	0.00	0.40	0.99	0.00
Calathus melanocephalus L.	C_melano	0.00	0.40	0.00	0.00
Calathus ambiguus (Paykull.1790)	C_amb	0.00	0.00	0.49	0.00
Carabus convexus Fabricius.1775	Ca_con	0.00	0.40	0.00	0.00
Carabus granulatus Linnaeus. 1758	Ca_gran	1.85	6.40	12.81	9.82
Carabus marginalis Fabricius.1794	Ca_marg	8.33	0.40	0.00	0.89
Carabus nemoralis O.F.Muller.1764	Ca_nemo	0.00	0.00	0.00	0.89
Carabus violaceus Linnaeus. 1758	Ca_viol	0.93	0.00	0.00	0.00
Curtonotus aulicus (Panzer.1797)	Cu_auli	0.00	0.00	0.49	0.00
*Cychrus caraboides (*Linnaeus.1758)	Cy_car	2.78	0.00	0.00	0.00
Harpalus rubripes (Duftschmid.1812)	H_rubri	0.93	0.00	0.49	0.00
Harpalus rufipes (De Geer.1774)	H_rufi	0.00	2.00	0.49	0.00
Harpalus signaticornis (Duftschmid.1812)	H_signa	0.93	0.00	0.00	0.00
Harpalus tardus (Panzer.1797)	H_tard	0.00	0.00	0.00	0.89
Nebria brevicollis (Fabricius.1792)	N_brev	0.00	0.00	0.49	0.00
Poecilus cupreus (Linnaeus.1758)	Po_cupr	11.11	19.60	7.88	8.04
Poecilus versicolor (Sturn)	Po_ver	55.56	46.00	56.16	44.64
Pterostichus melanarius (Illiger.1798)	P_melan	5.56	17.60	9.85	16.07
Pterostichus niger (Schaller.1783)	P_nige	7.41	1.20	0.99	4.46
Pterostichus nigrita (Paykull.1790)	P_nigr	0.00	0.00	0.49	1.79
Number of species		15	16	17	14
Number of individuals		108	250	203	112

tive composition of the analyzed *Carabidae* assemblages. Similar results of tests were reported by THIELE (1977), who concluded that the differences in the qualitative and quantitative composition of *Carabidae* living on light and heavy soils are larger than between assemblages of ground beetles dwelling on different types of crops. This dependence has also been confirmed by HOLOPAINEN et al. (1995), who studied communities of carabids in barley fields. Differences in the particle size distribution of soil seems to have influence on the number of captured carabid beetles. On sandy-loam soils, PAŁOSZ (2006) captured four times as many carabids as on sandy soil, without noticing any differences in the species composition between beetle communities from the two types of soils. In contrast, KOVAL and DUSEVA (2008) observed increased abundance of ground beetles on sandy soil compared to loamy soil. In addition, these authors found out that populations of *Carabidae* settling on the two types of soil were also different in the species composition and structure of dominance. In the present study, most of the individuals belonging to *Carabidae* were captured on the peat-muck soil (GMT1 – 250 individuals and GMT2 – 203 individuals). Significantly fewer individuals of the ground beetle family were captured on the peat soil (112 individuals) and the muckous soil (108 indiv.). The distribution of the numbers of captured species of *Carabidae* was similar. Most species were captured on the peat-muck soil in the two analzyed profiles (GMT1 – 16 and GMT2 – 17 species). The dominance structure is an important parameter in the evaluation of assemblages of *Carabidae* beetles. On the muckous soil, the superdominant species was *Poecilus versicolor* (55.56%) (Table 2). The eudominants included: *Poecilus cupreus*, which made up 11.11% of all captured individuals of *Carabidae*. The group of dominants consisted of 3 species: *Carabus marginalis*, *Pterostichus niger* and *Pterostichus melanarius*. On the peat-muck soils of both profiles, the superdominant species was *P. versicolor*, whereas the eudominant and dominant groups were composed of *P. cupreus*, *P. melanarius* and *Carabus granulatus*. On the peat soil, the composition of the species dominating in classes D6 – D4 was almost identical, except that another species, *Amara eurynota*, was added to the group of dominant species.

Our assessment of the dependences between the types of soil and the species of ground beetles captured on a given soil was based on ordinance analyses. The length of the gradient (SD = 3.756) established on the basis of the detrended correspondence analysis (DCA), implied a unimodal character of the distribution of data. The influence of particular properties of soil's surface layers on the captured ground beetles was evaluated according to the canonical correspondence analysis (CCA), the results of which suggested that the highest percentage of variability in the presence of the analyzed ground beetle species was described by an environmental variable, i.e. soil pH (Figure 1). This factor was most strongly correlated with the first ordinance axis and described 14.7% of the variability of the identified *Carabidae* species. The increasing soil pH was correlated with the occurrence of such species as *Carabus marginalis* and *Amara convexior*. The second ordinance

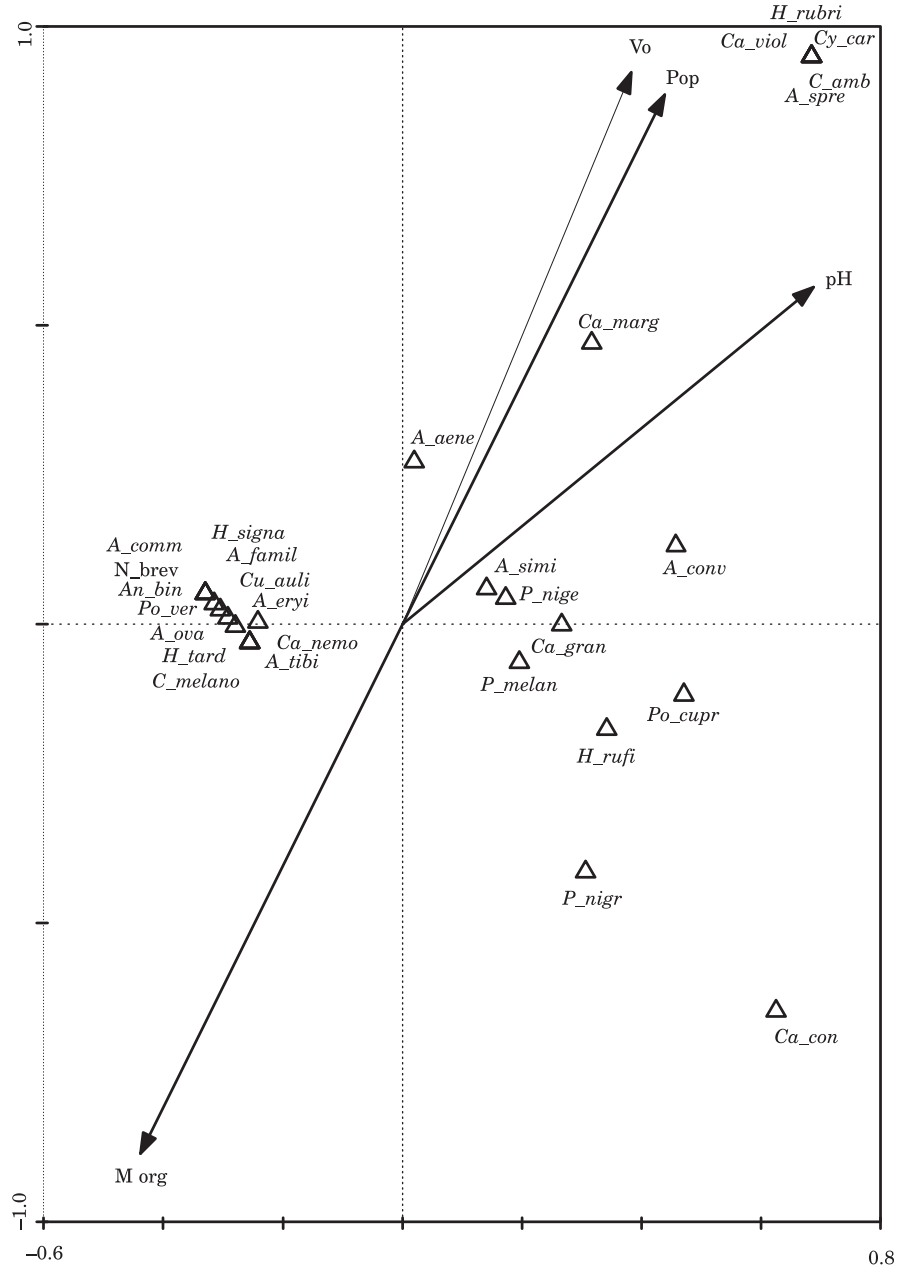

Fig. 1. Ordinance diagram of the canonical correspondence analysis (CCA) illustrating the dependence between presence of *Carabidae* species and parameters describing the analyzed types of soil. The statistical significance of cannonical axes was established according to Monte Carlo tests ($p<0.1$) Explanation of the abbreviations used in the diagram can be found under Tabs. 1 and 2

axis, which described smaller variability of the analyzed beetle assemblage than the first axis, was correlated with the bulk density of soil, its ash content and content of organic matter. Our search for the dependence between the type of soil and the species of *Carabidae* was based on the results of the canonical correspondence analysis (CCA). It has been found out that the soils representing the muck-peat type (GMT1 and GMT2) are wilingly penetrated by such species as *Carabus granulatus*, *Anisodactylus binotatus*, *Poecilus versicolor* and *Amara similata* (Figure 2). The muck type of soil (GM) was in turn associated with the presence of such species as *Amara ovata* and *A. eurynota*. *Harpalus rubripes* and *Pterostichus nigrita* correlated with another envioronmental factor, namely peat soils.

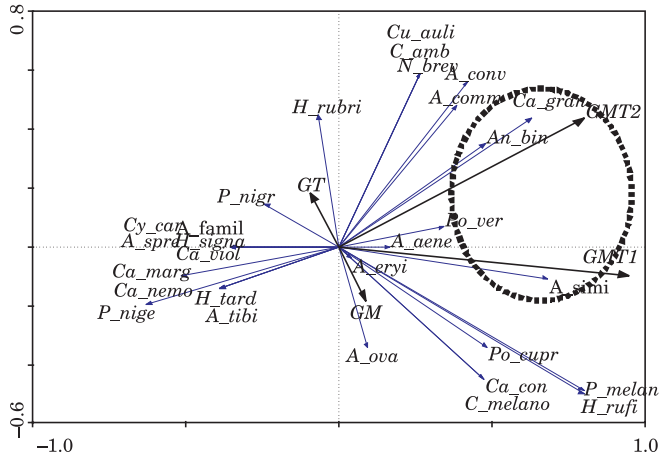

Fig. 2. Diagram of the detrended correspondence analysis (DCA) presenting the variability of the species composition depending on the analyzed soils.
(Explanation of the abbreviations of the species names used in the diagram can be found in Table 2)

The captured species of ground beetles were classified into specific ecological groups, according to their feeding, habitat or moisture requirements, type of development and geographical distribution. Based on the redundancy analysis (RDA) (the data were characterized by linear distribution), the authors tried to establish whether there was any dependence between the analyzed types of bog and post-bog soils and the presence of ground beetles belonging to specific ecological groups. It has been determined that the first ordinance axis correleated with the factor such as the muck-peat soils (GMT1 and GMT2) and peat soils (GT) (Figure 3). The muck-peat soils were correlated with the presence of hygrophilous carabid species, associated with peatlands and open areas. The muckous soils, however, were characterized by the presence of woodland species of ground beetles, mesoxerophilous species

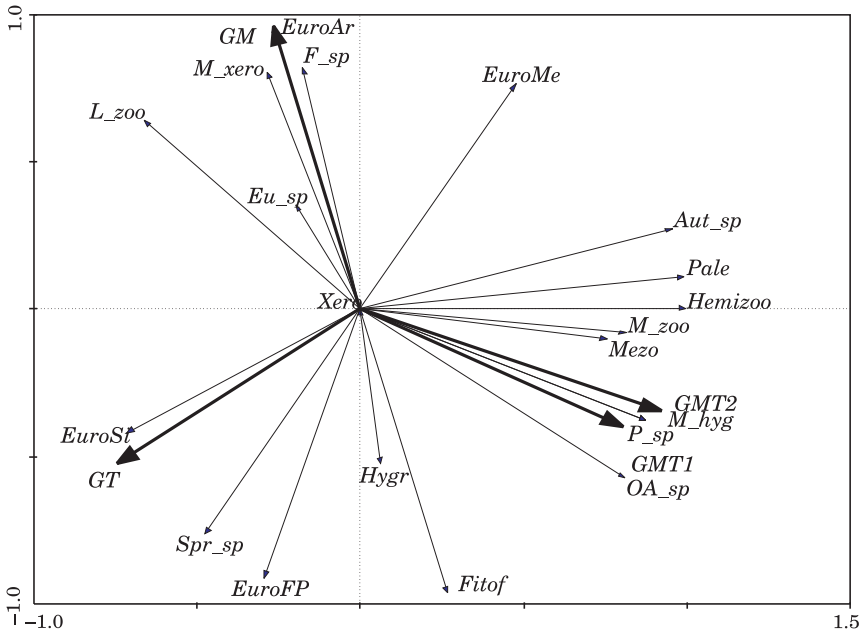

Fig. 3. Diagram of the redundency analysis (RDA) representing dependeces between the analyzed types of soil and *Carabidae* species ordered according to their ecological characteristics: L_zoo – Large zoophages, M_zoo – Medium zoophages, Hemizoo – Hemizoophages, Fitof – Phytophages, F_sp – Forest species, OA_sp – Open area species, P_sp – Peatbog species, Eu_sp – Eurytopic species, Xero – Xerophilic species, M_xero – Mesoxerophilic species, M_hygr – Mesohygrophilic species, Hygr – Hygrophilic species, Pale – Palaearctic species, EuroAR – Euroarctic species, EuroSi – Euro – Siberian species, EuroMe – Euro – Mediterranean species, EuroFP – European Forest Province species, Spr_sp – Spring species, Aut_sp – Autumn species

living in the Euro-Arctic area. The peat soils were strongly correlated with the presence of Euro-Siberian species. This dependence was described by ALESKANDROWICZ (2002) and NIETUPSKI et al. (2008), who drew attention to the fact that an increasing share of Euro-Siberian species on peatlands was encouraged by results of some types of man's activity, for example land drainage systems and cut meadows.

CONCLUSIONS

1. The type of soil proved to be a factor that significantly influenced the number of captured individuals representing the family of *Carabidae*.

2. The decreasing pH gradient of the analyzed soils as well as the increasing soil content of organic carbon in the muckous, peat-muck and peat soils were associated with the decreasing number of carabid individuals less tolerant to the changing moisture conditions (mesophilous species), which were replaced by hygrophilous specimens.

3. As the soil's acidity and content of organic substance were increasing, so did the number of mixophagous at the expense of predatory individuals.

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SOIL β-GLUCOSIDASE ACTIVITY UNDER WINTER WHEAT CULTIVATED IN CROP ROTATION SYSTEMS DEPLETING AND ENRICHING THE SOIL IN ORGANIC MATTER

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Abstract

β-glucosidase (E.C. 3.2.1.21), an enzyme involved in cellulose degradation, plays an important role in the soil organic carbon cycle. Cellulose is the most abundant organic compound in the biosphere so a product of its enzymatic hydrolysis is important as an energy source for soil microorganisms. Since β-glucosidase is very sensitive to different factors, determination of its activity might be helpful in soil quality monitoring. The objective of the study was to assess the effect of various doses of farmyard manure (FYM) and mineral nitrogen on β-glucosidase activity in soil samples taken under winter wheat cultivated in crop rotation systems depleting soil from organic matter (A) and enriching soil in organic matter (B). Soil samples were taken in 2002 from a two-factor fertilization experiment carried out as randomized sub-blocks cropped with winter wheat cultivated on lessivé soil. The experiment was located at the Experimental Station of the Institute of Tillage and Soil Science in Grabowo on the Vistula River. All fertilization combinations included FYM (0, 20, 40, 60 and 80 $t \cdot ha^{-1}$) and nitrogen fertilization (0, 40, 80 and 120 kg ha^{-1}). The activity of β-glucosidase was determined according to Eivazi, Tabatabai (1988). The enzyme activity ranged 3.604-7.041 mM pNP g^{-1} h^{-1} in soil samples taken from crop rotation A and between 4.931-7.445 mM pNP g^{-1} h^{-1} in those collected from the crop rotation enriching the soil in organic matter. These data were closely related to the applied FYM and nitrogen fertilization doses. Moreover, β-glucosidase activity depended significantly on sampling dates. Enzyme activity was closely connected with soil organic carbon and total nitrogen content, which was confirmed by highly significant correlation coefficients between these parameters (r=0.611-0.770 for $C_{org,}$ and r=0.844-0.912 for N_{og}; $p<0.01$ and $p<0.001$).

Key words: β-glucosidase activity, farmyard manure, nitrogen fertilization, lessivé soil.

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AKTYWNOŚĆ β-GLUKOZYDAZY GLEBOWEJ SPOD PSZENICY OZIMEJ W ZMIANOWANIU ZUBOŻAJĄCYM I WZBOGACAJĄCYM GLEBĘ W MATERIĘ ORGANICZNĄ

Abstrakt

Biorąca udział w rozkładzie celulozy β-glukozydaza (E.C. 3.2.1.21) odgrywa ważną rolę w obiegu węgla w glebie. Jak wiadomo, celuloza to jeden z najobficiej występujących związków organicznych w biosferze, a produkt jego hydrolizy enzymatycznej stanowi cenne źródło energii dla mikroorganizmów glebowych. Ponieważ β-glukozydaza jest bardzo czuła na działanie różnorodnych czynników, oznaczanie jej aktywności może być pomocne w monitorowaniu jakości gleby. Celem pracy było określenie wpływu zróżnicowanych dawek obornika i nawożenia azotowego na aktywność β-glukozydazy w próbkach gleby spod pszenicy ozimej uprawianej w zamianowaniu zubożającym (A) i wzbogacającym (B) glebę w materię organiczną. Próbki gleby do badań pobrano w 2002 r. spod pszenicy ozimej uprawianej na glebie płowej w dwuczynnikowym doświadczeniu nawozowym założonym metodą losowanych podbloków. Doświadczenie zlokalizowano w RZD w Grabowie nad Wisłą. W każdym z bloków zastosowano kombinacje nawozowe obornika (0, 20, 40, 60 i 80 t ha^{-1}) oraz nawożenia azotowego (0, 40, 80 i 120 kg ha^{-1}).

Aktywność β-glukozydazy oznaczono wg metody EIVAZI, TABATABAI (1988). Kształtowała się ona w zakresie 3,604-7,041 mM pNP g^{-1} h^{-1} w próbkach gleby z doświadczenia A oraz 4,931-7,445 mM pNP g^{-1} h^{-1} w próbkach z doświadczenia B i pozostawała w ścisłej zależności od zastosowanych dawek obornika i azotu mineralnego. Aktywność β-glukozydazy zależała także istotnie od terminu pobrania próbek glebowych. Ponadto aktywność oznaczanego enzymu była ściśle powiązana z zawartością C_{org} i N_{og}, o czym świadczą uzyskane wysokie współczynniki korelacji (r = 0,611-0,770 – dla $C_{org,}$ oraz r = 0,844-0,912 – dla N_{og}; p<0,01 i p<0,001).

Słowa kluczowe: aktywność β-glukozydazy, obornik, nawożenie azotowe, gleba płowa.

INTRODUCTION

A group of cellulolytic microorganisms producing a specific enzyme complex is responsible for cellulose degradation, the most abundant organic compound in the biosphere (RUSSEL et al. 2005). This complex of enzymes hydrolyzing cellulose consists of endocellulases (endo-β-1,4-glucanase), which randomly cleave β-1,4-glucosidic linkages in the cellulose chain, exocellulases, which release cellobiose (and other celloligosaccharides) from non-reducing ends of cellulose molecules and b-glucosidase (EC 3.2.1.21), which catalyses the degradation of cellobiose to two molecules of glucose and releases glucose from non-reducing ends of celloligosaccharides (RAPA, BEERMANN 1991). Thus, β-glucosidase activity plays a crucial role in the C cycle of soils and the product of its enzymatic hydrolysis is important as an energy source for soil microorganisms (TABATABAI 1994, BANDICK, DICK 1999, JIMČNEZ et al. 2007).

One of the main anthropogenic factors affecting soil enzymatic activity is fertilization, both organic and mineral. Organic fertilization, mainly with farmyard manure, beneficially increases organic carbon and nitrogen con-

centrations in soil and affects the quality and quantity of organic matter (JARECKI, KRZYWY 1991). Thus, fertilization has crucial influence on soil biological status and the enzymatic activity. Since b-glucosidases are proteins which are very sensitive to different natural and anthropogenic factors (BANDICK, DICK 1999, GIANFREDA, RUGGIERO 2006), determination of their activity might be helpful in monitoring soil quality, especially soil subject to differentiated organic and mineral fertilization in various crop rotations.

The objective of the study was to assess the effect of various doses of farmyard manure (FYM) and mineral nitrogen on β-glucosidase activity in soil samples taken under winter wheat cultivated in crop rotation systems depleting and enriching the soil in organic matter.

MATERIAL AND METHODS

Soil samples were taken in 2002 from a two-factor fertilization experiment carried out as randomized sub-blocks located at the Experimental Station of the Institute of Tillage and Soil Science in Grabowo-on-the-Vistula, cropped with winter wheat cultivated on lessivé soil. Soil material was collected from the surface horizon of soil from crop rotation systems depleting (A) and enriching (B) the soil in organic matter. Soil was sampled three times in each vegetation season of winter wheat (at the end of March – date I, mid-May – date II, mid-July – date III) and after harvest (the beginning of September –date IV). The crop rotation systems applied in both experiments are given below:

Year	Crop rotation A	Crop rotation B
2001	potato	potato
2002	winter wheat	winter wheat + white mustard spring
2003	spring barley	barley + red clover
2004	corn	red clover + grasses

The fertilization combinations of every sub-block included FYM (0, 20, 40, 60 and 80 t ha^{-1}) and nitrogen fertilization (0, 40, 80 and 120 kg ha^{-1}). β-glucosidase activity was determined according to EIVAZI and TABATABAI (1988). The method is based on the spectrophotometrical measure of *p*-nitrophenyl (*p*NP) released after 1 hour of incubation of soil samples at 37°C with *p*-nitrophenyl-β-D-glucopyranoside (*p*NPG) in modified universal buffer (pH 6.0) as the substrate. The enzyme activity was expressed as mmoles *p*NP released kg^{-1}dry soil per 1 hour (mM *p*NP kg^{-1} h^{-1}). Soil chemical properties, such as organic carbon content (C_{ORG}), total nitrogen content (N_{TOT}) and pH in 1 mol KCl dm^3, were determined according to standard methods accepted in soil science.

RESULTS AND DISCUSSION

Soil samples collected from both crop rotations had acid to slight acid reaction. The pH values measured in 1 mol $KCl \cdot dm^3$ ranged 4.5-6.1 for soil samples from crop rotation A, while the corresponding values for experiment B ranged 4.9-6.1. Total nitrogen content reached 0.889-1.005 g kg^{-1} in soil material collected from the experiment enriching soil with organic matter, and 0.826-1.029 g kg^{-1} in soil samples taken from crop rotation A (mean values for FYM and nitrogen fertilization doses). Increasing FYM doses significantly increased the total nitrogen content in the soil samples collected from both investigated crop rotations. However, no significant influence of nitrogen fertilization on total nitrogen concentration was shown in soil samples from either crop rotation.

β-glucosidase activity (Table 1) was higher in soil samples taken from plots of the experiment enriching the soil with organic matter as compared with the activity found in the soil material taken from the crop rotation system depleting the soil from organic matter. The results indicated the importance of incorporating plants of the fabae family in a crop rotation system and the influence of plant residues left behind in the soil as a result of intercrop cultivation on the soil's biological activity status.

The enzyme activity ranged from 3.604 to 7. 041 mM pNP g^{-1} h^{-1} in the soil samples taken from crop rotation A and from 4.931 to 7.445 mM pNP g^{-1} h^{-1} in those collected from the crop rotation enriching the soil with organic matter. These data were closely related with the FYM and nitrogen fertilization doses applied (Table 1). The enzyme activity was modified by farmyard manure fertilization to a higher degree than by mineral nitrogen. Organic fertilization caused an increase in β-glucosidase activity by enhancing the abundance of a microbial population, which is the main source of the enzyme in soil (Marcote et al. 2001, Böhme, Böhme 2006).

For most of the soil samples, it was shown that higher FYM doses were accompanied by higher β-glucosidase activities. The biggest difference between the enzyme activity in the control soil sample and samples from the plots fertilized with the maximum FYM dose (80 t ha^{-1}) was noted in experiment A (an increase from 41% to 46%, as dependent on N doses) in comparison with the corresponding samples from crop rotation B (an increase from 22% to 43%, as dependent on N doses).

No clear influence of the applied nitrogen doses on soil β-glucosidase was found. However, when FYM was not applied, increasing doses of nitrogen fertilization clearly elevated the enzyme activity in the soil samples from both crop rotations.

In the soil samples from both experiments, whenever FYM was applied, increasing N fertilization doses did not cause any increase in β-glucosidase

Table 1

Soil β-glucosidase activity and organic carbon content in soil samples taken from crop rotation systems A and B (mean values for sampling dates)

Parameters	FYM* (t ha^{-1})	Crop rotation system A					Crop rotation system B				
		Nitrogen fertilization** (kg ha^{-1})									
β-glucosidase	0	3.604	3.825	4.011	3.913	3.838	4.931	5.425	5.311	5.232	5.225
	20	4.513	4.432	4.445	4.625	4.504	6.554	5.911	6.173	6.811	6.362
	40	5.000	4.741	5.227	5.279	5.062	5.912	6.410	6.342	6.713	6.344
	60	7.041	6.910	6.713	6.532	6.799	7.337	6.931	6.968	7.248	7.121
	80	6.710	6.919	6.924	6.625	6.795	7.445	7.175	7.212	6.731	7.141
	mean	5.374	5.365	5.464	5.395	5.400	6.436	6.370	6.401	6.547	6.439
	$LSD_{0.05}$	factor I: 0.286 ; factor II: 0.139					factor I: 0.284 ; factor II: 0.187				
Organic carbon (g kg^{-1})	0	7.812	6.519	6.625	7.839	7.199	8.823	8.345	9.221	9.212	8.900
	20	6.241	6.909	6.421	7.279	6.713	9.554	9.615	9.832	10.34	9.835
	40	7.606	7.291	7.120	7.465	7.371	9.314	9.721	9.681	10.00	9.679
	60	8.715	8.204	7.820	8.125	8.216	9.991	10.12	9.665	9.441	9.804
	80	7.404	7.821	8.045	8.578	7.962	10.10	10.23	10.95	10.34	10.41
	mean	7.556	7.349	7.206	7.857	7.492	9.556	9.606	9.870	9.867	9.725
	$LSD_{0.05}$	factor I: 0.518 ; factor II: n.s.					factor I: 0.428 ; factor II: n.s				

*FYM – farmyard manure – factor I; **nitrogen fertilization – factor II; n.s. – differences not significant

activity, which in some cases even decreased significantly, especially when FYM was applied in the doses of 60 and 80 t ha^{-1}.

No clear tendency describing the influence of nitrogen fertilization on soil enzymatic activity has been reported in earlier papers. The way nitrogen fertilization affects soil enzymes depends, among other factors, on the kind of fertilization, dose of the fertilizer, the time of its application and the individual character of an enzyme (GIANFREDA, RUGGIERO 2006). For instance EIVAZI and TABATABAI (1990) showed that application of $N\text{-}NO_3$ in various doses partly inhibited β-glucosidase activity, while in a greenhouse experiment with corn, the enzymatic activity was directly proportional to the $N\text{-}NH_4NO_3$ amount applied over 164 days of the experiment (FAUCI, DICK 1994). According to DICK et al. (1988) enzymatic activity is not directly related to the N cycle (as the β-glucosidase activity). In their experiment, it did not correlate with nitrogen fertilizers used in various doses.

Moreover, β-glucosidase activity depended significantly on the sampling dates (Figure 1). The enzyme was less active in the soil samples taken on sampling dates I and II in the experiment where soil was enriched with organic matter as compared with the soil samples collected in the summer and autumn. The opposite was noted for the soil samples taken from crop rotation B, where the activity was the lowest in the samples collected on sampling dates III and IV.

No significant influence of nitrogen fertilization on organic carbon content was proved, although it was essentially modified by FYM doses in soil samples from both experiments (Table 1). In most soil samples, along with an increase in the FYM dose, the organic carbon content increased as well. Positive influence of farmyard manure fertilization on the organic carbon content, as well as the accumulation, mineralization and humification of

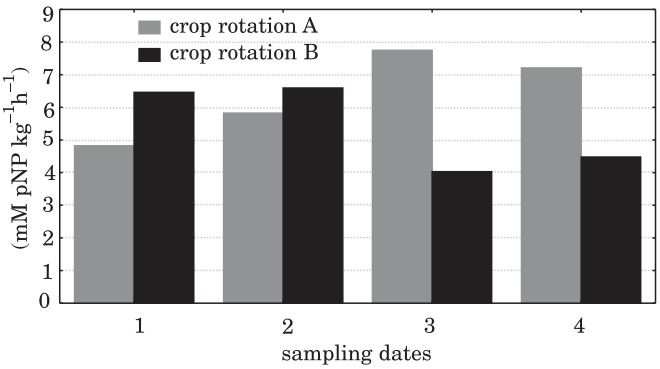

Fig. 1. β-glucosidase activity in soil samples taken from crop rotation systems A and B as dependent on sampling dates

organic matter, has been shown by many researchers (JARECKI, KRZYWY 1991, MAĆKOWIAK, ŻEBROWSKI 1999, MERCIK et al. 2004).

β-glucosidase activity, which plays a key role in the soil carbon cycling, was closely connected with the organic carbon content in the soil samples taken from both experiments on all sampling dates. Correlation coefficients between the discussed parameters ranged between $r = 0.611$ and $r = 0.770$, with $p < 0.05$ and $p < 0.001$, respectively. SIMILARLY, BÖHME and BÖHME (2006) obtained significant and positive correlation coefficients between β-glucosidase activity and organic carbon content in soil samples under spring barley and sugar beets cultivated in a long-term fertilization experiment ($r = 0.965$ and 0.954 with $p < 0.001$, respectively). Some other trials have also produced evidence for a significant relationship between soil β-glucosidase activity and organic carbon content (EIVAZI, TABATABAI 1990, BANDICK, DICK 1999). According to LANDGRAF and KLOSE (2002), soil β-glucosidase activity is closely related to the content of easily soluble organic carbon. Moreover, soil β-glucosidase activity was significantly and positively correlated with total nitrogen ($r = 0.844$-0.912, $p < 0.001$) and pH in KCl ($r = 0.632 = 0.773$; $p < 0.05$ and $p < 0.001$), but only in soil samples collected from systems depleting the soil from organic matter (B).

CONCLUSIONS

1. Higher β-glucosidase activity observed in the soil samples taken from the systems enriching the soil with organic matter as compared to the values measured in the crop rotation depleting the soil from organic matter indicated the importance of plant residues introduced into soil as a result of intercrop cultivation as well as the significance of cultivation of the fabae plants in the soil enzymatic activity status.

2. Organic carbon and total nitrogen content as well as soil β-glucosidase activity in soil samples taken from both crop rotations coincided with increasing FYM doses.

3. No clear tendency was observed in the β-glucosidase activity as influenced by increasing nitrogen fertilization doses. Moreover, nitrogen fertilization did not influence significantly organic carbon and total nitrogen content in the soil samples collected from both crop rotation systems.

4. While soil β-glucosidase activity was characterized by seasonal variability, there was no clear direction in the enzyme activity when the soil samples from both experiments were compared.

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REVIEW PAPER

BIOCHEMISTRY OF MAGNESIUM

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Abstract

Magnesium is essential for biochemical functions of cells. Since Mg^{2+} has a relatively low ionic radius in proportion to the size of the nucleus (0.86 versus 1.14 f Å for Ca^{2+}), it shows exceptional biochemical activity. Due to its physicochemical properties, intracellular magnesium can bind to the nucleus, ribosomes, cell membranes or macromolecules occurring in the cell's cytosol. It is indispensable for the nucleus to function as a whole and for the maintenance of physical stability as well as aggregation of rybosomes into polysomes able to initiate protein synthesis. Mg^{2+} can also act as a cofactor for ribonucleic acid enzymes (ribozymes) capable of specifically recognizing and cleaving the target mRNA. As an essential cofactor in NER, BER, MMR processes, Mg^{2+} is required for the removal of DNA damage. An activator of over 300 different enzymes, magnesium participates in many metabolic processes, such as glycolysis, Krebs cycle, β-oxidation or ion transport across cell membranes. Mg^{2+} plays a key role in the regulation of functions of mitochondria, including the control of their volume, composition of ions and ATP production.

Key words: magnesium, DNA repair process, enzyme, metabolic cycle, cellular respiration, calcium ion transport, potassium ion transport.

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Abstrakt

Magnez jest składnikiem niezbędnym dla zasadniczych funkcji biochemicznych komórki. Ponieważ Mg^{2+} ma relatywnie mały promień w stosunku do wymiarów jądra (0.86 i 1.14 Å odpowiednio dla Mg^{2+} i Ca^{2+}), wykazuje dużą aktywność biochemiczną. Dzięki właściwościom fizykochemicznym śródkomórkowy Mg^{2+} może wiązać się z jądrem komórkowym, rybosomami, błonami komórkowymi oraz makromolekułami cytosolu komórki. Magnez jest niezbędny dla funkcjonowania jądra komórkowego jako całości oraz utrzymania fizycznej stabilności i agregacji rybosomów do polisomów zdolnych do biosyntezy białka. Odgrywa on również rolę kofaktora katalitycznych cząsteczek RNA (rybozymów), odpowiedzialnych za specyficzne rozpoznawanie i fragmentację docelowego mRNA. Jako kofaktor w procesach: NER, BER, MMR, przyczynia się do usuwania uszkodzeń DNA. Magnez, będąc aktywatorem ponad 300 różnych enzymów, uczestniczy w przebiegu wielu szlaków metabolicznych, takich jak glikoliza, cykl Krebsa, β-oksydacja czy transport jonów poprzez błony komórkowe. Odgrywa on ponadto bardzo ważną rolę w regulowaniu funkcji mitochondriów, łącznie z regulacją ich wielkości, kompozycją jonów, a także bioenergetyką i regulacją produkcji ATP.

Słowa kluczowe: magnez, proces naprawy DNA, enzym, cykl metaboliczny, oddychanie wewnątrzkomórkowe, transport jonów wapnia, transport jonów potasu.

INTRODUCTION

The involvement of magnesium ions (Mg^{2+}) in metabolic processes is governed not only by their abundance in nature or relative amount in living organisms but also by their physicochemical characteristics.

Since Mg^{2+} has a relatively low ionic radius in proportion to the size of the nucleus (0.86 versus 1.14 f Å for Ca), it shows exceptional biochemical activity. Ionized Mg^{2+} usually coordinates with 6-7 molecules of H_2O, as in the case of $MgCl_2 \cdot 6\ H_2O$ or $Mg_2SO_4 \cdot 7\ H_2O$, while Ca and Ba combine with 1 or 2 mols of H_2O ($BaCl_2$ and $CaCl_2$ respectively). In comparison to calcium (Ca), the most abundant cation in the human body, Mg^{2+} displays higher affinity for oxygen donor ligands, that is negatively charged carboxylates and phosphates or enolate moieties (Wolf, Cittadini 2003). Mg-water coordination occurs in a typical octahedral conformation and thereby magnesium exhibits slower water exchange than other ions. Consequently, it is much bigger and more stable in comparison to Ca in biological systems (Wolf, Cittadini 2003, Wedekind et al. 1995)

Considering stereochemical properties, nickel (Ni) most closely resembles Mg^{2+} (it has atoms of the same size of and identical water exchange constant). However, Ni^{2+} cannot compete with Mg^{2+} in living organisms both due to its paucity and tendency to bind nitrogen rather than oxygen.

At the cellular level, magnesium ions compete not only with Ca but also with protons or amines ($-NH_2^+$). Protons are usually present in concen-

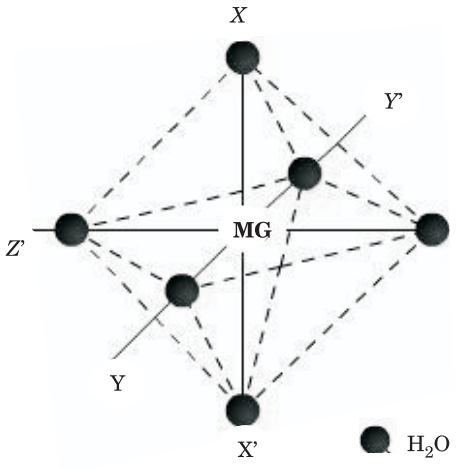


Fig. 1. Octahedral magnesium complexes (WOLF, CITTADINI 2003)

trations below 10^{-7} M at pH 7 and link up to phosphate groups with a pKa of 6.5, which is significantly lower than that of Mg-phosphate complexes. This suggests that Mg^{2+} is removed from ATP when pH falls to 6.0, causing significant modifications of Mg-dependent reactions:

$$Mg \cdot ATP + H^{+} \leftrightarrow H \cdot ATP + Mg^{2+}$$

Polyamines, organic derivatives of ammonia, exhibit high-affinity binding of polyanions, for instance nucleic acids, and dislodge Mg^{2+} bound therein (WOLF, CITTADINI 2003).

Due to its physicochemical properties, intracellular magnesium can bind to the nucleus, ribosomes, cell membranes or macromolecules occurring in the cell's cytosol.

Magnesium and DNA

More than half the magnesium contained in the nucleus is closely associated with nucleic acids and free nucleotides. Since nucleic acids are polyanions, they require counterions in order to neutralize negatively charged phosphate groups (WOLF, CITTADINI 2003, ANASTASSOPOULOU, THEOPHANIDES 2002). The intracellular concentrations of Na and Ca are low, therefore the binding of the metal with nucleic acids is dominated by K^{+} and Mg^{2+}. Free Mg^{2+} is the winner in this competition because it has more positive charges (+ II and +I for Mg and K, respectively) and higher hydration energy (WOLF, CITTADINI 2003).

In Mg-DNA interactions, metal ions interact with purine bases at the N7 site and pyrimidine bases at the N3 site by forming chemical bonds, Mg-

N7 and/or Mg-N3. They also interact with the negatively charged oxygen atoms of phosphate groups of nucleotide chains (ANASTASSOPOULOU, THEOPHANIDES 2002). These interactions play a significant role in the stabilization of the secondary and tertiary structure of DNA.

The pathway of binding divalent metal ions to guanosino-5-monophosphate is shown in Figure 2. The intrinsic structure of this complex results from the fact that one of the coordinated water molecules may be substituted by the N7 coordination site of the nucleotide or be hydrogen-bound to it, while another one may be involved in a hydrogen bond with O6, and yet others form more hydrogen bonds (ANASTASSOPOULOU, THEOPHANIDES 2002).

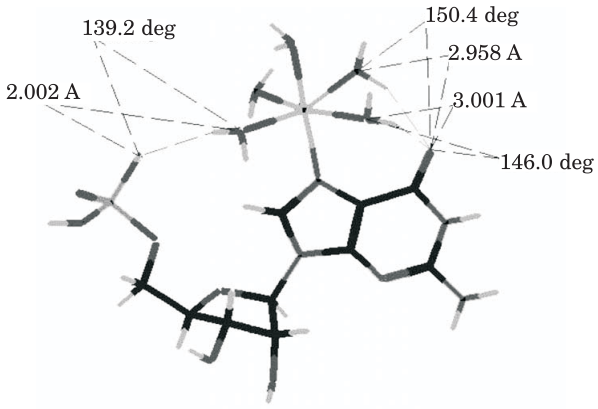


Fig. 2. Structure of magnesium hydrate complex with guanosino-5- monophosphate (the broken lines show hydrogen bonds together with the hydrogen bond distances and angles) (ANASTASSOPOULOU, THEOPHANIDES 2002)

Whether or not Mg^{2+} acts as a gene regulator remains unclear. Since the bioactivity of this cation is remarkable, it is reasonable to think that magnesium may act as a competitor to polyamines, which are currently recognized as potential regulators of the cell cycle (WOLF, CITTADINI 2003).

$$DNA^*(Mg^{2+})_n + m\text{-polyamine}^{x+} \longleftrightarrow (DNA^* \text{ polyamine}^{x+})_m + nMg^{2+}$$

Magnesium ions can affect the cellular cycle also in the form of Mg-ATP. This complex plays a key part in a phosphorylation cascade catalyzed by protein kinases. Alternatively, due to its capability to directly interact with proteins, Mg^{2+} can modulate histone phosphorylation (WOLF, CITTADINI 2003).

Irrespective of the above mechanisms, magnesium is indispensable for the functioning of the cell nucleus as a whole as it is involved in the activation of enzymes important for DNA repair (endonuclease) (WOLF, CITTADINI 2003, HARTWIG 2001, WOLF et al. 2003), replication (topoisomerase II (WOLF,

CITTADINI 2003, WOLF et al. 2003), polimerase I (WOLF, CITTADINI 2003) and transcription (rybonuclease H) (WOLF, CITTADINI 2003). Mg^{2+} is crucial for the physical integrality of double-stranded DNA.

In ribosomes, Mg^{2+} is associated with rRNA or proteins, which are essential for the maintenance of physical stability as well as aggregation of these structures into polysomes able to initiate protein synthesis. Cowan has shown that magnesium deficit leads to the cleaving of a ribosomal complex (COWAN 1995). Since the only function performed by ribosomes is protein biosynthesis, it is the presence of Mg^{2+} in ribosomes that conditions the shape of RNA structures by stimulating the transformation of amino acids into active forms, polypeptide synthesis and stabilization of a protein structure.

Moreover, magnesium can also act as a cofactor for ribonucleic acid enzymes (ribozymes) capable of specifically recognizing and cleaving the target mRNA. Ribozymes are chiefly used in steered therapy of neoplastic diseases (JOŚKO, KNEFEL 2003). It is believed that two metal ions (mostly Mg^{2+}, although Mn^{2+}, Zn^{2+}, Ca^{2+}, Co^{2+} or Na^{+} are also possible) are necessary for catalytic activity of hammerhead ribozymes. One metal ion activates the attacking hydroxyl group, and the other stabilizes the negative charge of the oxygen atom of the released group (ADAMALA, PIKUŁA 2004). While one experiment with minimal hammerhead domains has demonstrated that the efficiency of catalysis is highly dependent on the concentration of magnesium ions, another one has shown that this efficiency can be increased at low magnesium concentration through stabilization of catalytically active conformation by tertiary interactions between helices I and II. Apart from these electrostatic interactions, both free Mg^{2+} as well as the GTP-Mg complex play an important role in tubulin polymerization and, consequently, in chromosome segregation during mitosis (HARTWIG 2001).

Role of magnesium in genomic stability

In 1976 LOEB et al. noted that magnesium ions are indispensable for DNA replication fidelity. Although Co^{2+}, Mn^{2+} and Ni^{2+} ions can be substituted for Mg^{2+}, such an exchange causes a considerable decrease in the fidelity of the discussed process. A metal ion (A) binds to the 3'-hydroxyl group of a new synthesis strand, leading to the lowering of its pKa and thereby facilitating an attack on the á-phosphate of “arriving” dNTP. Another metal ion (B) facilitates the leaving of the â- and ă-phosphates as well as phosphodiester bond formation (HARTWIG 2001).

Role of magnesium in DNA repair processes

Damage to DNA can be caused by exogenous factors (e.g. ultraviolet or electromagnetic radiation, high temperature, viruses, polycyclic aromatic hydrocarbons, radiotherapy or chemotherapy) or endogenous factors (mainly

ROS – reactive oxygen species). In order to lower the frequency of mutation, cells have developed many different DNA-repair systems.

Nucleotide excision repair (NER) is an evolutionarily conserved DNA repair pathway, which repairs DNA damaged by various environmental mutagens. Photodimers, pirymidine, adducts as well as some of the damage repaired in the course of base excision repair (BER) can be removed from DNA (SANCAR 1994). The repair process is dependent on coordinated action of more than 20 different proteins. The majority of them are engaged in damage recognition and incision at both sides of the defect. Magnesium acts as a cofactor practically at every NER stage. Results of *in vitro* investigations have shown that this mechanism is completely inhibited in the case of absence and very high concentrations of magnesium. Experimental data have confirmed that the DNA-damage recognition protein UV-DDBP, the helicase XPD and the nuclease XPG are all magnesium-dependent. The element is required not only in enzymatic incision of DNA, but also in the processes of polymerization and ligation (HARTWIG 2001).

Reactive oxygen forms, normal products of cellular metabolism, lead to a wide variety of DNA modifications: destabilization of a DNA helix as well as degradation of protein-DNA crosslinks. Additionally, they are a major contributor to the oxidation of purine and pyrimidine bases, the damage of pentose ring, the hydrolysis of amine – or N-glycosidic bonds and phosphodiester bonds, hydrolytic deamination and methylation of oxygen or nitrogen atoms of DNA bases. At the extracellular level, ROS impair the function of blood platelets and induce protein, lipid or nucleic acid oxidation resulting in tissue destruction in many organs (CERIELLO, MOTZ 2004). It is believed that a mature organism can produce about 2 kilograms of superoxide anions per year, which can be transformed to H_2O_2 by dismutation reaction (ROSZKOWSKI 2002).

Endogenous damages of DNA are mainly repaired by the BER mechanism. According to current models, BER begins with a removal of modified nitrogenous base by a specific N-glycosidase generating an AP site, which is repaired by AP-endonucleases cleaving the phosphodiester bond at the AP 5' side and leaving a 3' hydroxy terminus, making the action of DNA polymerase and ligase possible (ROSZKOWSKI 2002). Contrary to DNA glycosidases, enzymes involved in later stages of BER always require magnesium. In hydrolytic nucleases, which are metal-ion-dependent (mainly magnesium-dependent), metal interacts with a substrate or is directly involved in cleavage of the phosphate-oxygen bond. In human apurinic/apyrimidinic endonuclease (HAP1), single magnesium ion combines with a defined Glu residue in the active center and aids the attack on the P-O3' bond by polarization of the P-O bond, perhaps by correctly orientating the phosphate group rather than directly participating in the nucleolysis reaction. HAP can also be activated by manganese or nickel ions, but its activity is considerably lower (by 50 and 90% respectively). Other examples of magnesium-dependent endonucle-

ases in BER include apurinic/apyrimidinic endonuclease, whose activity is associated with the 5-hydroxymethyluracil-DNA glycosylase, flap-endonuclease-1 (FEN-1) and a structure-specific endonuclease involved in DNA replication and DNA repair (HARTWIG 2001).

The third system, mismatch repair (MMR), has evolved to correct errors occurring during DNA replication or recombinaton of genes. Impairment of MMR leads to genomic instability, which creates favourable conditions for induction and development of carcinogenic processes. The most frequent mutations in the MMR system are those of genes from the MLH gene family. Constitutive mutations involving one allele of the MLH1 gene lead to tumor predisposition syndrome – hereditary nonpolyposis colorectal cancer (HNPCC type II) (COOK 2000) and can also be connected with Turcot syndrome. Additionally, the role of this gene in carcinogenic processes in other organs, especially in breast cancer, is also investigated (BRYŚ et al. 2004).

A study conducted by BAN and YANG (1998) has shown that MutL gene of *E. coli* is absolutely Mg-dependent and, in absence of magnesium ions, hydrolysis of the MutL-ATP complex can be observed. High homology between MHL and MutL suggests that magnesium is also indispensable for the activity of human MHL genes. Moreover, double-stranded DNA break repair induced by ionizing radiation or formed during meiosis has also been found to be Mg-dependent (HARTWIG 2001).

Magnesium and enzymes

An activator of over 300 different enzymes, magnesium participates in many metabolic processes, including transformation of proteins, lipids, carbohydrates and nucleic acids as well as electrolyte transport across cell membranes (WOLF, CITTADINI 2003).

Magnesium can originally bind to a substrate (by chelatation), producing a complex that is the correct substrate for enzyme or directly attach to enzyme, creating active structure able to affect a substrate. However, these mechanisms are combined with each other because ATPase affects the correct substrate (ATP-Mg) only if it is activated by another Mg^{2+} ion.

The general mechanisms of Mg^{2+} action as a cofactor can be described as follows (WOLF, CITTADINI 2003):

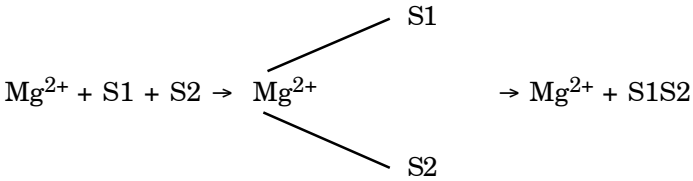
A. Magnesium is engaged in stabilization of an intermediate product:

$$Mg^{2+} + S \rightarrow Mg^{2+} - Y \rightarrow Mg^{2+} + P$$

B. Magnesium stabilizes a product leaving group:

$$Mg^{2+} + SX \rightarrow Mg^{2+} - XS \rightarrow Mg^{2+} X + S$$

C. Magnesium binds two reactive substrates simultaneously and facilitates reaction through the proximity effect:



The majority of enzymes can be also activated by metal ions other than Mg^{2+}, although such a replacement leads to reduced efficiency of enzymatic reaction.

Magnesium in metabolic cycles

In higher organisms, metabolic processes such as glycolysis, Krebs cycle, β-oxidation, active transport of ions or electrochemical coupling are regulated by Mg-dependent enzymes. The main domain of magnesium action is the activation of enzymes responsible for formation, storing and using of high-energy compounds. All reactions involving ATP require the presence of magnesium ions (Touyz 2004). An Mg^{2+} ion, coupled with oxygen atoms of phosphorus groups located at â and ă positions, protects APT molecules from enzymatic hydrolysis, while the dislocation of Mg^{2+} in the direction of á and â positions facilitates the hydrolysis of terminal phosphorus groups. Magnesium in the form of β, γ-Mg-ATP complex binds to active centers of many enzymes. The complexes of Mg-ATP are essential for catalytic activity of, e.g., phosphotransferases (kinases), nucleotidylotransferases and ATPases (Cowan 1995).

Magnesium ions activating adenylate cyclase control cyclic adenosine monophosphate (cAMP) synthesis. Adenylate cyclase activtion is crucial for the control of anaphylactic reactions because high intracellular cAMP and cGMP concentrations slow down or stop degranulation of mast cells. Consequently, the accessibility of magnesium to the enzyme can modulate cyclic nucleotide metabolisms in cells. Since Mg^{2+} deficit stimulates histamine release from mast cells by inhibition of cAMP production, it is believed that magnesium reduces the hypersensitivity reactions (Błach et al. 2007).

Perfect confirmation of the key role of cellular magnesium is glycolysis, especially in human erythrocytes, as many enzymes involved in this process are Mg-dependent. Moreover, removing extracellular Mg^{2+} or chelating intracellular Mg^{2+} markedly inhibits glycolysis and limits glucose transport by erythrocytes (Laughlin, Thompson 1996).

Numerous literature data suggest some correlation between glucose transport and changes in intro- or extracellular Mg^{2+} level resulting from hormonal stimulation of β-pancreatic islets (Henquin et al. 1983, Fagan, Romani 2000), hepatocytes (Fagan, Scarpa 2002, Gaussin et al. 1997) or cardiomyocytes (Romani et al. 1993, Romani, Scarpa 1990, Romani et al. 2000).

An increase in catecholamine or glucagon leads to secretion of glucose and Mg^{2+} from liver cells into the extracellular compartment (GAUSSIN et al. 1997). The presence of glucose transport inhibitors (ROMANI, SCARPA 1990) and the absence of extracellular Na^{+}, which hampers magnesium extrusion, also impair glucose output by liver cells. TORRES et al. (2005) have reported that hepatocytes from starved rats (after overnight fasting) accumulated approximately fourfold more Mg^{2+} than liver cells from fed animals. This clearly indicates that diminution of intrahepatic cellular glycogen or glucose level causes decreased ability of catecholamine or glucagon to mobilize Mg^{2+} from the hepatocyte.

In cardiac myocytes, parallel accumulation of glucose and Mg^{2+} is induced by insulin (HARTWIG 2001, ROMANI et al.1993, 2000). Insulin acts as an endogenous regulating factor of Mg^{2+} homeostasis. Flux concentrations of blood or tissue magnesium are dependent on the amount of insulin released from pancreatic islets and insulin immunity of tissues (ROMANI et al. 2000). Also in this case, the absence of extracellular glucose or the presence of glucose transport inhibitors hamper Mg^{2+} transportation, lower extracellular Mg^{2+} content and break the transport of glucose to myocytes (ROMANI et al. 1993). Some correlation between glucose and Mg^{2+} transport/utilization in rats rendered diabetic by streptozotocin injection has been confirmed by FAGAN et al. (2004). Rats experienced a 10% and 20% decrease in the liver magnesium level after 4 and 8 weeks, respectively, after the onset of the disease. CEFARATTI et al. (2004) have confirmed dminished accumulation of Mg^{2+} in liver blisters isolated from rats with experimental diabetes.

Since Mg^{2+} accumulation directly or indirectly influences protein kinase C activation, it is possible that in diabetic patients the enzymatic action is disturbed. TANG et al. (1993) have also observed selective alterations in the expression PKC and marked differences in the distribution of the various isoforms between membrane and cytosol fractions of hepatocytes with streptozotocin-induced animals.

Similar modifications in the distribution of PKC as well as the reduction of cell magnesium content have been observed in tissues of ethanol-fed rats (YOUNG et al. 2003). The total magnesium concentration in animal hepatocytes of the examined group was 26.8 ± 2.4 nM mg^{-1} proteins versus 36.0 ± 1.4 nM mg^{-1} proteins for the control group. In comparison to the control conditions, the Mg^{2+} level in hepatocytes from EtOH-treated samples did not increase following stimulation of protein kinase C by vasopressin or analogs of diacilglicerol (DAG). Moreover, the stimulation of α- or β-adrenoreceptors in alcohol supplemented animals, did not elicit Mg^{2+} extrusion from liver cells to the extacellular space.

KIMURA et al. (1996) have shown that in Mg-deficient rats concentration of blood glucose and plasma insulin both in overnight fasted and non-fasted individuals as well as in response to oral sucrose loading are impaired. After 8 weeks of low-Mg^{2+} diet, translocation of insulin-stimulated glucose trans-

porter 4 (GLUT4) to the adipocyte plasma membrane was significantly reduced. In addition, phosphorylation of insulin receptor was lower in Mg-deficient animals. On the other hand, wortmannin (WT) or another PI 3-kinase inhibitor blocked the insulin-stimulated activity of Na^+/Mg^{2+} exchange (Ferreira et al. 2004).

These data may suggest that Mg^{2+} absence induces alterations in glucose metabolism by reducing intestinal glucose absorption or glucose assimilation in liver and/or other tissues (Kimura et al. 1996).

Magnesium and cellular respiration

Magnesium maintains a mitochondrial respiratory coupling chain, in which phosphorylation and oxidation obtain high efficiency. Magnesium ions might be transported to the mitochondrial matrix across Mrs2p channel of the inner mitochondrial membrane, whose activity depends on both electric potential and Mg^{2+} concentration, but the electrophysiological profile of Mrs2p remains to be developed (Kolisek 2003).

The flux Mg^{2+} level in the mitochondrial matrix modulates á-ketoglutarate dehydrogenase (Chakraborti et al. 2002), pyruvate dehydrogenase and glutamate dehydrogenase (Panov, Scarpa 1996) activity. Alterations of the matrix Mg^{2+} concentration (coupled with alternatively to changes of Ca^{2+}) are reflected in the mitochondrial respiration rate.

Moreover, Lin et al. (1993) demonstrated that Mg^{2+} is an integral component of subunit IV of cytochrome c oxidase complex, the last enzyme of the respiratory chain catalyzing molecular oxygen reduction. The volume of an organelle is regulated by the matrix magnesium through direct control of the K^+ /H^+ antiporter, inhibition of mitochondrial inner membrane anion channel (IMAC) as well as through indirect modulation of the channel's permeability.

IMAC channels display selectivity among monovalent (Cl^-, HCO_3^-) as well as polyvalent (e.g. citrate) ions. These channels are probably also involved in the synchronization of oscillation in a mitochondrial membrane potential of isolated cardiomiocytes. Evidence has been provided that IMAC regulates the flow of anionic peroxidase from mitochondria during the ischaemic preconditioning (IPC) (Skalska et al. 2006). Although the IMAC control mechanism has not been completely elucidated, Beavis and Powers (2004) suggested that the matrix Mg^{2+} as well as the protons impair channel activity.

The IMAC activation precedes the opening of the mitochondrial permeability transition pore (PTP), thereby promoting cell death. The PTP opening is a direct cause of the death of neurons in a damaged brain or in cardiac myocytes during ischemia and reperfusion. PTP also plays a role in muscular dystrophy (DMD), caused by deficiency of collagen VI, as well as in hepatocytosis, inducted by cancerogenic factors (Skalska et al. 2006). The increase of the mitochondrial calcium pool facilitates the PTP opening whereas

a larger matrix Mg^{2+} concentration blocks this channel. Moreover, ZORATTI and SZABO (1995) showed that megachannels are inhibited by divalent cations, such as Mg^{2+} or Mn^{2+}, nucleotides: ADP and ATP as well as poliamines. DOLDER et al. (2003) established that magnesium plays an indirect role in modulating the PTP opening. They proved that creatine kinase can regulate the PTP size by tightly associating to the mitochondrial membrane and remaining in an active state. Impaired concentration of the extramitochondrial Mg^{2+} causes reduction of creatine kinase activity and increased pore permeability (DOLDER et al.).

Magnesium ions are also essential to glutathione synthesis, which can be confirmed by the fact that GSH level in the red blood cells of rats decreased after 2-3 weeks of a Mg^{2+}-deficient diet (WĘGLICKI et al. 1996). Glutathione depletion enforces reactive oxygen species accumulation, resulting in mitochondrial dysfunction, which is decisive in apoptotic cascade. The changes in the mitochondrial membrane's potential lead to the opening of megachannels in mitochondrial membranes, to alterations of membrane permeability, to translocation of cytochrome c and apoptosis inducing factor (AIF) from the mitochondria to the cytosol, which is the starting point for programmed cell death.

The above facts confirm the key role of Mg^{2+} in the regulation of mitochondrial function, including the control of their volume, composition of ions and ATP production.

Magnesium and calcium ion transport

Magnesium ions are important for maintaining cell homeostasis because they are essential to the stabilization of cell membranes, to the activation of sodium-potassium pump (Na-K-ATP-ase) or calcium pump (Ca-ATP-ase), and to the regulation of composition of intra- and extracellular liquid (HARTWIG 2001, COWAN 1995).

As calcium antagonist, magnesium increases the neuromuscular excitability and has an antispastic and anticonvulsive effect, impairing the contractibility of muscles.

As early as in in 1988, WHITE and HARTZELL showed that free intracellular magnesium can regulate the functioning of calcium channels. BARA and GUIET-BARA (2001) have confirmed that extracellular magnesium salts ($MgCl_2$ or, to a smaller extent, $MgSO_4$) reduce the influx of calcium through high-voltage channel Ca^{2+} type L in vascular smooth muscle cells (VSMCs) and vascular endothelial cells (VECs) of human plancenta (BARA, GUIET-BARA 2001), and consequently modulate the tonus of placental vessels. Mg^{2+} and GTP binding sites are assumed to reside in the intracellular C-terminal side of the a1 subunit of the channel. In basal conditions (i.e. the dephosphorylated channel and Mg^{2+} and GTP abundant on the intracellular side) Mg^{2+} and GTP binding to C-terminal inhibit the current conduction. A decrease in Mg^{2+} without intracellular GTP produces a current conducting state but

addition of GTP blocks the channel. Phosphorylation results in both Mg^{2+} and GTP blocks by unbinding these blocking substances through conformational change of the channel protein.

Serrano has described a similar blocking effect of extracellular magnesium on α1G T-type calcium channels, which play an important role in the mechanisms underlying thalamocortical oscillation (SERRANO et al. 2000). This is particularly essential because T channels are not blocked by classic calcium antagonists (except for mibefradil which is not used in clinical practice on account of undesirable action).

Whether or not Mg^{2+} ions modulate the action of store-operated calcium release-activated Ca^{2+} channels (CRAC), involved in regulation of inflammatory mediators production in allergic reactions as well as in differentiation and activation of T lymphocytes, is still not completely elucidated.

While the results of some experimental research have shown that intracellular magnesium modulates activity and selectivity of CRAC, others suggest that the channels regulated by intracellular Mg^{2+} are not CRAC channels but rather Mg-inhibited cation (MIC) channels that open as Mg^{2+} is washed out of the cytosol. MIC have been defined as another class of channels because they display different functional parameters from those displayed by CRAC in terms of inhibition (e.g. MIC are not blocked by SKF 96365 – the inhibitor of CRAC channels) or selectivity (unlike CRAC, MIC channels are permeable to Cs^+ ions; PCs/PNa = 0.13 vs. 1.2 for MIC) (PRAKRIYA, LEWIS 2000).

Studies carried out on rats with arterial hypertension have confirmed that extracellular Mg^{2+} imitates nifedipine in the process of reducing Ca^{2+} entry to vascular smooth muscle cells through store-operated channels (SOCs), resulting in the widening of circular vessels and a decrease in peripheral resistance as well as blood pressure (ZHANG et al. 2002).

Magnesium and potassium ion transport

Potassium channels play a crucial role in the regulation of membrane potential in smooth muscle cells and vascular tone.

As the equilibrium potential for potassium ions in vascular smooth muscle cells is more negative (-84 mV) than the cell's resting potential (-60 to -70 mV), the opening of potassium channels induces the K ion outflow from the cell. The loss of cations caused by an increase in the absolute value of membrane potential leads to the closing of L-type voltage-gated calcium channels (VGCC-L), to a decrease in intracellular calcium concentration as well as to relaxation of vessels. Blocking of potassium channels, however, lowers membrane potential, stimulates calcium ion inflow via voltage-gated ion channels (VDCC) and produces vessel contraction (BARANOWSKA et al. 2007).

TAMMARO et al. (2005) have provided evidence that intracellular Mg^{2+} ions affect voltage-dependent K channels (Kv), which regulate potassium ion dis-

tribution and cooperate with K_{Ca} channels in control of arterial vessels convolution in vascular smooth muscle cells. It was observed that an increase in the intracellular Mg^{2+} level slows down the K_V channel activation, causes inward rectification at positive membrane potentials and shifts voltage-dependent inactivation. The above results demonstrate that intracellular Mg^{2+} can act as a potent modulator of K_V channel in vascular smooth muscle cells, representing a novel mechanism for the regulation of K_V channel activity in the vasculature.

Cell magnesium also regulate the action of Ca^+-dependent K^+channels (BK_{Ca}), essential for modulating muscle contraction and neuronal activities such as synaptic transmission or hearinghttp://www.nature.com/nature/journal/v418/n6900/full/nature00941.html - B1 (SHI et al. 2002). Physiological activation of BK_{Ca} channels counteracts depolarization of cell membranes, contraction of blood vessels and increasing pressure (BARANOWSKA et al. 2007). Because of the importance of BK channels in neurotransmitter release and vascular tone, Mg^{2+} modulation of BK channels may play a substantial role in these pathophyisological processes. Mg^{2+} modulates their permeability by blocking the opening of a BK channel or by stimulation of channels independently from Ca^{2+} and voltage changes following binding to an open channel in different than Ca specific site or in no site (SHI et al. 2002). The structural separation between the binding site and the activation gate indicates that Mg^{2+} binding activates the channel by an allosteric mechanism; i.e., Mg^{2+} binding may cause a conformational change at the binding site that propagates to the activation gate for a channel opening (HUANGHE 2008).

Intracellular Mg^{2+} affects bioelectrical activity of the heart via regulation of inward rectifying potassium channels (K_{IR}), which are responsible for blocking outflow of K ions from cell and repolarization (BARANOWSKA et al. 2007).

Physiological concentrations of intracellular Mg-ADP complex regulate the sensitivity of ATP-sensitive potassium channels (K_{ATP}) to sulphonylurea derivatives. Sulphonylurea derivatives, used to treat type 2 diabetes, stimulate insulin secretion by blocking K_{ATP} channels in pancreatic β-cells. An intracellular Mg-ADP complex modulates sulphonylurea block, enhancing the inhibition of Kir6.2/SUR1 (β-cell type) and decreasing that of Kir6.2/SUR2A (cardiac-type) channels. This is important because the opening of K_{ATP} channels is regarded as an endogenous cardioprotective mechanism so the blocking effect of sulphonylurea derivatives in the cardiovascular system may have deleterious effects (REIMANN et al. 2003).

The influence of Mg^{2+} on K^+ channels is not limited to the cell membrane. BEDNARCZYK et al. (2005) have shown that matrix Mg^{2+} ions affect mitochondrial ATP-dependent potassium channel (K_{ATP}) in the heart, which plays a key role in protecting from ischemia/reperfusion. The ATP/Mg^{2+} complex inhibits K_{ATP} activity and free magnesium ions regulate both the channel conductance and open probability. Another study has suggested that mi-

toK_{ATP} channels make functional connection with mitochondrial pyruvate dehydrogenase forming a larger, multiprotein complex. A hypothesis has been formulated that enhanced activity of mitoK_{ATP} channel protects the heart muscle during myocardial ischemia as well as neurons, brain cells and skeletal muscle cells (SKALSKA et al. 2006).

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