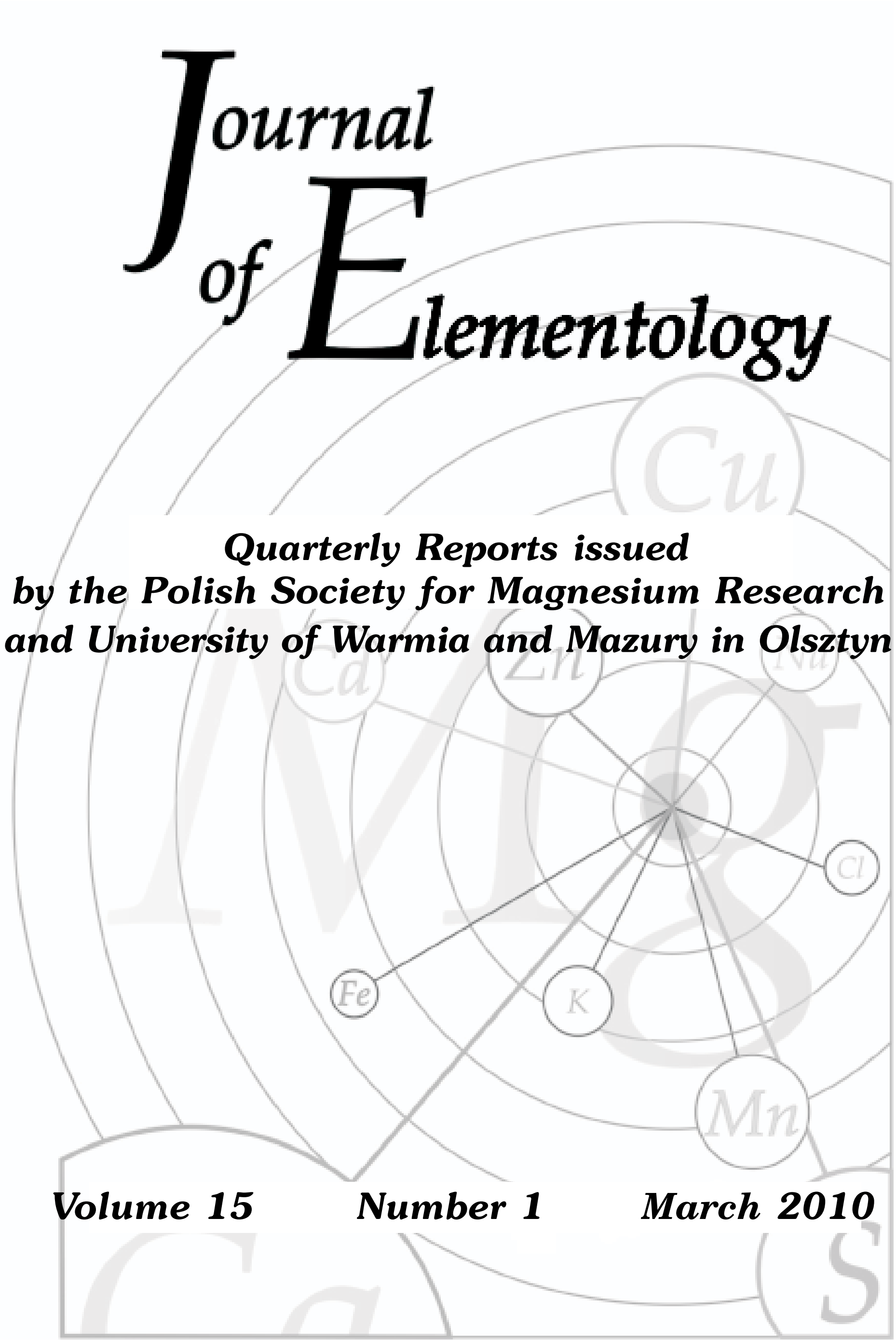

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University of Warmia and Mazury
Michała Oczapowskiego 8, 10-719 Olsztyn, Poland, phone: +48 089 5233231
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PLASMA CALCIUM AND MAGNESIUM CONCENTRATIONS IN PATIENTS WITH FRACTURES OF LONG BONES TREATED SURGICALLY

**Maysam Bacher, Małgorzata Sztanke,
Krzysztof Sztanke, Kazimierz Pasternak**

**Chair and Department of Medical Chemistry
Medical University of Lublin**

Abstract

Bone fractures, especially when treated surgically, are connected with changes in the homeostasis of the elements of which bone compounds consist, as well as the ones which participate in controlling processes. Such changes influence both the fracture itself and the operative procedure. For successful treatment of broken bones, it can be crucial to bring the homeostasis of bioelements back to the normal state.

Thus, the aim of this work has been to determine magnesium and calcium concentrations in plasma from patients with fractures of long bones, and to analyze changes in the examined elements depending on the time after the operative procedure, injury and patient's age.

The research was conducted on the blood of patients: 1) with long bone fractures treated surgically, 2) after a limb amputation because of a fracture or injury, 3) with long bone fractures and with multiorgan injury. With respect to the patients' age, there were 3 groups: 1) patients aged 18-40 years, 2) patients aged 41-60 years, 3) patients older than 60 years. In the postoperative period, the patients were on a strict postoperative diet, receiving only i.v. 0.9% NaCl and 5% glucose solutions.

Blood was taken from patients first directly after admittance to hospital and next on the first, third, fifth and seventh day after an operative procedure.

Plasma calcium and magnesium concentrations were determined by the method of emission spectrometry with induction plasma (ICP-OES). The results were worked out statistically. The differences $p<0.05$ were taken into consideration as statistically significant.

The research showed that in the blood plasma from patients with long bone fractures treated surgically, there were noticeable decreases of calcium and magnesium concentra-

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prof. dr hab. Kazimierz Pasternak, Chair and Department of Medical Chemistry Medical University of Lublin, Staszica 4, 20-081 Lublin, Poland, phone/fax 081 5357361, e-mail: kazimierz.pasternak@umlub.pl

tions mainly on the first, third and fifth postoperative day. These changes depended on the extend of an operative procedure, but did not depend on the patients' age. However, with respect to magnesium, its statistically significant decrease was noticed in the group of oldest patients.

In the postoperative period, it is especially important to pay attention to calcium and magnesium concentrations, and for patients over 60 years of age magnesium supplementation is necessary. Maintenance of macronutrient homeostasis in an organism can be crucial for the patient's convalescence process.

Key words: calcium, magnesium, long bone's fractures, surgical treatment.

OSOCZOWE STĘŻENIA WAPNIA I MAGNEZU U PACJENTÓW ZE ZŁAMANIAMI KOŚCI DŁUGICH LECZONYCH OPERACYJNIE

Abstrakt

Złamania kości, szczególnie leczone operacyjnie, wiążą się ze zmianami homeostazy pierwiastków wchodzących w skład związków budujących kości, a także biorących udział w procesach regulacyjnych. Wpływa na to zarówno samo złamanie, jak i zabieg operacyjny. Normalizacja zaburzeń w homeostazie biopierwiastków może mieć istotne znaczenie dla zrostu kości w procesie zdrowienia pacjenta ze złamaniem.

Celem pracy było oznaczenie stężeń magnezu i wapnia w osoczu krwi pacjentów hospitalizowanych z powodu złamań kości długich oraz analiza tych zmian w zależności od czasu po zabiegu operacyjnego leczenia złamania, rozległości urazu oraz wieku pacjenta.

Badania przeprowadzono na krwi pacjentów: 1) ze złamaniami kości długich leczonymi operacyjnie, 2) u których z powodu złamań i urazu konieczna była amputacja kończyny, 3) ze złamaniami kości długich, którym towarzyszył duży uraz wielonarządowy. Ze względu na wiek, pacjentów podzielono również na trzy grupy. Pierwszą grupę stanowiły osoby od 18. do 40. roku życia, drugą – od 41. do 60. roku życia, trzecią – powyżej 60. roku życia.

Od pacjentów pobierano krew bezpośrednio po przyjęciu do szpitala oraz w 1., 3., 5. i 7. dobie po zabiegu operacyjnym.

Oznaczenia stężeń pierwiastków w osoczu krwi wykonano metodą optycznej spektrometrii emisyjnej z plazmą indukcyjnie wzbudzoną (ICP-OES). Wyniki poddano analizie statystycznej. Za statystycznie istotne uznano różnice w przypadku $p < 0,05$.

Wykazano, że w osoczu pacjentów ze złamaniami kości długich leczonych operacyjnie stwierdzono zmniejszenie stężeń wapnia i magnezu głównie w 1., 3. i 5. dobie pooperacyjnej. Zmiany te zależały od rozległości zabiegu operacyjnego, a nie były zależne od wieku. Jednak w przypadku magnezu zaobserwowano statystycznie istotne zmniejszenie jego stężenia w grupie najstarszych pacjentów.

W okresie pooperacyjnym wskazane jest szczególne zwrócenie uwagi na stężenia wapnia i magnezu, a u pacjentów powyżej 60. roku życia konieczna jest suplementacja magnezu. Zapewnienie homeostazy makropierwiastków w organizmie może mieć wpływ na proces zdrowienia pacjenta.

Słowa kluczowe: wapń, magnez, złamania kości długich, leczenie operacyjne.

INTRODUCTION

Calcium makes up just 1.4-1.6% of body mass and it is mainly accumulated in the skeleton (99%). The remaining 1% of this element is in the blood plasma in three forms: in an ionized form – about 48%, bonded with proteins – 46%, in complexes with citrates and phosphates – 6%. Calcium salts are components of the skeleton and teeth, ensuring their proper resistance and hardness. It is calcium that many enzyme activity, muscle functions, humoural stimulus transmission, proper heart action, blood coagulability and iron absorption in organism depend on (Benech et al. 1998). Calcium is also an important component of cellular and intracellular membranes, which conditions their permeability. In order to provide adequate amounts and availability of calcium ions, an organism maintains the proper homeostasis, both at the cellular and systemic levels. Calcium deficiencies and disturbances in hormonal secretion processes, directly interfering with homeostasis of this element, are the causes for different pathologic syndromes. The main regulators of calcaemia are parathormone, vitamin D and calcitonin. Parathormone, which is synthesized by parathyroid glands, increases calcium release and its renal reabsorption, and it probably also enhances intestinal absorption of this element. Calcitonin decreases calcium release from the skeleton and increases its release by kidneys. Calcium stimulates the secretion of calcitonin. Active metabolites of vitamin D, especially 1,25-dihydroxycholecalciferol, increase intestinal absorption of calcium and its renal resorption (Kokot 2001, Lepage et al. 1998, Otto-Buczkowska 2002). According to Zdrojewicz and Belowska-Bień (2003), it is also a calcic receptor (CaR), which belongs to group C of receptors connected with protein G that plays an important role in the process of calcium homeostasis maintenance. This receptor is mainly situated on the surface of parathyroid glands, kidneys, intestines, bones and thyroid C cells, but also in tissues that are not connected with calcium metabolism, such as skin, the brain and the pancreas. Calcium receptor mutations are the reason for the whole family of existing disorders in calcium metabolism.

Main symptoms of reduced calcium concentration in blood, i.e. hypocalcaemia, are arrhythmia, intensified muscular excitability, anxiety, vertigo and stomachache. In the case of a prolonged calcium deficiency in an organism, processes intensifying its release from bones begin. If such a state lasts for a long time, then mechanical bone resistance decreases and bone decalcification is initiated. As a consequence, bones lose their resistance to injuries and the risk of fractures increases due to weaker bone structure and progressing osteoporosis. Prolonged calcium deficit can also cause dental caries, rickets and disorders in many other Processes (Allgrove 2003, Jakubas-Kwiatkowska et al. 2005, Takami, Iki 2005).

Magnesium also plays an important role in human physiology. It participates in many metabolic, regulatory and structural functions. Nowadays, it is claimed that this element plays a role as a cofactor of over three-hundred enzymes involved in biochemical reactions connected with storage and usage of energy, carbohydrate catabolism, protein and fat synthesis, membrane transport of sodium, potassium and calcium, proper nerve conduction and efficient functions of muscles, especially the cardiac muscle (AL GHAMDI et al. 1994, DROESTI 1995, 1991, MARCINKOWSKA-SUCHOWIERSKA 1991, PAPIERKOWSKI 2002).

Magnesium is also an element which is necessary for life. It is the second cation as regards the amount in a cell, and the fourth with respect to the amount in a whole organism (PASTERNAK 2000, TAKAMI, SHINNICHI 2005). There is about 1,000 mmol of magnesium in an adult human organism, out of which about 50-60% is found in bones. Next to calcium, magnesium is the main mineral component which is necessary for the proper formation of the skeletal structure (FORMANOWICZ, FORMANOWICZ 2004, ŁONIEWSKI 2003). On a cellular level, magnesium acts as an activator of many enzymes (NORONHA, MATUSCHAK 2002, PASTERNAK 2000, SARIS 2000, WOLF, CITTADINI 2003).

Magnesium homeostasis is supported by the state of balance in processes of supply, absorption, use and excretion of this element. The proper magnesium concentration in the plasma ranges from 0.7 to 1.2 mmol dm^{-3} (NORONHA, MATUSCHAK 2002), and owing to magnesium release from tissues, mainly bones, it is possible to keep it at this level even during high Mg deficiency. Thus, changes in magnesium concentration in the plasma do not inform us about its reserve in tissues, but can indicate magnesium metabolism (MARCINKOWSKA-SUCHOWIERSKA 1991, NORONHA, OGOŃSKI et al. 1997). Among clinical consequences of disturbed magnesium homeostasis, we can name arrhythmia and neurological disorders, both of which can cause higher mortality (ALLGROVE 2003, JOHNSON 2001, KOZIELEC, STAROBRAT-HEMERLIN 1998, PASTERNAK 2000). Hypomagnesaemia is most often caused by reduced magnesium supply with food, excessive excretion in urine, disturbances in its absorption in the alimentary tract and disorders in the distribution of this element in an organism. Clinical observations show that hypocalcaemia quite often accompanies hypomagnesaemia (OTTO-BUCZKOWSKA 2002).

Magnesium deficiency in an organism causes loss of muscular strength and inferior neuromuscular transmission, which leads to inferior movement coordination and a higher risk of a fall, which can be the main reason for bone fractures, especially fractures of forearm bones and the femoral bone. The risk of a fracture for women aged over 50 years is 39% (CZERWIŃSKI, BOROWY 2006). Insufficient magnesium supply to a human organism has been pointed recently as one of the risk factor for osteoporosis (GRAJETA 2003, KORKOSZ 2003, TAKAMI, IKI 2005). There are some mechanisms implied as responsible for depressing the skeletal mass when there is lack of magnesium in an organism. These are mainly mechanisms stimulated by nutrition and environmental conditions. Some research conducted on animals showed that magnesium deficiency causes inhibition of bone growth, acceleration of bone tissue resorption, decrease in bone capacity and increase in bone fragility (Korkosz 2003, Skibniewska et al. 1999).

Most elements in blood, especially calcium and magnesium ions, highly influence bone tissue metabolism. Bone is the kind of tissue where many metabolic processes continually occur. It is a live tissue, constantly changing into new one during reconstruction processes, which simultaneously take part in different places of the skeletal system (Galus 1994).

The skeletal system vulnerable to fractures, which are most often connected with injuries and the health state of this system. The extent of an injury, direction of the applied force and type of a bone are crucial for the type and extent of a resulting fracture. The structure of bones and the degree of bone saturation with both organic and inorganic compounds are an important factor that influences each bone's vulnerability and, at the same time, conditions its resistance. Bone fractures, especially the ones treated surgically, are connected with changes in the homeostasis of elements of which bone compounds consist as well as the ones which participate in controlling processes. Such changes influence both the fracture itself and the operative procedure. For successful treatment of broken bones, it can be crucial to bring the homeostasis of bioelements back to the normal state. Thus, the aim of this work has been to determine magnesium and calcium concentrations in the plasma from patients with long bone fractures and to analyze changes in the examined elements depending on the time after an operative procedure, extent of an injury and the patient's age.

MATERIAL AND METHODS

The research was conducted on blood samples taken from patients treated surgically because of long bone fractures in the Surgery Clinic of the Independent Public Clinical Hospital No1 in Lublin and in a hospital in Ryki. The research was conducted with the consent of the Ethics Board of the Medical Academy in Lublin (consent no KE-0254/102/2006).

The group of patients consisted of 51 people, 30 men and 21 women, of the average age 51.7±17.9 years. The patients were divided into 3 groups. The first group consisted of patients with long bone fractures treated surgically (34 patients); the second one of patients who had a limb amputated because of a fracture or injury (6 patients) and the third group comprised patients with long bone fractures and with multiorgan injury (11 patients). With respect of the patients' age, there were 3 groups distinguished from the research group: patients aged 18-40 years, aged 41-60 years and patients older than 60 years. In the postoperative period (two days), patients were on a strict postoperative diet, during which they only received intravenously 0.9% NaCl solution and 5% glucose solution. However, they did not receive any infusion solutions, which could have influenced magnesium and calcium concentrations.

Blood was taken from patients' elbow vein directly after admittance to hospital and then on the first, third, fifth and seventh day after an operative procedure. The blood was sampled directly to heparinized test tubes. Next, the sampled blood was centrifuged for 15 minutes, at the revolution of 3000xg, in a MPW type centrifuge. The plasma was separated and frozen at -20şC until the determinations.

Concentrations of the elements in the plasma were determined by emission spectrometry with induction plasma (ICP-OES), using a Liberty II model sequence spectrometer made by Varian Company. In this method, determination of a particular element was based on the measurement of the intensity of radiation emitted at a wavelength characteristic for a given element. For determination of calcium concentration the length 315.887 nm was applied, and for magnesium concentration the length 279.083 nm was used. The spectrometer was calibrated using multielement patterns by MERCK Company.

The results were worked out statistically with *Medistat 2.0* version programme. The results were analysed by t-Student test, and the comparison of groups of results was done on the basis of Cochran-Cox test. The differences $p<0.05$ were taken into consideration as statistically significant.

RESULTS AND DISCUSSION

Magesium and calcium concentrations in the tested groups of patients are presented in Figures 1-2 and Tables 1-3.

Plasma calcium and magnesium concentrations in patients with long bones' fractures are presented in Table 1.

The average calcium concentration in the analyzed postoperative periods did not differ from its concentration determined on admittance to hospital.

Similarly, magnesium concentration, although decreasing in the postoperative period (on the first, third and fifth day), did not show any statistically significant differences in comparison to its concentration determined on admittance to hospital.

The plasma calcium and magnesium concentrations in patients with long bone fractures who had a limb amputated are presented in Table 2.

In the analyzed patient group, the average calcium concentration fluctuated from 2.20 to 2.50 mmol dm^{-3}. The changes in the concentration of this element on the consecutive postoperative days were not statistically significant versus the concentration determined on admittance to hospital.

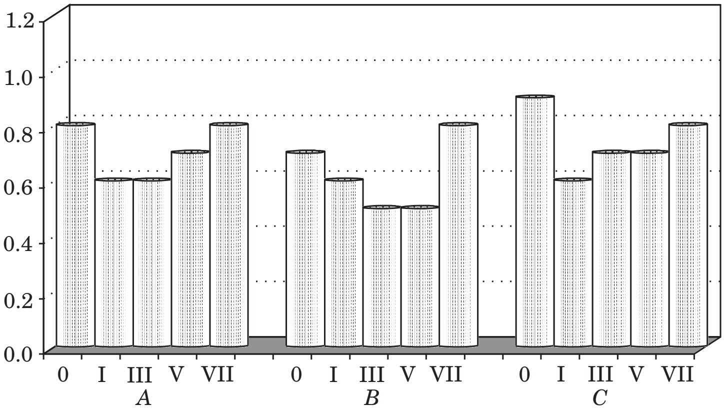

Fig. 1. Magesium concentrations (mmol·dm^{-3}) in tested groups of patients: *A* – patients with long bone fractures, *B* – patients with long bone fractures and limb amputation, *C* – patients with long bone fractures with extensive soft tissue injuries

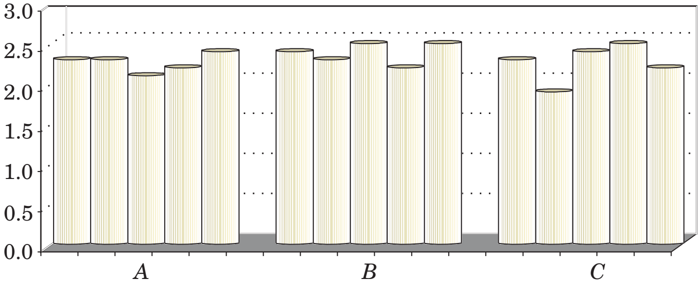

Fig. 2. Calcium concentrations (mmol·dm^{-3}) in tested groups of patients: *A* – patients with long bone fractures, *B* – patients with long bone fractures and limb amputation, *C* – patients with long bone fractures with extensive soft tissue injuries

Table 1

Plasma calcium and magnesium concentrations in patients with long bone fractures

Element (mmol dm^{-3})	Day				
	0	I	III	V	VII
	X±SD	*X*±SD	*X*±SD	*X*±SD	*X*±SD
Calcium	2.30±0.17	2.30±0.35	2.10±0.08↓	2.20±0.38↓	2.40±0.36↑
Magnesium	0.80±0.18	0.60±0.09↓	0.60±0.08↓	0.70±0.16↓	0.80±0.09

* – difference statistically important in comparison to its concentration on admittance to hospital

↑ – increase in comparison to its concentration on admittance to hospital

↓ – decrease in comparison to its concentration on admittance to hospital

Table 2

Plasma calcium and magnesium concentrations in patients with long bones fractures who had a limb amputated

Element (mmol dm–^{3})	Day				
	0	I	III	V	VII
	X±SD	X±SD	X±SD	X±SD	X±SD
Calcium	2.40±0.39	2.30±0.37↓	2.50±0.28↑	2.20±0.46↓	2.50±0.18↑
Magnesium	0.70±0.16	0.60±0.08↓	0.50±0.07*	0.50±0.17↓	0.80±0.08↑

* – difference statistically important in comparison to its concentration on admittance to hospital
↑ – increase in comparison to its concentration on admittance to hospital
↓ – decrease in comparison to its concentration on admittance to hospital

A decrease in the magnesium concentration relative its concentration on admittance to hospital was noticed on the first, on the third and on the fifth postoperative day. The lowest magnesium concentration was noticed on the third postoperative day, which was statistically significant as compared to the concentration of this element on admittance to hospital.

The plasma calcium and magnesium concentrations in patients with long bone fractures with extensive soft tissue injuries are presented in Table 3.

Table 3

Plasma calcium and magnesium concentrations in patients with long bones fractures with extensive soft tissue injuries

Element (mmol dm^{-3})	Day				
	0	I	III	V	VII
	X±SD	X±SD	X±SD	X±SD	X±SD
Calcium	2.30±0.47	1.90±0.26↓	2.40±0.37↑	2.50±0.40↑	2.20±0.48↓
Magnesium	0.90±0.16	0.60±0.07↓	0.70±0.18↓	0.70±0.08↓	0.80±0.16↓

* – difference statistically important in comparison to its concentration on admittance to hospital
↑ – increase in comparison to its concentration on admittance to hospital
↓ – decrease in comparison to its concentration on admittance to hospital

The average calcium concentration fell to the lowest value on the first postoperative day, when it was 1.90 mmol dm^{-3}. The changes in the calcium concentration on each analyzed postoperative day were not statistically significant, as compared to the concentrations determined on admittance to hospital.

Magnesium concentrations were low. The highest concentration of this element was noticed on admittance to hospital; afterwards it continued to decline down to 0.60-0.70 mmol dm^{-3}. These changes, however, were not significant in comparison to the magnesium concentration on admittance to hospital.

Calcium and magnesium concentrations for particular age groups are presented in Figures 3-4 and Tables 4-6.

Table 4

Calcium and magnesium concentrations in the youngest age group (18-40 years)

Element (mmol dm^{-3})	Day				
	0	I	III	V	VII
	X±SD	*X*±SD	*X*±SD	*X*±SD	*X*±SD
Calcium	2.30±0.25	2.10±0.16 ↓	2.00±0.17* ↓	2.20±0.27 ↓	2.20±0.29 ↓
Magnesium	0.90±0.17	0.60±0.07*	0.70±0.07 ↓	0.70±0.18 ↓	0.70±0.09 ↓

* – difference statistically important in comparison to its concentration on admittance to hospital

↑ – increase in comparison to its concentration on admittance to hospital

↓ – decrease in comparison to its concentration on admittance to hospital

Table 5

Calcium and magnesium concentrations in patients at the age from 41 to 60 years

Element (mmol dm^{-3})	Day				
	0	I	III	V	VII
	X±SD	*X*±SD	*X*±SD	*X*±SD	*X*±SD
Calcium	2.30±0.28	2.10±0.15 ↓	2.00±0.36 ↓	2.40±0.49 ↑	2.40±0.27 ↑
Magnesium	1.10±0.18	0.80±0.08 ↓	0.70±0.16 ↓	0.70±0.07 ↓	0.70±0.09 ↓

* – difference statistically important in comparison to its concentration on admittance to hospital

↑ – increase in comparison to its concentration on admittance to hospital

↓ – decrease in comparison to its concentration on admittance to hospital

Table 6

The concentrations of calcium and magnesium in the oldest age group (over 60 years)

Element (mmol dm^{-3})	Day				
	0	I	III	V	VII
	X±SD	*X*±SD	*X*±SD	*X*±SD	*X*±SD
Calcium	2.40±0.51	2.10±0.38 ↓	2.10±0.29 ↓	2.40±0.28	2.30±0.37 ↓
Magnesium	1.00±0.17	0.60±0.09*	0.60±0.07*	0.70±0.07*	0.80±0.16 ↓

* – difference statistically important in comparison to its concentration on admittance to hospital

↑ – increase in comparison to its concentration on admittance to hospital

↓ – decrease in comparison to its concentration on admittance to hospital

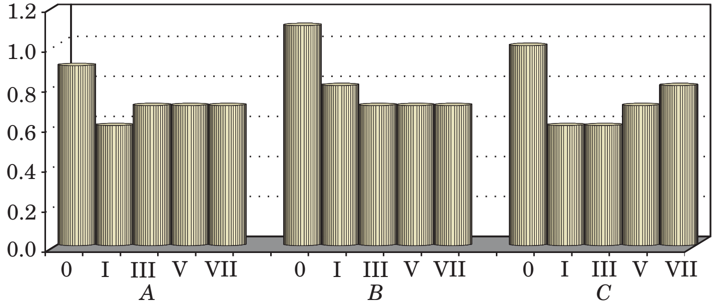

Fig. 3. Magnesium concentrations (mmol·dm^{-3}) for particular age groups of patients: *A* – patients at the age of 18–40 years, *B* – patients at the age of 41–60 years, *C* – patients at the age above 60 years

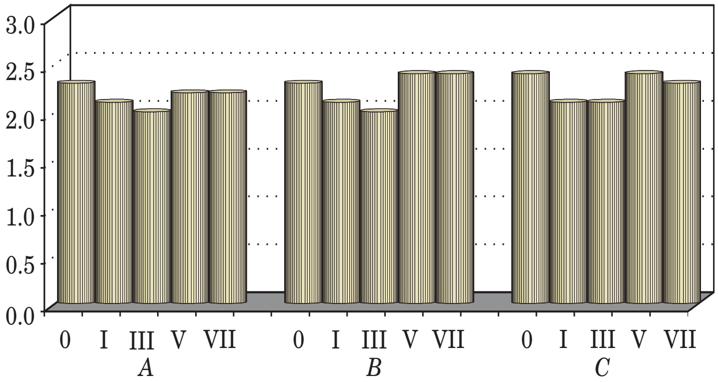

Fig. 4. Calcium concentrations (mmol·dm^{-3}) for particular age groups of patients: *A* – patients at the age of 18–40 years, *B* – patients at the age of 41–60 years, C – patients at the age above 60 years

Calcium and magnesium concentrations in the youngest age group (18-40 years) are presented in Table 4.

In the youngest group of patients (18-40 years), a statistically significant decrease, relative to the values determined on admittance to hospital, in the calcium concentration was noticed only on the first postoperative day.

Similarly, the magnesium concentration for this age group showed a very small decreasing tendency. The lowest concentration of this element was noticed on the first postoperative day, and it statistically significant as compared to its concentration on admittance to hospital.

Calcium and magnesium concentrations in patients 41 to 60 years old are presented in Table 5.

In this age group, a decrease in the calcium concentrations was noticed on the first and on the third postoperative day, but these changes were not statistically important as compared to the concentrations determined on admittance to hospital.

Magnesium concentrations for this age group, in comparison to the initial values, were lower on every postoperative day, but the differences were not statistically significant.

Calcium and magnesium concentrations in the oldest age group (over 60 years) are presented in Table 6.

In the oldest age group, calcium concentrations were the lowest on the first and on the third postoperative day, but the differences were not significant.

In this group, low magnesium concentrations were noticed. On every analyzed postoperative day, the concentrations of this element were lower than on admittance to hospital. On the first, third and fifth postoperative day, these differences were statistically important.

Calcium and magnesium are among the macroelements that play a very important role in both water-electrolyte balance maintenance and cell excitability (KOKOT, 2007). Therefore, much attention is paid to their concentration in any clinical research (KOKOT 1998, TOMASZEWSKI 2001). Disorders in concentrations of these elements can be caused by many factors, including operative procedures, which are connected with blood loss and systemic fluid depletion.

Magnesium concentration in the blood plasma analyzed on admittance to hospital was quite low, reaching particularly low concentrations in patients whose injuries were so extensive that they had a limb amputated. For these patients, the magnesium concentration was close to the low normal range and fell to the lowest value on the third postoperative day. This decrease was statistically significant as compared both to its concentration on admittance to hospital and to the normal values. Literature data support the results of this analysis, as there are reports on both low contents of magnesium in food (KOZIELEC, STAROBRAT-HEMERLIN 1998, KREINHOFF et al. 1990, PAPIERKOWSKI 2002) and on its loss in stressful situations (FLORIAŃCZYK 1997, PASTERNAK 2000). Due to the fact that magnesium is an intracellular cation, with only about 1% of its total content in an organism found extracellularly, the determination of its concentration can be only an indicator of magnesium metabolism. For the sake of maintaining normomagnesaemia, during a decrease in the magnesium concentration in the blood plasma, the element is released from tissues, mainly from bones, the liver or kidneys, so that hypomagnesaemia appears only when there is high deficiency of magnesium in tissues (PASTERNAK 2000, WALASEK 1998).

Moreover, loss of magnesium connected with a stressful situation, such as a fracture or an operative procedure, is very important for an organism. This claim is supported by other authors (PARTYKA, SITKOWSKA-RYSIAK 2001).

Worth attention is the fact that a statistically significant decrease in the magnesium concentration on the first, third and fifth postoperative day was revealed in the oldest group (over 60 years). It could have been caused by reduced ability to maintain magnesium homeostasis and higher susceptibility to stress. The results reported by other authors support the observation that the blood plasma of elderly people contains less magnesium (DURLACH 1991, FLORIAŃCZYK 1997, PASTERNAK 2000).

CONCLUSIONS

1. In the blood plasma from patients with bone fractures treated surgically, decrease in calcium and magnesium concentrations was determined on the first, third and fifth postoperative day.

2. The concentrations of the analyzed macroelements (calcium, magnesium) in the blood plasma after an operative procedure changed slightly.

3. The changes in the concentration of the macroelements did not depend on the patients' age, although for magnesium, a reduction in its concentration was noticed in the group of the oldest patients (over 60 years).

4. For patients over 60 years of age, magnesium supplementation seems necessary.

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DESORPTION OF PHOSPHATE (V) IONS FROM BROWN SOIL

Jadwiga Bartoszewicz, Elżbieta Karp

Chair of Chemistry
University of Warmia and Mazury in Olsztyn

Abstract

The objective of the study was to determine, under laboratory conditions, the rate of phosphate (V) ions leaching down brown soil horizons under the influence of redistilled water of pH 6.2. Laboratory tests were conducted to determine the effect of mineral and organic fertilization on phosphate (V) ion concentrations in brown soil horizons before and after extraction with water. The content of desorbed PO_4^{3-} ions was determined in percolating water samples. The results indicate that the quantity of desorbed phosphate (V) ions was affected by the type and rate of fertilization as well as by the dose of the applied solvent. The highest desorption of phosphate (V) ions from the brown soil profile was reported in the treatment fertilized with manure + PK, while the lowest desorption was observed in the plot fertilized with NPK. The maximum desorption of phosphate (V) ions was noted in soil layers at the depths of 0-25 cm and 26-50 cm. In all filtrate samples, PO_4^{3-} values significantly exceeded the minimum quantity required to initiate eutrophication. The highest content of phosphate (V) ions, at 64.8 mg $PO_4^{3-}\cdot kg^{-1}$ soil, was determined in percolating water from the treatment fertilized with slurry rate II (123.8 $t\cdot ha^{-1}$). Percolating water samples collected in the non-fertilized (control) plot were least abundant in phosphate (V) ions (21.7 mg $PO_4^{3-}\cdot kg^{-1}$ soil).

Key words: phosphorus, leaching, fertilization, soil.

WYMYWANIE JONÓW FOSFORANOWYCH (V) Z GLEBY BRUNATNEJ

Abstrakt

Celem pracy było zbadanie, w warunkach laboratoryjnych, intensywności przemieszczania się jonów fosforanowych (V) w głąb poszczególnych warstw profilu gleby brunatnej pod wpływem wody redestylowanej o pH=6,2. Zakres badań obejmował określenie wpływu nawożenia mineralno-organicznego na zawartość jonów fosforanowych (V) w poszczegól-

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prof. dr hab. Jadwiga Bartoszewicz, Chair of Chemistry, University of Warmia and Mazury in Olsztyn, pl. Łódzki 4, 10-957 Olsztyn, Poland, phone./fax: 89 523 35 25

nych warstwach gleby brunatnej przed i po ekstrakcji wodą. Oznaczono także ilość zdesorbowanych jonów PO_4^{3-} w zebranych wodach przesiąkowych. Stwierdzono, że ilość zdesorbowanych jonów fosforanowych (V) zależała od rodzaju i dawki nawożenia oraz ilości zastosowanego rozpuszczalnika. Największą desorpcję jonów fosforanowych (V) z profilu gleby brunatnej uzyskano z obiektu nawożonego obornikiem + PK, natomiast najmniejszą z obiektu nawożonego NPK. Maksimum desorpcji jonów fosforanowych (V) stwierdzono z warstw 0-25 cm i 26-50 cm. We wszystkich zebranych przesączach oznaczone wartości PO_4^{3-} przekraczały znacznie minimalną ilość potrzebną do zapoczątkowania eutrofizacji. Największą zawartość jonów fosforanowych (V), wynoszącą 64,8 mg $PO_4^{3-} \cdot kg^{-1}$ gleby, stwierdzono w wodach przesiąkowych pochodzących z obiektu nawożonego II dawką gnojowicy (123,8 $t \cdot ha^{-1}$). Najmniejszą ilość analizowanych jonów (21,7 mg $PO_4^{3-} \cdot kg^{-1}$gleby) oznaczono w wodach przesiąkowych zebranych z obiektu nie nawożonego (kontrolnego).

Słowa kluczowe: fosfor, wymywanie, nawożenie, gleba.

INTRODUCTION

The rapid growth in production and the mass scale application of phosphorus fertilizers in the 1960s and 1980s resulted in vast accumulation of phosphorus in soil and its migration into the environment (SAPEK 2001, GASSNER, GRZEBISZ 2003). The problem of excessive phosphorus deposition is also encountered in Poland, and it is of economic importance as it deteriorates the quality of water resources, which are already scant. Despite the high cost of water protection projects, water contamination with phosphorus was not abated in the following years (KOC, SKWIERAWSKI 2003). A detailed study investigating the significance of the sources of phosphorus migration was carried out in the European Union in the early 1990s, and it revealed that more than 50% of phosphorus found in surface waters of the EU countries originated from farming (GRZEBISZ, POTARZYCKI 2003). Surface leaching is the main factor responsible for phosphorus migration to surface water. Leaching down through the soil profile is also an important contributor to this process, in particular in sandy soils and substrates with large soil macropores (MCGECHAN 2003). For this reason, any convenient and economically viable method of long-term fertilization requires effective phosphorus immobilization to ensure that the nutrient requirements of crops are met but phosphorus desorption from the soil under the influence of atmospheric precipitation is prevented. The above is also an important consideration in the prevention of groundwater eutrophication (MOSKAL et al. 1999, SAPEK, URBANIAK 2001).

The objective of the study was to determine, in laboratory conditions, the rate of phosphate ion (V) leaching down through brown soil horizons under the influence of redistilled water, and to estimate the effect of varied mineral and organic fertilization on the leaching of PO_4^{3-} ions away from brown soil.

MATERIALS AND METHODS

Soil samples were collected after the 2003 harvest from experimental plots established in 1972 by the Chair of Environmental Chemistry, at the Experimental Station in Tomaszkowo near Olsztyn. They comprised brown soil developed from slightly loamy sand, of good rye complex and quality class IVb. The rates of organic fertilizers were set based on the total nitrogen and organic carbon content determined every year prior to their application. Three fertilization systems were compared in the experiment: organic, mineral and combined organic and mineral.

Eight fertilizer combinations were applied in the study: no fertilization, slurry rate I, slurry rate I + PK, slurry rate II, slurry rate II + PK, manure, manure + PK, NPK. Pig manure and slurry were used as organic fertilizers. Slurry was administered at two rates: rate I – equivalent to the manure rate as regards the amount of nitrogen introduced into the soil, and rate II – equivalent to the manure rate as regards the amount of carbon introduced into the soil. Organic fertilizers were supplemented with mineral (phosphorus and potassium) fertilizers at half the rate applied in the treatment fertilized with NPK. The nitrogen content of mineral fertilizers corresponded to the nitrogen content of manure and slurry rate I. The levels of phosphorus and potassium fertilization were adjusted so as to meet the nutrient requirements of particular crop species (SĄDEJ 2000). Soil texture (size fractions) was determined by the method suggested by Pruszyński (Table 1).

Table 1

Soil size fractions according to Pruszyński's method

Brown soil							
Soil horizon (cm)	fraction group*	percentage content of soil fractions, in mm					
		1.0-0.1	0.1-0.05	0.05-0.02	0.02-0.005	0.005-0.002	< 0.002
0-25	sls	55	32	6	3	3	1
26-50	lss	50	33	10	3	2	2
51-75	ssl	45	40	9	2	3	1
76-100	mhl	44	39	9	3	2	3

*Explanatioms: sls – slightly loamy sand, lss – loose silty sand, ssl – silty sandy loam, mhl – medium-heavy loam

Because of the low mobility of phosphate (V) ions, their migration into the soil profile to a depth of 1 m was investigated in this study. Soil samples were collected from four horizons: 0-25 cm, 26-50 cm, 51-75 cm and 76--100 cm. The samples were air-dried, purified of plant debris, sifted through a 1 mm mesh sieve and placed in glass columns 93 cm high and 5 cm in internal diameter, with a water-permeable layer of silica sinter at the bottom. The soil's original structure was preserved while filling the columns to create a natural testing environment. Every column was filled with layers of soil to a height of 80 cm (20 cm for every horizon: 0-25; 26-50, 51-75 and 76-100). The weight of soil samples was 1,760 g on average in each column. Redistilled water 6.2 in pH, stored in a corked bottle with CaO, was passed through the soil layers in columns in order to moisten them. The water had similar properties to rain water. On successive days of the experiment, filtrate samples (200 ml on average) were collected in a controlled environment and analyzed by molybdate and vanadate photoelectric colorimetry to determine phosphate (V) ion concentrations. The average total volume of percolating water samples from each column was 6.5 dm^3. The samples were preserved with toluene and refrigerated until analysis. The content of phosphate (V) ions was determined by the Egner-Rhiem method in every soil layer, for every fertilization system, before and after extraction with water.

The results were processed statistically by correlation analysis, linear regression analysis and the relevant parametric significance tests. The significance of correlations was determined with the use of Student's t-test. Correlation coefficients were compared with a normal distribution test. Regression functions were compared by Fisher F-test. The formulated hypotheses were verified at a significance level of α=0.05. Statistical calculations and data presentations were performed with Statistica PL and Excel applications.

RESULTS AND DISCUSSION

The effect of multi-year fertilization on phosphate (V) ion concentrations in each brown soil horizon is presented in Figure 1. The observed values were compared following desorption with water with pH of 6.2 (Fig. 1b). Among the treatments with equal amounts of nitrogen introduced to soil, the highest levels of phosphate (V) ions were noticed for the treatment fertilized with manure. The reported values were 1.5-fold lower than in the NPK-fertilized plot. The highest accumulation of the analyzed ions in the manure-fertilized treatment was observed in the ploughed layer (482 mg $PO_4^{3-} \cdot kg^{-1}$ soil) and in the subsoil horizon (442 mg $PO_4^{3-} \cdot kg^{-1}$ soil). In the plots fertilized with slurry rate I and NPK, the highest phosphate (V) ion content was observed in the ploughed layer (436.8 and 403 mg $PO_4^{3-} \cdot kg^{-1}$ soil, respectively). Similar results were reported by Grzebisz et al. (1992), Strączyńska (1998), Koper et al. (2002), Wacławowicz (2002), Sienkiewicz (2003), Zimny, Kuc (2005), Kalebasa et al. (2005) and Toor et al. (2005). The passage of water caused the highest desorption of PO_4^{3-} ions in the treatment ferti-

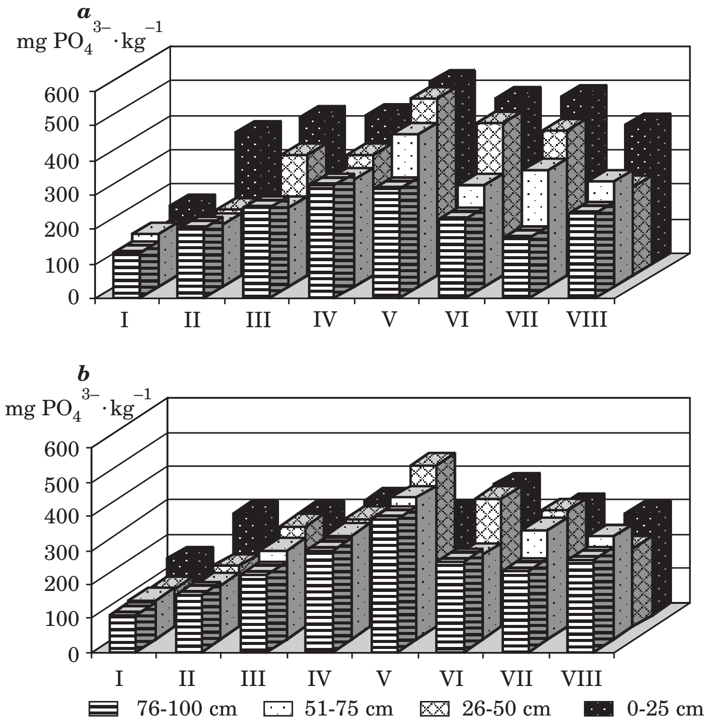

Fig. 1. Phosphate (V) ion concentrations in brown soil: *a* – before desorption; *b* – after desorption with water, I – control, II – slurry rate I, III – slurry rate I + PK, IV – slurry rate II, V – slurry rate II +PK, VI – manure, VII – manure + PK, VIII – NPK

lized with slurry rate I (213 mg $PO_4^{3-} \cdot kg^{-1}$ soil), corresponding to 21% of the total phosphate (V) ion content of the entire soil profile. The analyzed ions were leached out from each horizon, mostly from the ploughed layer (32.52%) and the 76-100 cm horizon (17.76%) – Table 2. In the remaining plots, the quantity of phosphate (V) ions desorbed from the entire soil profile was 12% in the manure-fertilized treatment and 9% in the NPK-fertilized treatment. In the plot with mineral fertilization, the highest quantity of phosphate (V) ions was leached out from the ploughed layer and from the subsoil horizon. Ion sorption in the 76-100 cm horizon reached 10.44% PO_4^{3-}.

The results indicate that smaller quantities of phosphate (V) ions were leached out by distilled water from brown soil with mineral fertilization (NPK), compared to plots fertilized with slurry rate I and manure. The observations made by other authors confirm that the rate of translocation into deeper horizons of the soil profile is higher in respect of phosphate (V) ions from organic than from mineral fertilizers (WITTHON et al. 1991, MOSKAL et al. 1999, FROSSARD et al. 2000, HOODA et al. 2001, SAPEK, URBANIAK 2001, TOOR et al. 2004, MARSHALL, LABOSKI 2006).

Table 2

Phosphate (V) ion desorption from brown soil fertilized with slurry rate I, slurry rate I + PK, slurry rate II, slurry rate II + PK, manure, manure + PK

Soil horizon (cm)	Control	Slurry rate I	Slurry rate I + PK	Slurry rate II	Slurry rate II + PK	Manure	Manure + PK	NPK
		PO_4^{3-} desorption (%)						
0-25	33.33	32.52	30.82	22.67	38.03	21.11	34.35	26.58
26-50	13.24*	6.99	15.65	8.85	7.83	14.55	18.53	10.47
51-75	10.89*	12.03	15.98*	2.15	6.34	15.91	5.14	0.44
76-100	-	17.76	13.27	10.16	23.31*	16.47*	36.92*	10.44*

*PO_4^{3-} sorption (%)

The treatments fertilized with slurry rate II and manure, equivalent in terms of the amount of carbon introduced into soil, were characterized by a similar content of phosphate (V) ions throughout the entire soil profile, which reached 355 mg $PO_4^{3-} \cdot kg^{-1}$ soil in the plot fertilized with slurry rate II and 361 mg $PO_4^{3-} \cdot kg^{-1}$ soil in the manure-fertilized plot. In comparison with soil fertilized with slurry rate II, the concentrations of the analyzed ions in the manure-fertilized treatment were higher by 50.9 mg $PO_4^{3-} \cdot kg^{-1}$ soil in the ploughed layer and by 93.8 mg $PO_4^{3-} \cdot kg^{-1}$ soil in the subsoil horizon. Soil fertilized with slurry rate II contained more phosphate (V) ions than the manure-fertilized plot, by 17 mg $PO_4^{3-} \cdot kg^{-1}$ soil in the 51-75 cm horizon and by 101.8 mg $PO_4^{3-} \cdot kg^{-1}$ soil in the deepest (76-100 cm) horizon. This suggests that the migration of phosphate (V) ions into the deepest horizon below the depth of 100 cm is higher in soils fertilized with slurry rate II than in manure-fertilized soils. The above could be attributed to the fact that slurry has an average 97% water content. Similar results were reported by KOPER (1994) and VADAS (2006). The use of distilled water resulted in the desorption of phosphate (V) ions in the first three horizons of soil fertilized with manure, while in the deepest horizon, ion sorption reached 37 mg $PO_4^{3-} \cdot kg^{-1}$ soil (16.47%). PO_4^{3-} ions were leached out from all horizons in plots fertilized with slurry rate II.

Slurry rate II increased phosphate (V) ion concentrations in the entire brown soil profile by an average of 30% in comparison with treatments fertilized with slurry rate I. In the ploughed layer, the quantity of phosphate (V) ions reached 436 mg $PO_4^{3-} \cdot kg^{-1}$ soil after the application of slurry rate I and 431 mg $PO_4^{3-} \cdot kg^{-1}$ soil after the application of slurry rate II. In the subsoil fertilized with slurry rate II, the content of the analyzed ions increased by 156 mg $PO_4^{3-} \cdot kg^{-1}$ soil. In the two remaining horizons (51-75 cm and 76-100 cm), phosphate (V) ion levels increased by 130 mg $PO_4^{3-} \cdot kg^{-1}$ soil on average in comparison with plots fertilized with slurry rate I.

An average of 11% phosphate (V) ions from the entire soil profile was desorbed with distilled water in the plot fertilized with slurry rate II, and 21% in the treatment fertilized with slurry rate I. PO_4^{3-} desorption in the 51-75 cm horizon fertilized with slurry rate II was five-fold lower than in the same horizon fertilized with slurry rate I.

The application of supplementary phosphorus-potassium fertilizers at half the NPK rate increased phosphate (V) ion concentrations throughout the entire brown soil profile fertilized with slurry rate I and II, by 64 and 81.7 mg $PO_4^{3-} \cdot kg^{-1}$ respectively. In soil fertilized with slurry rate I, supplementary PK fertilization increased the levels of phosphate (V) ions by 159 mg $PO_4^{3-} \cdot kg^{-1}$ soil in the 26-50 cm horizon, by 48 mg $PO_4^{3-} \cdot kg^{-1}$ soil in the 51-75 cm horizon, and by 58 mg $PO_4^{3-} \cdot kg^{-1}$ soil in the 76-100 cm horizon. In soil fertilized with slurry rate II, PK supplementation increased phosphate (V) ion concentrations in the above three horizons by 44, 164.8 and 131 mg $PO_4^{3-} \cdot kg^{-1}$ soil, respectively. PK supplementary fertilization did not increase the PO_4^{3-} content of manure-fertilized brown soil.

The concentrations of phosphate (V) ions in percolating water samples from each brown soil plot are presented in Figure 2. These data indicate that the average content of phosphate (V) ions in the total volume of filtrates (6.5 dm^3) collected in fertilized treatments after the use of water was twice as high as in the filtrate from the control treatment. The phosphate (V) ion content of filtrate samples obtained from fertilized plots increased with the quantity of water used. The above points to the possibility of increased PO_4^{3-} desorption in fertilized soil even when water volume exceeds 6.5 dm^3.

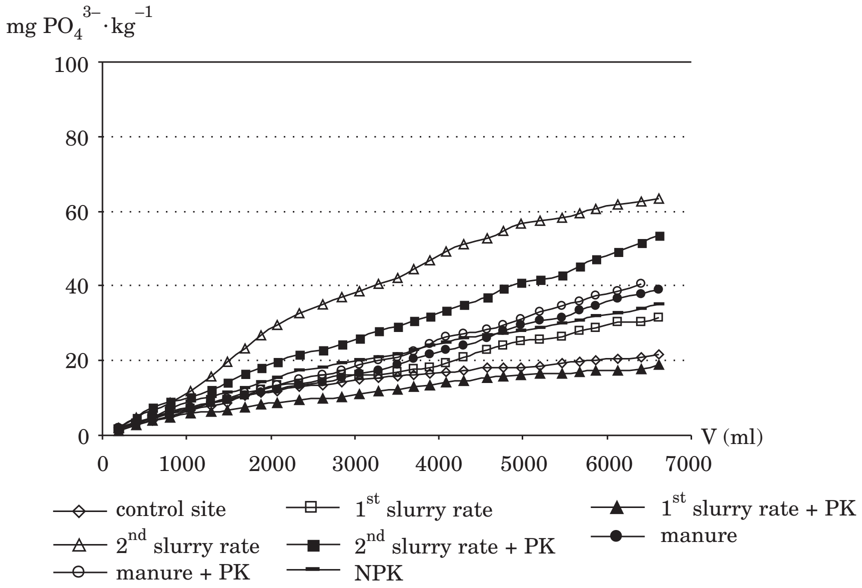

Fig. 2. Phosphate (V) ion concentrations in filtrate samples from brown soil

Table 3

The effect of soil horizon (measurement depth) on phosphate ion concentrations before and after desorption with water
Evaluation of correlations (correlation analysis and linear regression analysis) in experimental plots

Plot	Collection	Parameter		Correlation		Regression function $y = a + b \cdot x$	
		x	s	r	p	a	b
I control	before	314.23	67.11	-0.713	0.047	349.55	-1.583
	water	295.47	11.28	0.208	0.621	291.53	0.078
II 1st slurry rate	before	252.59	114.14	-0.753	0.031	396.80	-2.842
	water	199.33	59.51	-0.816	0.014	280.78	-1.605
III 1st slurry rate + PK	before	316.58	83.19	-0.886	0.003	440.21	-2.436
	water	270.35	29.96	-0.932	0.001	317.21	-0.923
IV 2nd slurry rate	before	355.44	48.91	-0.840	0.009	424.36	-1.358
	water	313.23	15.03	-0.992	<0.001	338.25	-0.493
V 2nd slurry rate + PK	before	437.18	79.18	-0.822	0.012	546.41	-2.152
	water	393.29	68.79	0.404	0.320	346.58	0.920
VI manure	before	361.80	111.43	-0.976	<0.001	544.31	-3.596
	water	317.92	65.93	-0.861	0.006	413.19	-1.877
VII manure + PK	before	354.10	123.83	-0.972	<0.001	556.13	-3.981
	water	304.85	42.13	-0.724	0.042	356.04	-1.009
VIII NPK	before	301.50	67.14	-0.773	0.024	388.63	-1.717
	water	274.03	30.64	-0.038	0.929	275.97	-0.038

The highest content of phosphate (V) ions, 64.8 mg $PO_4^{3-} \cdot kg^{-1}$ soil, was determined in the total volume of percolating water (6.5 dm^3) samples collected in the plot fertilized with slurry rate II. The lowest concentrations of the analyzed ions (21.7 mg $PO_4^{3-} \cdot kg^{-1}$ soil) were observed in the control treatment. In filtrate samples (total volume of 6.3 dm^3) collected from plots fertilized with manure, slurry rate I and mineral fertilizers, equivalent in terms of the amount of nitrogen introduced into soil, the highest concentrations of phosphate (V) ions were desorbed in manure-fertilized soil (36.5 mg $PO_4^{3-} \cdot kg^{-1}$ soil), followed by treatments fertilized with NPK (33 mg $PO_4^{3-} \cdot kg^{-1}$ soil) and slurry rate I (30 mg $PO_4^{3-} \cdot kg^{-1}$ soil). The desorption of phosphate (V) ions reached 50.8 mg $PO_4^{3-} \cdot kg^{-1}$ soil in the NPK-fertilized plot, and 41 mg $PO_4^{3-} \cdot kg^{-1}$ soil in the plot fertilized with slurry rate I. In treatments fertilized with slurry rate II and manure, equivalent with respect to the amount of carbon introduced into soil, the highest phosphate

Table 4

Phosphate ion concentrations in filtrate samples after desorption with water
Evaluation of correlations (correlation analysis) in experimental plots

Plot	Water	
	r	*p*
I = control	0.968	<0.001
II = 1st slurry rate	0.995	<0.001
III = 1st slurry rate + PK	0.992	<0.001
IV = 2nd slurry rate	0.988	<0.001
V = 2nd slurry rate + PK	0.999	<0.001
VI = manure	0.996	<0.001
VII = manure + PK	0.998	<0.001
VIII = NPK	0.993	<0.001

*if $p \leq 0.05$ ($p \leq 0.10$), then regression functions are not parallel

(V) ion concentrations were noticed in filtrate samples from the plot fertilized with slurry rate II, being 60% higher in comparison with the manure-fertilized treatment. The content of PO_4^{3-} ions in filtrate samples from the plots fertilized with manure and with slurry rate II increased with the amount of water used (pH=6.2).

The content of phosphate (V) ions determined in the total volume of filtrate (6.5 dm^3 on average) samples collected in plots fertilized with two different slurry rates was on average twice as high in the plot fertilized with slurry rate II as in the treatment fertilized with slurry rate I. Phosphate (V) ion concentrations in filtrate samples collected from soil fertilized with slurry rate II increased along with an increase in the volume of water to an average of 5 dm^3. The above points to the continual activation and leaching out of PO_4^{3-} ions from plots fertilized with slurry rate II. The content of phosphate (V) ions was lower in filtrate samples collected in the plot with phosphorus-potassium fertilization.

The results, including mean (x) and standard deviation (s) values, obtained for fertilized brown soil plots before and after desorption with water are presented in Table 3. The table presents the correlations (r) between phosphate (V) ion concentrations in each layer and the soil horizon (measurement depth), and the significance (p) of the determined correlations. The parameters of a simple regression function were also determined. As shown by the presented data, most of the correlations are statistically significant ($p \leq 0.05$). Table 4 presents the correlations between phosphate (V) ion concentrations in filtrate samples after desorption with water and the quantity of water used. All correlation coefficients were found to be statistically significant.

CONCLUSIONS

1. Long-term mineral and organic fertilization leads to increased accumulation of phosphate (V) ions in brown soil. The highest ion concentrations were determined in plots fertilized with slurry rate II + PK, manure, and manure + PK.

2. The highest desorption of phosphate (V) ions with water was noticed in brown soil fertilized with manure + PK, and the lowest – in the NPK-fertilized treatment.

3. The maximum desorption of phosphate (V) ions with water was observed in soil horizons at the depths of 0-25 cm and 26-50 cm.

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THE INFLUENCE OF PREGNANCY AND LACTATION ON THE MAGNESIUM AND CALCIUM CONCENTRATION IN GOATS' BLOOD SERUM

Maria Brzezińska, Monika Krawczyk

Chair of Physiology
University of Szczecin

Abstract

The aim of this work was to trace differences in magnesium and calcium concentrations (both total and ionic form) between goats which were in late pregnancy and lactating goats.

The study involved 30 goats. Blood samples were taken three times from each goat. Total concentrations of magnesium and calcium were determined with the colorimetric method and (A – 1.581 mmol dm^{-3}; B – 2.052 mmol dm^{-3}; C – 2.112 mmol dm^{-3}). Ionic form of calcium based on using ion–selective analysis (A – 1.219 mmol dm^{-3}; B – 1.126 mmol dm^{-3}; C – 1.123 mmol dm^{-3}).

The study showed that mean value of both the total of calcium from each goat did not reach the reference level for this species. Ionic form of calcium was within the lever limit of the physiological norm.

The content of magnesium in all the groups was within the range of reference concentrations (A – 1.051 mmol dm^{-3}; B – 1.165 mmol dm^{-3}; C – 1.117 mmol dm^{-3}).

Key words: goats, pregnancy, lactation, macroelements.

WPŁYW CIĄŻY I LAKTACJI KÓZ NA STĘŻENIE MAGNEZU I WAPNIA W SUROWICY

Abstrakt

W pracy prześledzono różnice stężeń magnezu i wapnia (całkowitego i zjonizowanego) między kozami będącymi w ciąży a kozami laktującymi.

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dr hab. Maria Brzezińska, prof. US, Chair of Physiology, University of Szczecin al. Piastów 40 B, bl. 6, 71-065 Szczecin, Poland, phone (91) 444-27-94

Badaniem objęto 30 kóz. Krew od zwierząt pobierano 3-krotnie. Stężenie magnezu i wapnia całkowitego oznaczono metodą kolorymetryczną, natomiast stężenie wapnia zjonizowanego – metodą jonoselektywną.

Stwierdzono, że średnia zawartość wapnia całkowitego w badanych grupach kóz nie osiągnęła norm referencyjnych dla tego gatunku (A – 1,581 mmol dm^{-3}, B – 2,052 mmol dm^{-3}, C – 2,112 mmol dm^{-3}). Poziom zjonizowanych form wapnia był korzystniejszy u wszystkich objętych badaniem kóz, choć jego wartości znajdowały się w dolnych granicach referencyjnych (A – 1,219 mmol dm^{-3}, B – 1,126 mmol dm^{-3}, C – 1,123 mmol dm^{-3}). Wyniki stężeń wapnia w surowicy krwi nie zawsze stanowią dobre odzwierciedlenie stopnia pokrycia zapotrzebowania zwierząt na ten pierwiastek, ponieważ podlega on stosunkowo precyzyjnej regulacji homeostatycznej. Dlatego optymalny poziom Ca w surowicy krwi nie jest równoznaczny z dostateczną jego ilością w organizmie. Niższy od norm świadczy jednak o jego niedoborze.

Poziom magnezu u wszystkich badanych kóz podczas całego okresu doświadczenia mieścił się w normie (A – 1,051 mmol dm^{-3}, B – 1,165 mmol dm^{-3}, C – 1,117 mmol dm^{-3}), co może świadczyć o dostatecznym doborze pokarmu pod względem zapotrzebowania zwierząt w ten makroelement.

Słowa kluczowe: kozy, ciąża, laktacja, makroelementy.

INTRODUCTION

Magnesium is the fourth most common and the second (next to potassium) most abundant intracellular cation in organisms (PASTERNAK 1999). This element plays an important role in the regulation of many significant biological processes, and especially in enzymatic reactions. Magnesium balance is maintained in the organism via the intestinal absorption processes and precisely controlled renal excretion. Magnesium absorption from food in the alimentary tract is very low in comparison with absorption of other elements. Magnesium absorbed in the blood in the form of Mg^{2+} enters a pool from which it is later accumulated in bones and other tissues. Under proper conditions, part of magnesium assimilated by tissues returns to the blood pool, from which it is excreted through the intestine with faeces (so-called endogenous magnesium) and through the kidneys with urine. A considerable amount of magnesium absorbed from food and released from tissues into the blood is excreted together with milk during intensive lactation, which predisposes lactating females to hypomagnesaemia. Determination of magnesium requirement is difficult and raises many objections. It depends mainly on the amount of magnesium absorbed in the alimentary tract and excreted with urine. Magnesium requirement increases, for example, during pregnancy and lactation (GABRYSZUK 1992).).

Magnesium ions are physiological antagonists of calcium ions. Calcium in organisms can be divided into two pools: an easily exchangeable calcium pool and a much bigger pool of non-exchangeable calcium. The former is responsible for the regulation of Ca^{2+}concentration in the blood plasma, the latter – for the resorption of calcium from bones and its re-incorporation.

Calcium stored in the bone tissue is an important reserve used in times of deficiency and that is why in physiological conditions its concentration in the blood remains at a steady level, because whenever calcium in food is deficient, it permeates from the bones into the blood. From 30% to 80% of calcium provided with food is absorbed. Calcium is absorbed during the active transportation of Ca^{2+} ions in the upper part of the small intestine, where there is also some calcium absorption through passive diffusion. Calcium absorption is adjusted to the organism's requirements. It increases when there is a deficiency of Ca^{2+} ions, and decreases when there are too many of these ions (BARANOW-BARANOWSKI et al. 2001). Regarding calcium requirements of animals, their physicological state should be considered. During pregnancy and lactation, for example, calcium requirement is high. Calcium requirement grows rapidly right after the delivery, which is connected with the excretion of considerable amounts of this element with milk (GABRYSZUK 1992).

This paper aims at the determination of differences in magnesium and calcium (total and ionized) concentration in pregnant goats and lactating goats maintained on the same feeding regime. Another objective was to compare and interpret the results for the purpose of their application in breeding.

MATERIALS AND RESEARCH METHODOLOGY

The research was carried out on 30 Saanen goats form a private farm in Świerznica near Rąbin. All of the animals were clinically healthy, kept under identical hygienic conditions and fed identical fodder doses (oats – 0.7 kg/a goat/a day, hay – *ad libitum*, 20% high-protein concentrate for goats – 0.21 kg/a goat). The research material was the venous blood which was taken three times:

group 1 – blood taken on 16.12. 2002 – pregnant goats, the second half of pregnancy,

group 2 – blood taken on 06.02.2003 – goats in the initial lactation period,

group 3 – blood taken on 02.04.2003 – goats in the advanced lactation period.

The following biochemical marking was carried out in the blood serum:

1) the colorimetric analysis without deproteinization was used to determine the total magnesium concentration; Bio-Merieux reagents and calmagite EGTA as an indicator were applied;
2) the colorimetric analysis without deproteinization was also used for determination of the total calcium concentration; Bio-Merieux reagents and methylene blue as an indicator were applied;
3) ionized calcium concentration was marked by means of the ion-selective method with the application of the electrolyte analyser AVL 9180.

Table 1 and Figures 1-3 present the results of the research: arithmetic means and standard deviations as well as the results of a comparison of the arithmetical means obtained with an aid of Statistica 6.0 software.

Table 1

Magnesium and calcium concentration in the blood serum

Group	Total magnesium (mmol dm^{-3})			Total calcium (mmol dm^{-3})			Ionized calcium (mmol dm^{-3})		
	1	2	3	1	2	3	1	2	3
N	10	10	10	10	10	10	10	10	10
X	1.051	1.165	1.117	1.581	2.052	2.112	1.219	1.126	1.123
SD	0.23	0.17	0.03	0.69	0.22	0.32	0.05	0.07	0.06
Maximum	1.52	1.42	1.16	3.14	2.44	2.56	1.31	1.21	1.25
Minimum	0.70	0.85	1.08	0.69	1.78	1.65	1.13	1.02	1.04
Reference value	0.74-1.62			2.20-3.05			1.12-1.32		
Statistically significant differences	–			1:2 ->0.01 1:3 ->0.01			1:2-> 0.01 1:3-> 0.01		

N – number of subjects, *X* – arithmetic mean, SD – standard deviation, 1 – goats in advanced pregnancy, 2 – goats in initial lactation period, 3 – goats in advanced lactation period

RESULTS AND DISCUSSION

In this research, magnesium concentration in the blood serum in all of the tested goat groups was within the standard limits accepted for the species; it is presented in Table 1 and Figure 1. In the first test, the average magnesium concentration was 1.051 mmol dm^{-3}, in the second test: 1.165 mmol dm^{-3}, and in the third test: 1.117 mmol dm^{-3}. There were no statistically significant differences between the average magnesium concentrations during comparable testing periods. It may be assumed that the goats' physiological state had little influence on the level of magnesium in the blood serum, which was probably owing to the proper feeding during the entire pregnancy and in the preceding period. The assessment of magnesium requirement on the basis of its content in fodder is possible only conditionally, since it is the effective use of magnesium that plays a decisive role in this respect. Magnesium absorption can be lower when there is too much K^+ (KRUCZYŃSKA, MOCEK 1997, BARANOW-BARANOWSKI et al. 2001). Magnesium requirement increases, for instance, during pregnancy and lactation. Magnesium deficiency can occur in ruminants fed with large amounts of low magnesium fodder or with fodder containing much of magnesium antagonists (KANIA 1998).

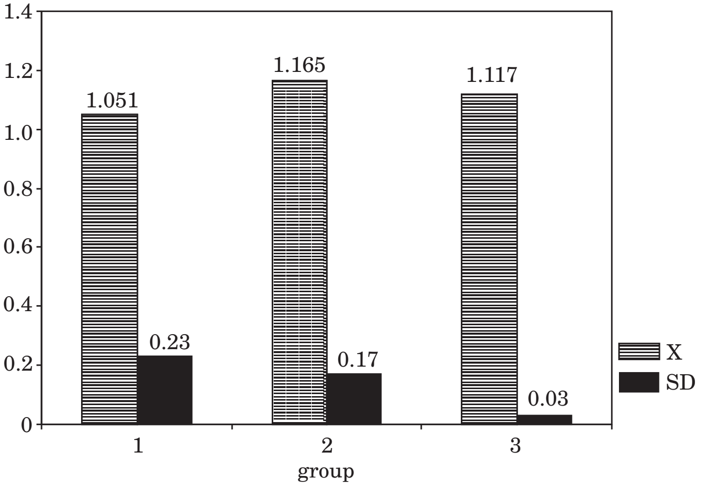

Fig. 1. Magnesium concentration (mmol dm^{-3}) in blood serum

Both the level of total calcium and ionized calcium in the animals' blood was tested. Total calcium concentration showed considerable fluctuations in all tested groups; it is presented in Table 1 and Figure 2. None of the average values of total calcium in the comparable tests was within the reference value limits (Winnicka 2002). The results indicate total calcium deficiency in the pregnant goats' blood serum. The average total calcium value was the lowest during pregnancy – group 1.

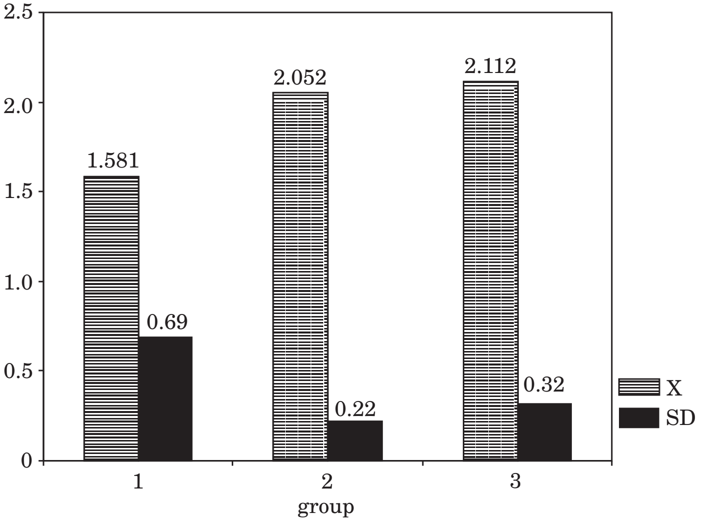

Fig. 2. Total calcium concentration (mmol dm^{-3}) in blood serum

The average values of ionized calcium concentration were even lower (Table 1, Figure 3). Ionized calcium concentration in the first test was on average 1.219 mmol dm^{-3}, in the second test: 1.126 mmol dm^{-3}, and in the last test: 1.123 mmol dm^{-3}. It is probable that the decrease in the ionized calcium level was the result of progressing lactation. The results of Ca concentration in the blood serum do not always correspond well to the degree to which the animals' calcium requirement is covered because it is the subject to a relatively precise homeostatic regulation. Thus, the optimum calcium level in the blood serum is not equivalent to its sufficient amount in the organism. If it is lower than the reference values, it proves calcium deficiency (Klata et al. 2000). It should be remembered that it is not recommended to enrich a feeding dose with calcium a few weeks before the delivery because increased supply and absorption of calcium activates a mechanism inhibiting the release of endogenous calcium. However, immediately before the delivery, at the time of activation of the lactiferous gland, it is necessary to introduce changes in feeding and add calcium preparations (Gabryszuk 1988). Such high levels of calcium supply should be maintained until the end of the lactation period because towards the end of this period calcium absorption from a dose increases considerably (Gabryszuk 1992).

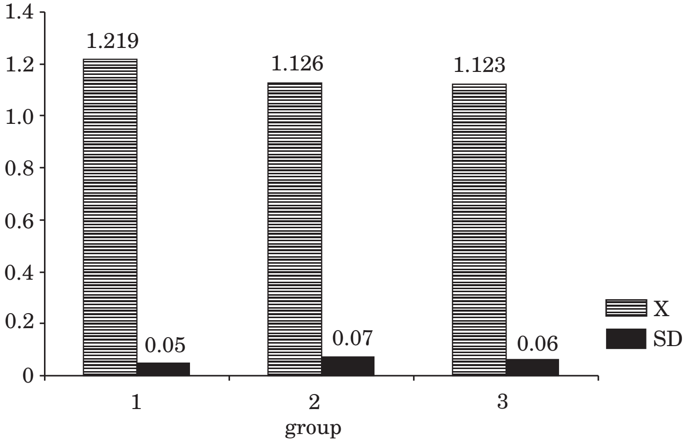

Fig. 3. Ionized calcium concentration (mmol dm^{-3}) in blood serum

CONCLUSIONS

1. In the evaluated physiological periods, the average content of total magnesium in goats' blood serum was proper. No significant fluctuations were observed in this respect, which can prove that the magnesium requirement was covered.

2. The average total calcium concentration in the goats' blood serum in the evaluation periods was below the reference values accepted for this species.

3. Calcium concentration in pregnant goats was the lowest and statistically significant, which may indicate a deficiency of this element in the diet, especially noticeable during pregnancy.

4. On the basis of the available literature, it can be assumed that ionized calcium concentration in goats is strictly related to total calcium concentration. The level of this biologically active form was more favourable in all of the evaluation periods, which may prove a more effective homeostasis mechanism of the biologically active forms of calcium in periods of increased requirement (pregnancy, the peak lactation period).

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FUNGI COLONIZING SOIL FERTILIZED WITH COMPOSTED SEWAGE SLUDGE AND MUNICIPAL WASTE

Bożena Cwalina-Ambroziak[1], Teresa Bowszys[2],
Jadwiga Wierzbowska[2]

[1]Chair of Phytopathology and Entomology
[2]Chair of Agricultural Chemistry and Environmental Protection
University of Warmia and Mazury in Olsztyn

Abstract

A strict field experiment was conducted in 2004-2007 on grey-brown podzolic soil originating from light silty loam, which was 5.04 in pH, highly abundant in P, moderately abundant in K, Zn, Mn and very poor in Mg. The experiment was established in a random block design with four replications. In a four-year crop rotation system, the following sequence of crops was grown: industrial potato, fodder spring barley, winter oilseed rape and winter wheat. The experiment consisted of treatments with farmyard manure and composted sewage sludge (Biohum, Polepszacz, Tyrowo) as well as composted municipal waste (Dano, green waste). The manure and organic fertilizers were applied once (10 t d.m. ha^{-1}) or twice (2·5 t d.m. ha^{-1}). For comparison, unfertilized plots (control) and NPK fertilized plots were used.

The phytopathological assays were completed in order to determine the effect of natural and organic fertilization on the structure of soil fungal communities. In a laboratory, soil samples were made into suspensions of the dilution from 10^{-2} to 10^{-4}. Fungi were cultured for 5 days on Martin substrate in Petri dishes at 22°C. The grown fungal colonies were calculated into g dry mass and inoculated onto agar slabs for later species identification.

The results suggest positive influence of fertilization with FYM, composted sewage sludge and composted municipal waste on the chemical properties and biotic relationships in soil, i.e. on the growth of fungi which can act antagonistically towards pathogenic species, suppressing at the same time populations of pathogenic fungi. Highest counts of pathogenic fungi were found in unfertilized soil (in the control treatment). The most desirable effect was produced by FYM applied once in a dose of 10 t d.m. ha^{-1}.

Key words: organic fertilization, zinc, manganese, soil, pathogens, saprophytes.

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dr hab. Bożena Cwalina-Ambroziak, prof. UWM, Chair of Phytopathology and Entomology , University of Warmia and Mazury in Olsztyn, Prawocheńskiego 17, 10-722 Olsztyn, Poland, e-mail: bambr @uwm.edu.pl

GRZYBY KOLONIZUJĄCE GLEBĘ NAWOŻONĄ KOMPOSTAMI Z OSADÓW ŚIEKOWYCH I ODPADÓW KOMUNALNYCH

Abstrakt

W latach 2004-2007 przeprowadzono ścisłe doświadczenie polowe na glebie płowej wytworzonej z gliny lekkiej pylastej, pH 5,04 oraz wysokiej zawartości P, średniej K, Zn i Mn oraz niskiej Mg. Eksperyment założono w układzie losowanych bloków, w trzech powtórzeniach. W czteroletnim płodozmianie uprawiano kolejno: ziemniak przemysłowy, jęczmień jary paszowy, rzepak ozimy i pszenicę ozimą. Eksperyment obejmował obiekty z obornikiem i kompostowanymi osadami ściekowymi (Biohum, Polepszacz, Tyrowo) oraz kompostowanymi odpadami komunalnymi (Dano, zieleń miejska). Obornik i nawozy organiczne stosowano jedno- (10 t s.m.$\cdot$ha^{-1}) lub dwukrotnie w zmianowaniu (2$\cdot$5 t s.m.$\cdot$ha^{-1}). Obiekty porównawcze stanowiły poletka bez nawożenia (kontrola) i z nawożeniem mineralnym NPK.

Badania fitopatologiczne wykonano w celu określenia wpływu nawożenia naturalnego i organicznego na strukturę zbiorowiska grzybów glebowych. W laboratorium z pobranych próbek gleby sporządzano zawiesiny wodne o rozcieńczeniu od 10^{-2} do 10^{-4}. Grzyby hodowano w płytkach Petriego, na podłożu Martina, przez 5 dni w temperaturze 22°C. Wyrosłe kolonie grzybów przeliczano na g suchej masy, po czym przeszczepiano na skosy agarowe z zamiarem późniejszej identyfikacji gatunkowej.

Wyniki badań wskazują na pozytywny wpływ zastosowanego nawożenia obornikiem i kompostami z osadów ściekowych i odpadów komunalnych na właściwości chemiczne i stosunki biotyczne w glebie, tj. wzrost liczebności grzybów o uzdolnieniach antagonistycznych względem gatunków patogenicznych, a jednocześnie redukcję populacji tych ostatnich. Najwięcej patogenów zasiedlało glebę nienawożoną (w obiekcie kontrolnym). Najbardziej dobroczynne działanie wykazał obornik zastosowany jednorazowo w dawce 10 t s.m.$\cdot$ha^{-1}.

Słowa kluczowe: nawożenie organiczne, cynk, mangan, gleba, patogeny, saprotrofy.

INTRODUCTION

Beside very small quantities used to reclaim degraded land or re-fill excavation cavities, most sewage sludge, including municipal waste and green waste, is deposited at landfills. However, the high content of organic substance and some mineral components that sewage sludge has makes it suitable for agricultural applications, especially as a substance amending poorly fertile soils. Increased content of organic carbon, owing to the availability of substrates and their improved utilization, enhances the physicochemical properties of soil (Garcia et al. 2000, Hue 1988). Moreover, the value of composts as fertilizer is a consequence of their beneficial influence on the soil's biological life. After all, carbon is known to be a source of nutrition and energy for soil microorganisms. In soil which is fertilized with organic substance, the concentration of macro- and micronutrients rises (Warman, Termeer 2005). There are many references (Ritz et al. 1997, Arti Bhatia Pathak, Joshin 2001) which prove that organic fertilizers help establish proper biotic relations in soils, i.e. rising counts of valuable bacteria and fungi which are antagonistic to plant pathogens, and this supports the biological protection of crops.

Owing to the enhanced microbial activity, the soil enzyme activity increases (de CAIRE et al. 2000). The suitability of composts for biological control of plant pathogens depends on the compost maturity, chemical composition and physical structure (BENDING et al. 2004). The mechanisms which are taken advantage of in this type of plant protection include competition for C and Fe, antibiosis, parasitism and induction of systemic resistance of plants (HORBNY 1990). Many reports (KUTTER et al. 1988, GORODECKI, HADAR 1990) state that natural and organic fertilizers, including sewage sludge and municipal waste-based composts, have a limiting effect on the development of soil pathogens of the genera *Pythium, Phytophthora* and *Fusarium* as well as the species *Rhizoctonia solani*. This study has been carried out to compare the effect of mineral fertilization versus application of FYM or various types of organic fertilizers on the content of zinc and manganese in soil as well as the structure of soil fungal communities.

MATERIAL AND METHODS

In 2004-2007, a strict field experiment was conducted at the Agricultural Experimental Station in Bałcyny. Plots covering 15 m^3 were established on grey-brown podzolic soil formed from light silty loam of class III, complex 4. Before the experiment was established, samples of soil, FYM and composts were taken. Soil was 5.04 in pH, highly abundant in available forms of P, moderately abundant in K, poorly abundant in Mg and moderately abundant in available Zn and Mn.

The experiment was established according to a random block design with three replications. In a four-year crop rotation cycle the following crops were sown: industrial potato, fodder spring barley, winter oilseed rape and winter wheat. The experimental design comprised the following treatments: I – control (no fertilization), II – NPK mineral fertilization, III – farmyard manure 10 t d.m. ha^{-1}, IV – farmyard manure 2·5 t d.m. ha^{-1}, V – Biohum 10 t d.m. ha^{-1} (sewage sludge composted with straw), VI – Biohum 2·5 t d.m. ha^{-1}, VII – Polepszacz 10 t d.m. ha^{-1} (dried and granulated sewage sludge), VIII – Polepszacz 2·5 t d.m. ha^{-1}, IX – Tyrowo 10 t d.m. ha^{-1} (sewage sludge composted alone), X – Tyrowo 2·5 t d.m. ha^{-1}, XI – Dano 10 t d.m. ha^{-1} (compost from unsegregated municipal waste), XII – Dano 2·5 t d.m. ha^{-1}, XII – composted green waste 10 t d.m. ha^{-1}, XIV – composted green waste 2·5 t d.m. ha^{-1}. The composts as well as FYM were applied once during the rotation cycle in a dose of 10 t d.m. ha^{-1} (under potato), or twice, 5 t d.m. ha^{-1} each time (under potato and winter oilseed rape). In the plots fertilized with organic substances and FYM, nitrogen was balanced up to 150 kg ha^{-1} (2004) and to 120 kg ha^{-1} (2005), depending on the concentration of total nitrogen in soil. Spring barley and winter wheat received only mineral fertilization. The concentrations of macro- and micronutrients in the applied organic fertilizers is presented in Table 1.

The phytopathological tests involved isolation of fungi from soil. For this purpose, in the first week of August in each year of the experiment, soil samples were taken from the depth of 10 cm, from three points on each plot. Portions of 10 g were weighted out from aggregated and mixed soil samples and placed in 250 cm^3 flasks. Afterwards, 90 cm^3 of sterile water was added to each flask. The samples were shaken for 20 minutes, and the suspension (concentration 10^{-1}) was used to obtain subsequent dilutions: 10^{-2} and 10^{-4}. A portion of 1 cm^3 of the suspension and 20 cm^3 of Martin nutrient were placed on each Petri plate. After 5-day incubation at 22°C, the grown colonies of fungi were calculated into g dry mass and then inoculated onto agar slabs for species identification according to the keys prepared by Booth (1971), Ellis (1971) and Domsch et al. (1980).

Table 1

Chemical composition of sewage sludge and composts used for the experiment

Specification	Unit	Sewage sludge			Municipal waste	
		with straw (Biohum)	dried and granulated (Polepszacz)	composted (Tyrowo)	Dano	green waste
Dry matter	%	58.68	85.14	49.31	74.64	78.88
C	%	18.47	38.27	21.39	13.62	8.02
N	$g \cdot kg^{-1}$	10.71	18.82	46.61	8.93	5.33
C/N		17.3	21.3	4.6	15.3	15.1
P	$mg \cdot kg^{-1}$	7.52	2.81	29.93	3.94	4.10
K	$mg \cdot kg^{-1}$	1.35	1.83	2.52	6.31	2.91
Mg	$mg \cdot kg^{-1}$	2.11	3.42	7.83	3.32	2.95
Zn	$mg \cdot kg^{-1}$	109.51	270.40	1310.02	679.50	301.03
Mn	$mg \cdot kg^{-1}$	210.60	228.01	300.51	273.64	326.81

RESULTS AND DISCUSSION

The soil-amending substances tested in our experiment significantly modified the soil content of available forms of zinc, but did not change the soil abundance class (Figure 1). After four years, it was evident that the most beneficial influence on the concentration of this element in soil, as compared with the control (7.3 mg kg^{-1}), was produced by composted sewage sludge from Tyrowo (15.8 mg kg^{-1}). In the soil samples from the other treatments, the available zinc content was on average around 10 mg kg^{-1}.

Microorganisms play an important role in the cycle of oxidation and reduction of manganese. Soil contamination with this element is associated with its form rather than the amount. Excess of easily soluble manganese

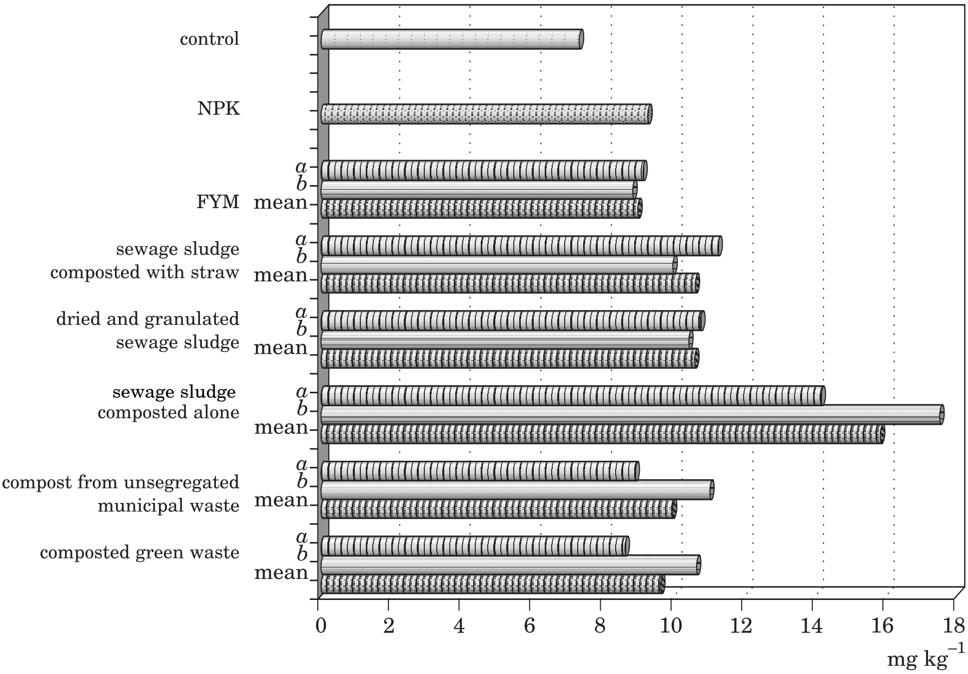


Fig 1. Zink content in the soil after 4-year testing cycles (*a* – 10 t d.m. ha^{-1}; *b* – 2·5 t ha^{-1})

can occur in soils fertilized with municipal waste. Although the amounts of manganese in all the tested fertilizers were approximately the same, their influence on the level of this element in soil was not unambiguous. After four years of the tests, the concentration of manganese in soil ranged from 116.2 (unfertilized treatment) to 156.2 mg kg^{-1} (composted sewage sludge from Tyrowo), but in none of the treatments the level of manganese exceeded the one claimed to be average for this type of soil (Figure 2). The form of the tested fertilizers rather than the way they had been applied had a more profound effect on the content of elements in soil.

The phytopathological tests demonstrated more colony forming units of fungi in the soil from the treatments where organic fertilizers were applied (Biohum in both application variants, Dano in a split dose of 5 t ha^{-1} and green waste in a single dose of 10 t ha^{-1}) than the ones fertilized with FYM, mineral fertilizers or the control treatment (Figure 3).

A study completed by WARMAN and TERMEER (2005) implies that under the influence of composted and fresh sewage sludge, the soil becomes enriched with Zn and Mn, and the mobility of these elements is higher in loamy than in sandy soil (SABIENE et al. 2002). Increasing rates of sewage sludge led to a growth in the population of microorganisms, including fungi, and the enhanced enzymatic activity of soil (AWAD, FAWZY 2004). KUBATOVA et al.

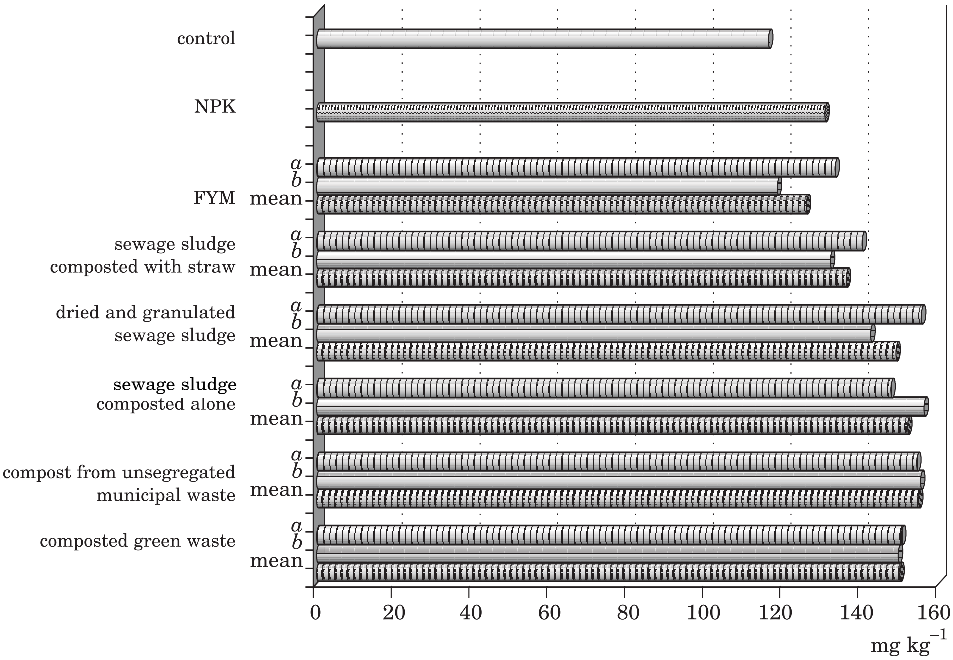


Fig. 2. Manganese content in the soil after 4-year testing cycle (a – 10 t d.m. ha^{-1}; b – 2·5 t ha^{-1})

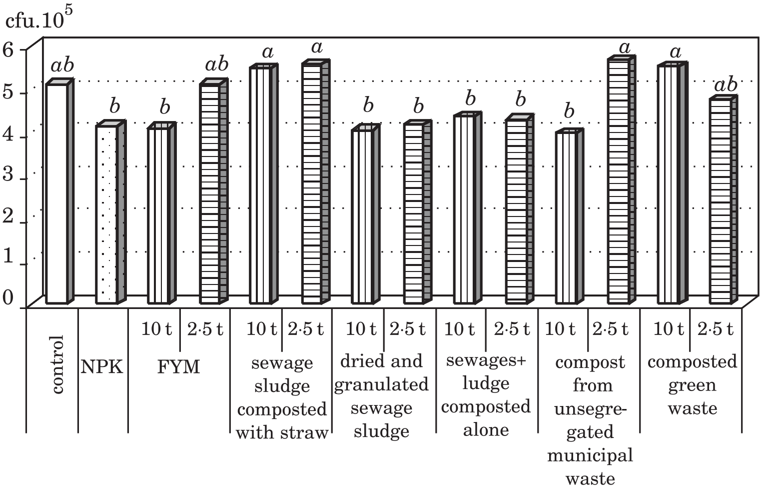


Fig. 3. Number of colony-forming units of fungi in the soil (data marked by the same letters do not differ statistically)

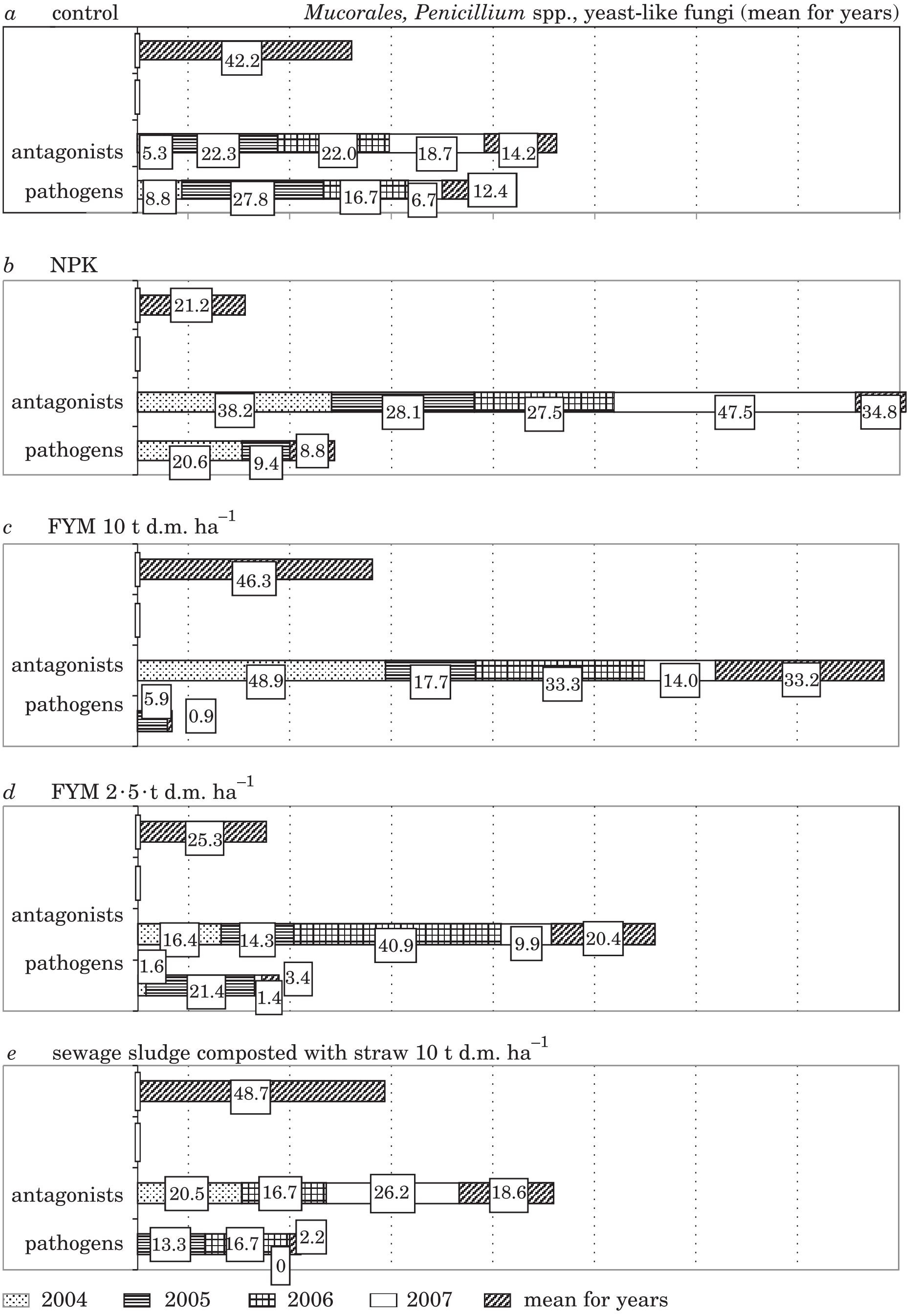
(2002) identified in soil rich in Zn and Mn such fungi as *Paecilomyces lilacinus*, *Mucor hiemalis*, *Mortierella aplina*, *Coniothyrium fuckeli*, species *Trichoderma*, *Penicillium* and *Fusarium*. Pratt (2008) in turn reports that the level of Zn in soil fertilized with waste from hog production tended to be higher than in unfertilized soil, and the level of Mn was rarely or never higher. The above authors also claim that the composition of a fungal assemblage in soil is constant and independent from organic fertilization.

In our own studies, the community of soil fungi was represented by 67 species of fungi as well as non-sporulating cultures and yeast-like fungi. Among the potential pathogens, species of the genus *Fusarium* were identified as well as the species *Alternaria alternata*, *Aureobasidium bollei*, *A. pullulans*, *Botrytis cinerea*, *Colletotrichum coccodes* and *Sclerotina sclerotinum*. Soil from the control treatment had the highest population of fungi (12.4% share in the microbial community; Figure 4a). At the same time, this population contained a low percentage of antagonistic fungi of the genera *Gliocladium*, *Paecilomyces* and *Trichoderma* (14.2%). It was evident that biotic relations in soil fertilized exclusively with NPK mineral fertilizers were superior to those discovered in the control plot (Figure 4b).

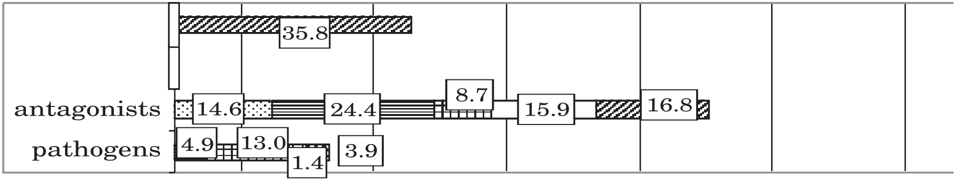
Mineral and organic fertilization determines the quantitative and qualitative composition of a community of soil microorganisms, and consequently has influence on the activity of biological life in soil (Shannon et al. 2002, Larkin et al. 2006, Mader et al. 2006, Korniłłowicz-Kowalska et al. 2008).

Our own research has demonstrated that FYM fertilization stimulated the growth of antagonistic fungi while suppressing counts of pathogens (Figures 4c,d). *Fusarium* spp. fungi, in the absence of any other pathogenic fungi, colonized, in the first two years of the experiment only, soil in the treatment where a single application of FYM was performed. In turn, antagonistic fungi appeared in high numbers in all the years of the experiment, with the peak occurrence of 50% in the first year.

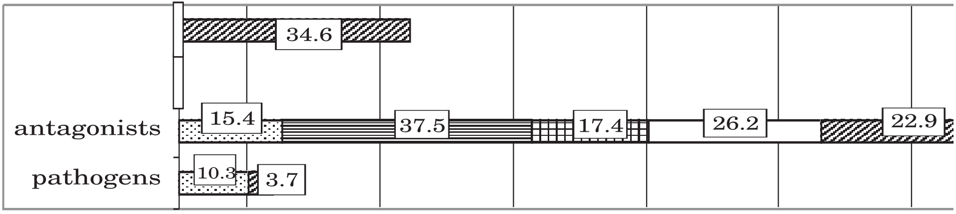
Among the composted sewage sludge and municipal waste (Figures 4 e-n), the most positive effect on the structure of soil fungal assemblages was produced by composted and dried sewage sludge applied as a single dose under potato (Figures 4g). Pathogenic fungi were isolated from the soil sampled from that treatment only in the first year of the experiment. This was probably due to the fact that antagonistic fungi occurred numerously during the growing seasons in the subsequent years. A comparable influence was produced by Polepszacz (applied twice during the crop rotation) (Figures 4h) and Biohum (in both application variants) – Figures 4e,f. In the soil enriched with these fertilizers, the share of pathogenic fungi would not normally exceed 6%, whereas the percentage of antagonistic fungi ranged from 11 to 19%. The least positive conditions for the health of crops were established in the soil enriched with the compost from Tyrowo (Figures 4i,j) and composted green waste supplied in a split dose (Figures 4n). There, the counts of determined pathogens, including *Fusarium* spp., was the highest. In the same treatments, soil had the highest amount of available forms of zinc.


a control
Mucorales, Penicillium spp., yeast-like fungi (mean for years)
42.2
antagonists
5.3
22.3
22.0
18.7
14.2
pathogens
8.8
27.8
16.7
6.7
12.4
b NPK
21.2
antagonists
38.2
28.1
27.5
47.5
34.8
pathogens
20.6
9.4
8.8
c FYM 10 t d.m. ha⁻¹
46.3
antagonists
48.9
17.7
33.3
14.0
33.2
pathogens
5.9
0.9
d FYM 2·5·t d.m. ha⁻¹
25.3
antagonists
16.4
14.3
40.9
9.9
20.4
pathogens
1.6
21.4
1.4
3.4
e sewage sludge composted with straw 10 t d.m. ha⁻¹
48.7
antagonists
20.5
16.7
26.2
18.6
pathogens
13.3
16.7
0
2.2
2004
2005
2006
2007
mean for years

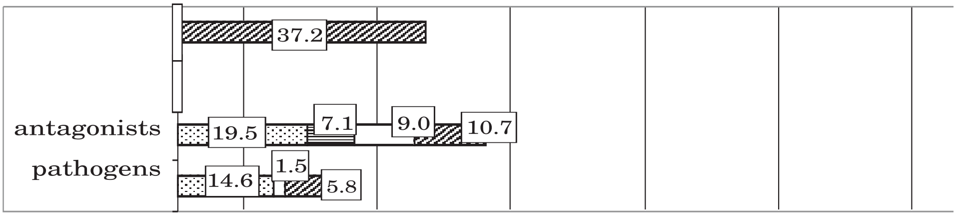
f sewage sludge composted with straw 2·5 t d.m. ha^{-1}



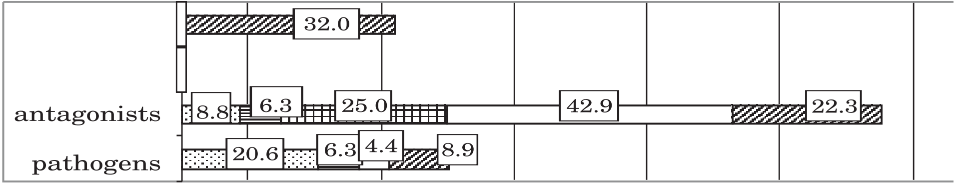
g dried and granulated sewage sludge 10 t d.m. ha^{-1}



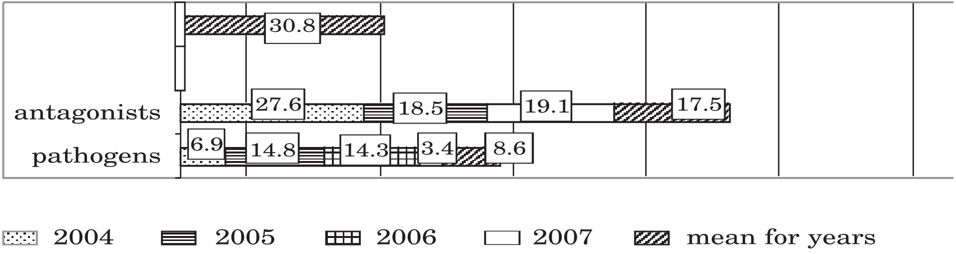
h dried and granulated sewage sludge **2·5** t d.m. ha^{-1}



i sewage sludge composted alone 10 t d.m. ha^{-1}



j sewage sludge composted alone 2·5 t d.m. ha^{-1}



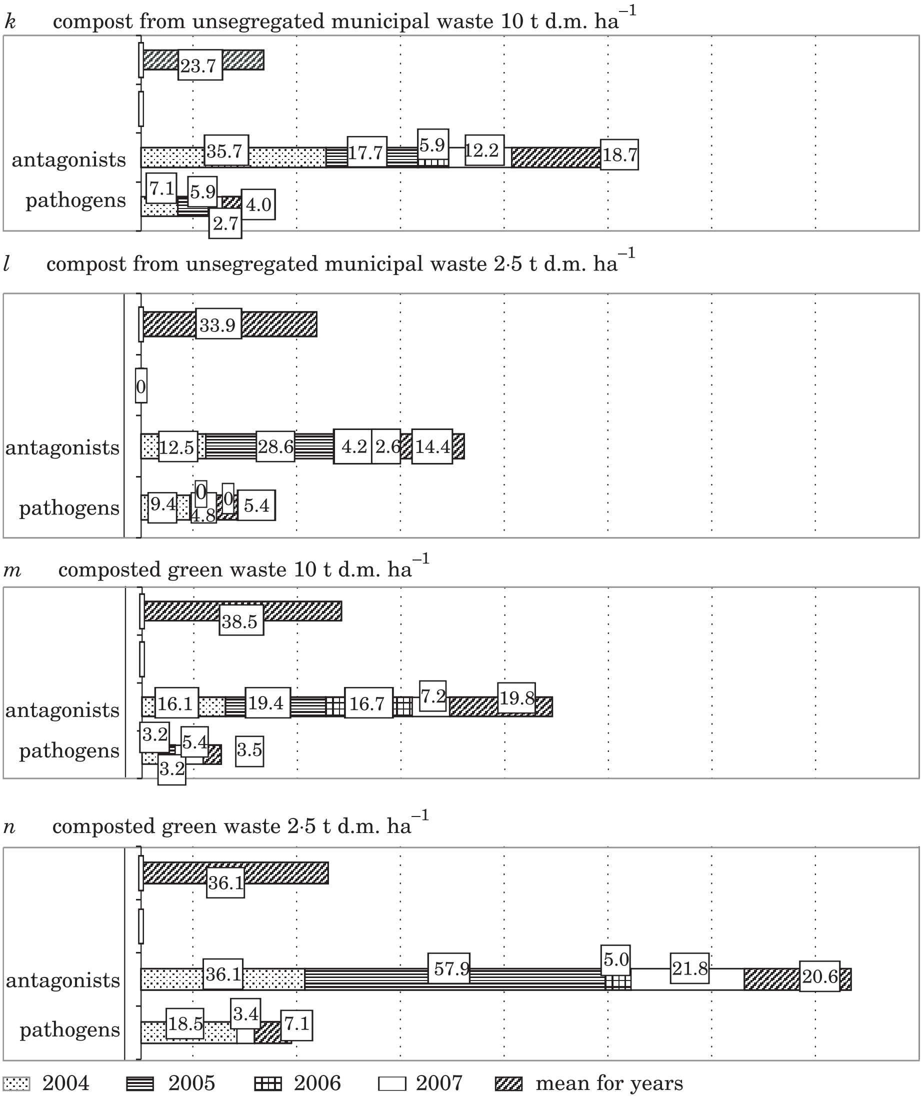


Fig. 4. Structure of fungi isolated from soil

Kato et al. (1981) report that application of composts favoured development of *Fusarium* spp. fungi, and attributed this finding to the fact that the C:N ratio was low and the content of N-NH$_4$ high. Contrary to that, Lewis et al. (1992) claim that organic fertilization with composts, including composted sewage sludge, significantly depressed the range of presence of *Pythium*, *Phytophthora*, *R. solani* and *Fusarium* in soil cropped with different plants.

In the present study, the highest counts of fungi of the genus *Trichoderma* were determined in the soil fertilized with sewage sludge-based composts. At the same time, the soil from these treatments was characterized by the highest concentrations of zinc and manganese. Modifications in a soil fungal community leading towards increased counts of beneficial microorganisms are highly desirable, as they condition the biological control of plant pathogens. PANDEY et al. (2006) concluded that organic fertilization of soil favoured the development of fungi which acted as antagonists or parasites of plant pathogens. The presence and number of *T. viride*, as NAAR and BIRO (2006) reported, was positively correlated with the content of Zn in soil. The stimulating activity of the above microelements on the development of species of the genus *Trichoderma* has been verified by *in vitro* tests.

KREDICS et al. (2001) as well as SHANINA KALIM and GANDHI (2003) implied even that Zn and Mn (provided they occur in an optimum rate in soil) can protect some crop species against pathogens which belong to the genus *Rhizoctonia*. However, very high doses of Zn inhibited the enzymatic activity of antagonists which being tolerated by the pathogens of the genus *Pythium* (NAAR 2006), and could even stimulate production of oospores of this fungus-like organisms (ALTAF HUSSAIN BAIG 2003).

On average, the share of species of the order *Mucorales* in the communities of soil fungi assayed during the four years of the study did not exceed 15% in any of the application variants, except the treatments fertilized with Biohum. Good conditions for the growth and development were established for yeast-like fungi and the ones belonging to the genus *Penicillium*. The latter were most often isolated from the soil fertilized twice with Dano, and the lest frequently – from the soil enriched once with FYM or Biohum. At the same time, the smallest and the biggest counts of fungi of the genus *Trichoderma* were isolated from these treatments, respectively. It has been reported that the development of saprophytes of the genus *Penicillium* is suppressed by the above antagonists (DOMSCH et al. 1980).

The results of our experiment seem to suggest that FYM and composts used as fertilizers have positive influence on the chemical and biological properties of soil.

CONCLUSIONS

1. The use of biowaste for soil fertilization increase soil concentration of available forms of Zn and Mn, compared to mineral fertilization or FYM application.

2. Farmyard manure and composts applied in a single dose of 10 t d.m. ha^{-1} rather than a split dose of 2·5 t d.m. ha^{-1} are more efficient at stimulating the development of fungi which act antagonistically towards pathogens; at the same time they are better at suppressing counts of pathogens.

3. Most of potential pathogens colonized unfertilized soil.

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INFLUENCE OF ANTHROPOPRESSION ON CONCENTRATION OF BIOGENIC COMPOUNDS IN WATER OF SMALL PONDS IN FARMLAND

Małgorzata Gałczyńska, Marcin Kot

Chair of General and Ecological Chemistry
West Pomeranian University of Technology in Szczecin

Abstract

A rapidly diminishing number and degradation of water ponds cause many negative effects in the structure of agricultural ecosystems, by limiting surface retention and increasing migration of chemical compounds from the basin. The goal of this research was to find the influence of anthropopression on the chemical composition of water in field ponds. Analysis of some chemical and physical parameters was carried out on water samples collected once a month from 5 field ponds located in the District of Pyrzyce, Commune of Żabowo, between March and October 2004. The five water ponds differed with respect to the spatial development of their surroundings and their degradation. The concentration of N-NH_4^+, N-NO_2^- i N-NO_3^- oraz PO_4^{3-} were measured according to the Polish Standards. The level of oxygen, temperature and water pH were measured immediately after collecting each sample of water. The analyzed field ponds were different in respect of the degree of degradation processes. What was characteristic of the five ponds was a large variation in concentrations of biogenic compounds in water, depending on the form of anthropopression and vegetation in and around the ponds. The high level of PO_4^{3-} and N-NH_4^+ in water and the succession of water plants prove that ponds 2, 3 and 4 are highly eutrophicated. Due to the extremely high concentration of PO_4^{3-} in the water, field pond 2, located near a former state farm, can be classified as hypertrophic. This pond also had the biggest oxygen deficit.

Key words: nitrogen, phosphorus, mid-field water ponds.

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dr inż. Małgorzata Gałczyńska, Chair of General and Ecological Chemistry, West Pomeranian University of Technology in Szczecin, ul. Słowackiego 17, 71-434 Szczecin, Poland, phone: (091)449 6322, e-mail: Malgorzata.Galczynska@zut.edu.pl

WPŁYW ANTROPOPRESJI NA STĘŻENIA ZWIĄZKÓW BIOGENNYCH W WODACH MAŁYCH ZBIORNIKÓW NA TERENACH UŻYTKÓW ROLNYCH

Abstrakt

Szybkie zanikanie i degradacja oczek wodnych pociąga za sobą wiele negatywnych zjawisk w strukturze agroekosystemów, zmniejsza retencję powierzchniową i powoduje wzrost migracji składników ze zlewni. Celem pracy było ustalenie wpływu antropopresji na skład chemiczny wód śródpolnych oczek wodnych. Materiał do analiz wybranych wskaźników chemicznych i fizycznych wód stanowiły próbki wodne pobierane raz w miesiącu z 5 oczek śródpolnych położonych w powiecie pyrzyckim, w gminie Żabowo, w okresie od III do X 2004 roku. Badane zbiorniki różniły się nie tylko sposobem zagospodarowania terenu w bezpośrednim ich sąsiedztwie, ale również formą dewastacji. Oznaczenia stężeń: $N\text{-}NH_4^+$, $N\text{-}NO_2^-$ i $N\text{-}NO_3^-$ oraz PO_4^{3-} wykonano zgodnie z Polskimi Normami. W terenie, bezpośrednio po poborze prób, zmierzono stężenie tlenu, temperaturę i pH wód. Ustalono, że analizowane śródpolne oczka wodne różniły się stopniem zaawansowania procesów degradacji. Cechą charakterystyczną badanych obiektów była duża zmienność stężeń związków biogennych w wodzie zależna od formy antropopresji oraz roślinności występującej w zbiorniku i wokół niego. Wysoki poziom stężenia PO_4^{3-} i $N\text{-}NH_4^+$ w wodach oraz sukcesja roślinności wodnej świadczą o zaawansowanym procesie eutrofizacji szczególnie oczek nr 2, 3 i 4. Bardzo duże stężenie ortofosforanów(V) w wodach oczka nr 2, położonego przy zabudowaniach dawnego PGR-u, można uznać za hipertroficzne. W wodach tych stwierdzono też największy deficyt tlenowy.

Słowa kluczowe: azot, fosfor, śródpolne oczka wodne.

INTRODUCTION

Among many different glacial forms in the area of Pomorze Szczecińskie, there are numerous water ponds, which can now be called biotope ‘islands’ in the otherwise monotonous agricultural landscape, but they are also part of ecological corridors for migrating animals. In agricultural areas, such closed water ecosystems are exposed to a risk of area and point contamination (e.g. illegal rubbish dumping or municipal sewage discharge). A rapid decrease in the number of ponds and their degradation cause a chain of negative changes in agroecosystems, by decreasing surface retention and increasing migration of nutrients from the basin (Pieńkowski 1996, Kochanowska, Raniszewska 1999, Koc et. al. 2001, Pieńkowski, Podlasiński 2001).

The goal of this research was to find the influence of anthropopression on the chemical composition of water in field ponds.

MATERIAL AND METHODS

Water samples taken from five field ponds in Żabowo, District of Pyrzyce, provided material for analyses of selected chemical and physical parameters.

The ponds differed with respect of the use of their surroundings and form of degradation.

Pond 1 had highly developed water vegetation and open water surface. Around the pond there were no bushes or trees which could limit intensive transpiration from the water surface. At the time of the study, wheat was planted around the pond. A rubble dump was situated near the pond and in the pond itself food waste and used tires were seen. The general morphometric parameters of the pond are as follows: 1.2 m depth, 30 m^2 surface square footage, 0.5 m depth. After five months of research the pond's water disappeared.

Pond 2 was located close to farm buildings of a former state farm. This location means that the pond had been probably used for flushing dung. Although there were many bushes growing around the pond, its water table was not shaded. The whole pond was overgrown with dense *Lemna minor,* which proves the hypertrophic character of the pond; rubbish and animals' bones were noticed on the surface. Canola was planted around the pond. The pond was 512 m^2 in square footage. There was a 4-meter gap between the pond's border and the water surface. Water depth was 0.2-0.3 m. The pond had a very high level of the bottom sludge.

Pond 3 differed significantly from the others because it was separated from the cultivated surface with a dense border of trees and bushes. The pond's border was situated higher than the ground level of the field. This suggested limited surface flow. Near the pond, there was an illegal rubbish dump and a rubble dump (including empty bags of fertilizers and pesticides). In the absence of plants in the water, there were only algae. The pond's surface decreased from 8 m^2 to 1 m^2 during the research's time. The pond's area was covered with dense bushes and trees. There was a 4-meter gap between the pond's border and the water surface.

Pond 4 was located in some fallow land, about 150 m from expressway E-65. Around the pond there were trees and bushes, which shaded the water's surface. Lack of water plants was characteristic for this pond. Used car tires and plastic bags were seen in the water. General morphometric parameters of the pond: long shape, 18 m length, 3 m depth, 3.5 m width. There was water at the pond's bottom, which disappeared after four months of the research.

Pond 5 was located on the outskirts of the village of Ryszewko. There was a road, some houses, a milk collection site (closed at the time) and farmland in its close neighborhood. This pond had the highest water potential among all the examined ponds. There was a large number of algae in water and a broad belt of *Phragmites australis* covering 20% of the surface. The surface square footage was 372 m^2 and the water depth was 0.5-1.0 m.

Water samples for chemical and physical analyses were collected once a month. The tests were carried out from March to October 2004. Chemical determinations of N and P were obtained by colorimetry. In water samples, concentrations of ammonium nitrogen, nitrite nitrogen, nitrate nitrogen and orthophosphates were measured according to the Polish Standards (PN-76/ /C-04576.01, PN-73/C-04576.06, PN-82/C-04576.08, PN-EN 1189:2000). Immediately after taking the samples, oxygen concentration, temperature and water pH were measured. All measurements were repeated three times.

RESULTS AND DISCUSSION

The analyzed parameters of water (Table 1) were different depending on dates of sample collection and pond location.

The average water temperature was 13.9°C, which on the one hand was good for dissolution of chemical compounds and growth of water organisms, but on the other hand contributed to the depletion of oxygen in water. In ponds 3 and 4, the temperature of water was lower than in the other ponds because of the shadow cast by trees and bushes. Very shallow waters tend to warm up and cool down fast, depending on the term of the year and insolation.

Higher variation of the results was found in the analysis of oxygen's concentration in the water. The average results for each pond were higher than those measured by Skwierawski and Szyperek (2002) for twenty ponds (4.0-6.8 mg $O_2 \cdot dm^{-3}$). In pond 2, oxygen deficit was noticed (less than 2-3 mg $O_2 \cdot dm^{-3}$), caused by degradation of organic compounds with the use of oxygen, which occurred in water and in bottom sediments. Moreover, dense *Lemna minor* covering the whole water surface limited water surface movements and, in consequence, oxygen diffusion.

The reaction of water in the examined ponds was slightly acidic or neutral, between pH 6.4 and 7.1. The results are significantly lower than the average results measured by Skwierawski and Szyperek (2002), who determined pH 7.06 for waters in farmland, pH 7.29 for fallow land, pH 7.76 for build-up areas and pH 6.97 for forests. It was also noticed that the highest pH values are measured in spring, after which they gradually decrease to reach the spring values in autumn. This, however, is more of a general tendency as in some ponds the water pH was unstable.

Non-organic nitrogen in the ponds could have originated from mineral and organic fertilizers flushing from the fields, from degradation of organic compounds (food waste) and from empty bags of fertilizers.

For each pond, the average values of nitrogen ammonium significantly differed from those measured by Koc et al. (2001). The average concentration of $N\text{-}NH_4^+$ was 0.661 $mg \cdot dm^{-3}$ (range 0.23-1.12) for ponds on farmlands, 0.560 $mg \cdot dm^{-3}$ (0.22-1.66) for build-up areas, and 0.886 $mg \cdot dm^{-3}$ (0.34--1.26) for forests. According to Skwierawski and Szyperek (2002), the average value of $N\text{-}NH_4^+$ for ponds located on farmland was 0.58 $mg \cdot dm^{-3}$,

Table 1

Chemical and physical proporties of the investigated waters in 2004

No. mid-field ponds		1	2	3	4	5	1÷5
$N\text{-}NH_4^+$ $mg \cdot dm^{-3}$	mean	0.27	1.88	0.94	2.33	0.39	1.16 SD 1.44
$N\text{-}NO_2^-$ $mg \cdot dm^{-3}$	mean	0.003	0.019	0.012	0.039	0.023	0.019 SD 0.026
$N\text{-}NO_3^-$ $mg \cdot dm^{-3}$	mean	0.24	0.40	0.41	0.96	0.14	0.43 SD 0.38
PO_4^{3-} $mg. \cdot dm^{-3}$	mean	0.4	84.2	1.0	1.0	1.1	17.6 SD 17.4
mg $O_2 \cdot dm^{-3}$	mean	5.1	2.5	7.1	5.1	6.0	5.2 SD 2.0
Temp. °C	mean	14.1	16.2	12.9	12.1	14.3	13.9 SD 4.3
pH	mean	7.0	6.9	7.1	6.4	7.0	6.9 SD 0.5
Number of sampling		5	8	8	4	8	33

0.54 $mg \cdot dm^{-3}$ for build-up areas and 0.81 $mg \cdot dm^{-3}$ for forests. The following aspects affected the value of $N\text{-}NH_4^+$ concentration in the examined water ponds (Figure 1): oxygen conditions, temperature and growth stage of water plants. In the ponds where highly developed water plants prevailed, nitrogen ammonium ions reached concentration higher than 1 $mg \cdot dm^{-3}$. High concentration of nitrogen ammonium ions in waters of pond 2 was caused by a large amount of organic compounds and low concentration of oxygen in the water. In pond 4, the concentration of $N\text{-}NH_4^+$ measured in June reached 6.82 $mg \cdot dm^{-3}$, which was probably caused by a lower level of water and the presence of wild animals in the pond. In pond 1 (which dried out in July), no growth in the concentration of nitrogen ammonium ions was noticed after the disappearance of water because water plants restricted the concentration of this form of nitrogen during the time of the research. In pond 5, a low concentration of ammonium ions was observed. Among all the ponds, this one contained most water, so its ability to overcome possible contamination was the highest. The extensive area of *Phragmites australis* limited the biogenic content in the pond, as can become a 'filter' for possible contaminants (KOC, SZYPEREK, 2001). KOCHANOWSKA and RANISZEWSKA (1999) point out that lack of plant buffer around a water pond causes loss of nutrients in surface soil, from which they are flushed with water to the pond. The wide and dense belt of trees and bushes near pond 3 had a good influence on the pond by limiting the concentration of nitrogen ammonium ions, despite the fact that empty mineral fertilizer bags were present in the pond.

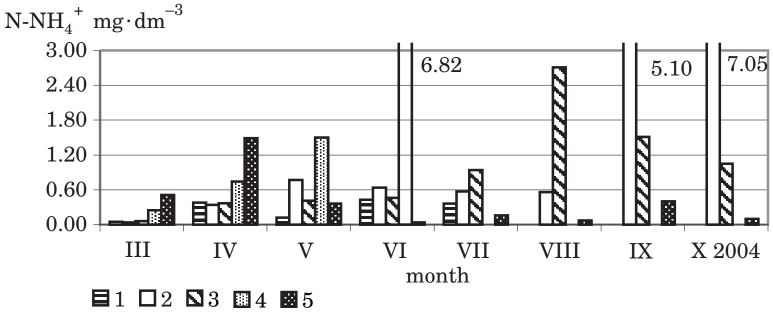

Fig. 1. Changes in the content of N-NH_4^+ in water of mid-field ponds during the vegetation season in 2004

Nitrogen nitrate in soil and as well in water takes part in biological sorption only. Thus, it can be immobilized in the water environment, being good nourishment for the growth and development of biomass of different organisms, especially plants. The smallest average concentration of nitrogen nitrate was measured in the water of pond 5, followed by pond 1, and then ponds 2 and 3; the highest value was measured in pond 4 (Table 1). The average values of nitrogen nitrate concentrations measured in this research were close to those reported by Koc et al. (2001), which were 0.201 mg·dm^{-3} (range 0.007–0.431) for water ponds on farmlands, 0.697 mg·dm^{-3} (0.005--2.430) for rough grazing meadows and 0.182 mg·dm^{-3} (0.006–0.670) for build-up areas. Skwierawski and Szyperek (2002) measured slightly smaller concentrations of nitrogen nitrate for ponds of in the Olsztyn Lake District: 0.41 mg·dm^{-3} for farmlands, 0.16 mg·dm^{-3} for rough grazing meadows, 0.12 mg·dm^{-3} for wasteland, and 0.25 mg·dm^{-3} for build-up areas. The highest nitrogen nitrate concentration was observed in pond 5 in March, after which it decreased rapidly (Figure 2). The decrease in its concentration was caused by increased photosynthesis, growth and development of water plants and change in dissolved oxygen concentration in the water. In pond 1, on the last sampling date, no increase of nitrogen nitrate concentration was noticed although the level of water was lower. It was also associated with absorption of this form of nitrogen by water plants. In general, it is thought that during the time of vegetation the level of N-NO_3^- in water declines to rise in the winter. A similar pattern was noticed for ponds 2 and 3, where *Lemna minor* along with trees and bushes contributed to the reduction of the N-NO_3^- concentration. Such correlation can be distorted by an influx of sewage, containing high quantities of nitrogen compounds. In the water of pond 4, a very high concentration of nitrogen nitrate was measured in the last month of water sampling (June), attributed to the lowering of the water level. An increase of this form of nitrogen was observed in most of the ponds between March and May 2004, which was probably caused by the surface flow from farmland due to intensive precipitation.

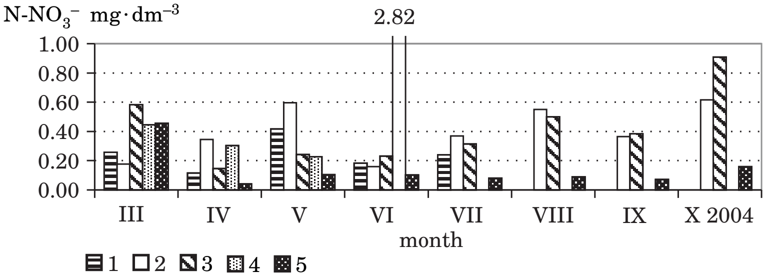

Fig. 2. Changes in the content of N-NO$_3^-$ in water of mid-field ponds during the vegetation season in 2004

Nitrogen nitrite is a transitory form in a complex biochemical process of organic nitrogen transformation and its formation depends on accessibility to oxygen and on the number of nitrobacteria. For oxidation of 1 g of N, 4.6 g of O_2 is needed, which is possible only when water is properly oxygenated. The average values of nitrogen nitrite for the examined ponds (Table 1) are very similar to those measured by Skwierawski and Szyperek (2002) in field ponds in Olsztyn Lake District: 0.010 mg N-NO$_2^-$·dm^{-3} for water ponds on farmlands and 0.025 mg N-NO$_2^-$·dm^{-3} for ponds in build-up areas. Koc et al. (2001) measured average annual concentrations: 0.009 mg N-NO$_2^-$·dm^{-3} (range 0.004–0.018) for water ponds on farmlands and 0.020 mg N-NO$_2^-$·dm^{-3} (range 0.003–0.095) for build-up areas. The highest concentration of nitrogen nitrite was found in pond 4 in June 2004 (Figure 3), which reflects the high levels of other forms of nitrogen discussed before (0.138 mg N-NO$_2^-$·dm^{-3}). The causes of the observed changes are the same. In pond 5, the nitrogen nitrite concentration declined five-fold from May to June and reached the value of 0.02 mg N-NO$_2^-$·dm^{-3}, which was probably due to enhanced nitrification process because the water had the highest oxygen level. The most stable object in terms of N-NO$_2^-$ concentration was pond 1, which also contained the least of this form of nitrogen. For the water of other

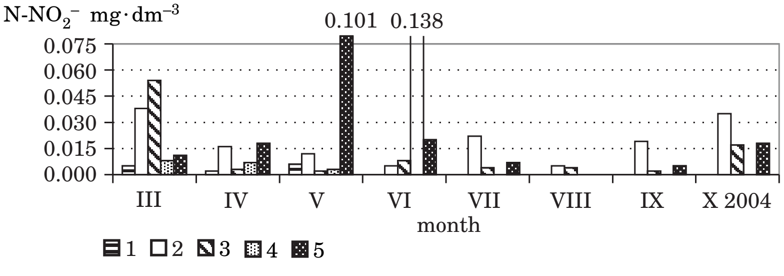

Fig. 3. Changes in the content of N-NO$_2^-$ in water of mid-field ponds during the vegetation season in 2004

ponds (2 and 3), the average concentration of N-NO_2^- was at the level of 0.012 and 0.019 mg·dm^{-3}, which corresponded to the measured dissolved oxygen concentrations in this water.

The analysis of the per cent shares of nitrogen forms (Figure 4) shows that nitrogen ammonium dominated in water samples taken from all of the ponds.

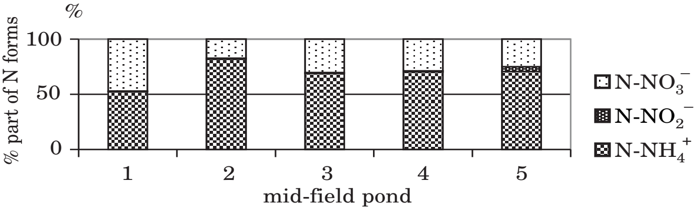

Fig. 4. Share of mineral nitrogen forms in water of mid-field ponds

Phosphorus is crucial for the growth of organisms and can be an element limiting the primary production of surface water. In the water of the five analyzed ponds, flushing rainwater with mineral and organic fertilizers from the fields, decomposing organic materials (food waste) and fertilizer remains from empty bags dumped near the ponds could be possible phosphorus sources. While analyzing the results, significant differences between summer and spring sampling dates were found. The highest concentration of PO_4^{3-} was noticed in pond 2 in July 2004 (189.4 mg·dm^{-3}). This value was a hundred-fold higher than in the other ponds (Figure 5).

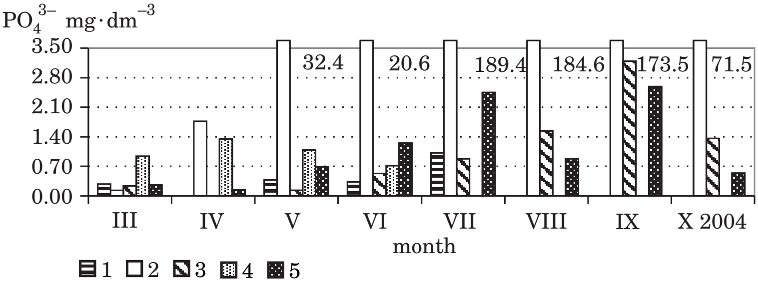

Fig. 5. Changes in the content of PO_4^{3-} in water of mid-field ponds during the vegetation season in 2004

The average values of concentrations and ranges for the other ponds were higher than those measured in water of ponds in Olsztyn Lake, which is: 0.241 mg PO_4^{3-}·dm^{-3} (range 0.003-0.900) for ponds on farmlands and 0.327 mg mg PO_4^{3-}·dm^{-3} (range 0.39-0.97) for build-up areas (Koc et al. 2001). Higher values of concentrations of orthophosphates were measured by Durkowski and Woroniecki (2001), who examined water ponds and fish ponds located in villages of Western Pomerania, i.e. 4.796 mg $PO_4^{3-} \cdot dm^{-3}$ (range 0.002-27.8). The upper range in that research can be compared to the results for pond 2, but is still several fold lower. According to Gałczyńska et al. (2005), the average concentration of orthophosphates at the level of 1.7 mg $PO_4^{3-} \cdot dm^{-3}$ was measured in the water of a field pond near Krąpiel in Western Pomerania. The highest level of orthophosphates was characteristic for pond 2, located near a former state farm. This was due to a rapid decrease in the water level in summer, as well as their recirculation from the bottom sludge, which is much faster under anaerobic conditions.

The process of releasing phosphorus from bottom sludge depends on oxygen conditions, redox potential at the border of water and sludge, reaction and, to some extent, on the concentration of iron and calcium ions in sludge and in the water above it. It has to be stressed that the highest values of orthophosphates were measured in summer, and such a situation occurs in polymictic lakes, where the concentration of phosphorus in spring is usually higher than in summer due to release of this element by interstitial waters and rapid mineralization of organic material in sludge at high temperatures (Koc, Skwierawski 2004, Skwierawski 2004). Water movements in polymictyc ponds, caused by wind, make the surface part of the bottom sludge, saprophel, diffuse into water. This slime, because of its chemical content and structure, causes immediate increase of oxygen consumption and dramatically reduces water clarity. Both of these phenomena initiate self-destruction of the biocenosis of a water pond because competitiveness of macrophytes and all animals, from zooplankton to fish, immediately declines. Under such conditions, atrophy of whole plant and animal species is observable, with dead plant and animal bodies secondarily increasing the deposit of saprophel so that the vicious circle starts again. Macro-hydrophytes in ponds 1 and 5 played a crucial role in creating a dynamic exchange of phosphorus compounds between water and sludge. The average concentration of orthophosphates in the water of these ponds was 0.4 and 1.1 $mg \cdot dm^{-3}$, respectively. On the one hand, sludge is a source of biogenic compounds for rooted plants, participating in the recycling of phosphorus accumulated in deeper layers, which theoretically are not involved in the circle, (Pieczyńska 1988, 1993). On the other hand, some plants rooted in the bottom cause oxygenation of the core of sludge deposits and limit water surface movements, improving conditions of sedimentation and limiting resuspension (Pieczyńska 1988, Wiśniewski 1995). In the water of ponds 3 and 4, the surrounding trees and bushes took part in reducing the concentration of orthophosphates by absorbing biogenic compounds.

CONCLUSIONS

1. The examined field water ponds in Pyrzyce Plain (Równina Pyrzycka) differ in the processes of degradation which occurred in these water bodies.

2. There was a large variation in the concentration of biogenic compounds in the water of the five ponds, depending on the form of athropopression and vegetation in each pond and its surroundings.

3. The high level of PO_4^{3-} and $N\text{-}NH_4^+$ in the water of the ponds and succession of water plants prove that water ponds 2, 3 and 4 are highly eutrophicated.

4. The extremely high concentration of PO_4^{3-} in the water of pond 2, located near a former state farm, can be classified as hypertrophic. This pond also demonstrated the highest oxygen deficit.

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ASSESSMENT OF THE INFLUENCE OF SEWAGE SLUDGE FERTILIZATION ON YIELD AND CONTENT OF NITROGEN AND SULPHUR IN MAIZE (*ZEA MAYS* L.)

Krzysztof Gondek

Chair of Agricultural Chemistry
University of Agriculture in Krakow

Abstract

The effect of sewage sludge fertilization on nitrogen and sulphur content in maize was assessed in a pot experiment conducted in 2003-2005. The experimental design comprised the following treatments in four replications on three soils: treatment without fertilizer – (0); mineral fertilization – (NPK); farmyard manure – (FYM); sewage sludge A – (SSA); a mixture of sewage sludge A with peat – (MSSA); sewage sludge B – (SSB) and a mixture of sewage sludge B with peat – (MSSB). The tests were conducted on weakly loamy sand (SI), sandy silt loam (SII) and medium silt loam (SIII), which were collected from the arable layer (0-20 cm) of ploughed land in the vicinity of Krakow. Sewage sludge which originated from two municipal mechanical and biological wastewater treatment plants, and their mixtures with peat (the materials were mixed in a 1:1 weight ratio in conversion to dry mass of organic matter) were used in the experiment. After wet mineralization of maize biomass in concentrated sulphuric acid, nitrogen was determined using Kjeldahl method in a Kjeltec II Plus apparatus. Sulphur was assessed after material mineralization in a concentrated nitric acid using the ICP–AES method in a JY 238 Ultrace apparatus. Fertilization with sewage sludge and sludge mixture with peat acted significantly better (as noted during the three-year experimental period) on maize yields than fertilization with mineral salts. In comparison with organic material and farmyard manure applied to soil, fertilization with mineral salts significantly increased nitrogen content in maize biomass. Sulphur content grew markedly in maize biomass fertilized with sewage sludge in comparison with the concentration of this element assessed in plants treated with farmyard manure. Values of the N:S ratio in aerial parts of maize from organic material treatments was within the optimal value range. The widest N:S ratio was assessed in the aerial parts and roots of maize receiving mineral fertilizers (NPK).

Key words: sewage sludge, maize, yielding, nitrogen, sulphur.

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dr inż. Krzysztof Gondek, Chair of Agricultural Chemistry, University of Agriculture in Krakow, Al. Mickiewicza 21, 31-120 Kraków, Poland

OCENA ODDZIAŁYWANIA NAWOŻENIA OSADAMI ŚCIEKOWYMI NA PLONOWANIE ORAZ ZAWARTOŚĆ AZOTU I SIARKI W KUKURYDZY (*ZEA MAYS* L.)

Abstrakt

Ocenę wpływu nawożenia osadami ściekowymi na zawartość azotu i siarki w kukurydzy przeprowadzono w doświadczeniu wazonowym w latach 2003-2005. Schemat doświadczenia, w czterech powtórzeniach, obejmował następujące obiekty na trzech glebach: obiekt bez nawożenia – (0); nawożenie mineralne – (NPK); obornik – (FYM); osad ściekowy A (SSA); mieszanina osadu ściekowego A z torfem – (MSSA); osad ściekowy B – (SSB) oraz mieszanina osadu ściekowego B z torfem – (MSSB). Do badań użyto piasek słabo gliniasty (SI), glinę piaszczystą pylastą (SII) i glinę średnią pylastą (SIII), które pobrano z warstwy ornej (0-20 cm) pól uprawnych z okolic Krakowa. W badaniach zastosowano osady ściekowe pochodzące z dwóch różnych komunalnych oczyszczalni mechaniczno-biologicznych oraz ich mieszaniny z torfem (materiały zmieszano w stosunku wagowym 1:1 w przeliczeniu na suchą masę materiałów organicznych). W biomasie kukurydzy – po mineralizacji na mokro w stężonym kwasie siarkowym – azot oznaczono metodą Kjeldahla na aparacie Kjeltec II Plus. Siarkę – po mineralizacji materiału w stężonym kwasie azotowym – oznaczono metodą ICP-AES na aparacie JY 238 Ultrace. Nawożenie osadami ściekowymi i mieszaninami osadów z torfem działało istotnie lepiej (w trzyletnim okresie badań) na plony kukurydzy niż nawożenie solami mineralnymi. W porównaniu z zastosowanymi doglebowo materiałami organicznymi i obornikiem, nawożenie solami mineralnymi istotnie zwiększyło zawartość azotu w biomasie kukurydzy. Istotnie zwiększyła się zawartość siarki w biomasie kukurydzy nawożonej osadami ściekowymi, w porównaniu z zawartością tego pierwiastka oznaczonego w roślinach nawożonych obornikiem. Wartości stosunku N : S w częściach nadziemnych kukurydzy z obiektów nawożonych materiałami organicznymi mieściły się w zakresie wartości optymalnych. Najszerszy stosunek N:S stwierdzono w częściach nadziemnych i korzeniach kukurydzy nawożonej mineralnie (NPK).

Słowa kluczowe: osady ściekowe, kukurydza, plonowanie, azot, siarka.

INTRODUCTION

Biomass production leads to soil depletion of available forms of nutrients. Fertilization is a basic method applied to maintain their optimal content in soil.

Many research projects have revealed considerable fertilizer value of sewage sludge (MAZUR 1996, PETERSEN 2003, AKDENIZ et al. 2006, LAVADO 2006, TOGAY et al. 2008). Suitability of sewage sludge for soil and plant fertilization results from its considerable concentration of organic substance and nutrients (JARAUSCH-WEHRHEIM et al. 2001, BARAN et al. 2002). When adhering to all the rules of safe application, sewage sludge can supplement or replace farmyard manure.

Among biogenic substances, sewage sludge contains the highest amounts of nitrogen and phosphorus (MAZUR 1996, KALEMBASA et al. 1999). CZEKAŁA (2002) and DEWIL et al. (2008) demonstrated that considerable quantities of sulphur are additionally accumulated in sewage sludge. Most plant nutrients, including nitrogen and sulphur in sewage sludge, occur as organic compounds. Having been introduced to soil, sewage sludge undergoes complex transformations, which do not determine its bioavailability.

Under the soil and climatic conditions occurring in Poland, nitrogen is the main factor affecting the amount and quality of plant yields. Nitrogen concentrations in plants do not pose any major hazard if balanced fertilizer doses are used (Kopeć et al. 1996). The need to fertilize plants with sulphur is mainly due to the role of this element in nitrogen metabolism, which may markedly improve yield biological value (Marska, Wróbel 2000, Scherer 2001).

The use of sewage sludge causes changes in physical, chemical and biological soil properties, which, depending on grain size distribution of soil, can differently influence nitrogen and sulphur bioavailability and may affect biological value of crop yield. The aim of the research was to assess how fertilization with sewage sludge and its mixtures with peat affect yield of maize, cultivated on soils different in grain size distribution, and its content of nitrogen and sulphur.

MATERIAL AND METHODS

The effect of sewage sludge fertilization on nitrogen and sulphur content in maize was assessed in a pot experiment conducted in 2003-2005. The experimental design comprised the following treatments in four replications on three soils: treatment without fertilizers – (0); mineral fertilization – (NPK); farmyard manure – (FYM); sewage sludge A – (SSA); a mixture of sewage sludge A with peat – (MSSA); sewage sludge N – (SSB) and a mixture of sewage sludge B with peat – (MSSB). The term “fertilizer treatments” used in the paper refers to all treatments except the object without fertilizers (0). The following soil material was used in the experiment: weakly loamy sand – (SI), sandy silt loam – (SII) and medium silt loam – (SIII), which were collected from the arable layer (0–20 cm) of ploughed land in the vicinity of Krakow. The sewage sludge, obtained from two municipal mechanical and biological wastewater treatment plants, and its mixtures with peat (the materials were mixed in a 1:1 weight ratio in conversion to dry mass of organic matter) were used in the experiment. Peat, which had 408 $g \cdot kg^{-1}$ dry mass content, contained 88 $g \cdot kg^{-1}$ ash, 34.4 g $g \cdot kg^{-1}$ N, 0.91 g P, 1.14 g K and 2.48 g $S \cdot kg^{-1}$ d.m. The chemical composition of the other organic materials and soil (values converted into dry mass determined at 105°C) is specified in Tables 1 and 2.

PCV pots used for the experiment contained 5.50 kg of air-dried soil material. Before the experiment, the soils were gradually moistened to 30%

Table 1

Chemical composition of materials used in experiment

Determination		FYM	Sewage sludge (SSA)	Sewage sludge + peat (MSSA)	Sewage sludge (SSB)	Sewage sludge + peat (MSSB)
Dry matter, $g \cdot kg^{-1}$		189	310	343	418	372
pH (H_2O)		6.22	6.12	5.57	5.73	5.20
Organic matter, $g \cdot kg^{-1}$ d.m.		679	353	652	552	771
Total forms						
N	$g \cdot kg^{-1}$ d.m.	25.1	17.2	25.5	42.4	38.4
S		7.24	8.81	6.23	14.62	7.85
P		22.60	5.48	3.00	19.32	0.76
K		26.69	2.71	1.88	2.81	1.64
Cr	$mg \cdot kg^{-1}$ d.m.	6.07	19.74	10.25	37.88	17.47
Zn		531	899	488	1684	821
Pb		3.99	65.9	38.2	29.4	17.5
Cu		338.0	78.3	40.6	119.4	51.8
Cd		1.28	2.71	1.45	2.25	1.03
Ni		11.74	13.32	7.14	25.36	12.07
Hg		trace	3.58	1.80	2.29	1.07

Table 2

Some properties of soils before the establishment of the experiment

Determination			Soil		
			(SI)	(SII)	(SIII)
Granulometric composition Ø	1.0 - 0.1 mm	%	78	42	28
	0.1 - 0.02 mm		13	33	29
	< 0.02 mm		9	25	43
pH KCl			6.21	5.69	5.30
Hydrolitic acidity		$mmol(+) \cdot kg^{-1}$ d.m.	11.2	23.4	33.2
Sum of alkaline cation			39.9	86.8	128.4
Total N		$g \cdot kg^{-1}$ d.m.	0.36	1.25	1.72
Organic C			9.37	13.36	17.68
Total S			0.16	0.28	0.32

of maximum water capacity. After moistening, sandy silt loam and medium silt loam were limed to fulfill the conditions stated in the Regulation of the Minister of the Natural Environment on municipal sewage sludge (*Regulation...* 2002). The dose of sludge was applied separately in each pot. Chemically pure CaO was used and its dose was calculated on the basis of soil hydrolytic acidity. Afterwards, all soils were left for 4 weeks and water loss was occasionally supplemented. After that, organic materials were introduced in the amount corresponding to a dose of 1.20 g $N \cdot pot^{-1}$. Phosphorus and potassium were supplemented in all treatments (except the control) to a level equal the one achieved with the organic materials. In the first year, 1.26 g $P \cdot pot^{-1}$ was used as an aqueous solution of $Ca(H_2PO_4)_2 \cdot H_2O$ and 1.48 g $K \cdot pot^{-1}$ as an aqueous KCl solution. An identical nitrogen dose was applied to the mineral (NPK) treatment as an aqueous solution of NH_4NO_3, and equal P and K doses, respectively in form of $Ca(H_2PO_4)_2 \cdot H_2O$ and KC, were added. Equal fertilizer component amounts, i.e. 0.80 g N; 0.20 g P and 1.40 g $K \cdot pot^{-1} \cdot year^{-1}$ as chemically pure salts, were used in the second and third year of the experiment.

Each year of the experiment, cv. San maize (FAO 240) was cultivated as a test plant and 5 plants per pot were left. Maize was harvested at the 7–9 leaves stage. The plant vegetation periods were respectively: 47 days in the first year, 66 days in the second and 54 days in the third. Throughout the experiment, the plants were watered with distilled water to 50% of maximum water capacity.

After the harvest, the plants were dried (at 70°C) to constant weight and the yield of dry mass of shoots and roots was determined. The plant material was comminuted in a laboratory mill and wet mineralized in concentrated sulphuric acid. Afterwards, nitrogen was determined using Kjeldahl method (Ostrowska et al. 1991) in a Kjeltec II Plus apparatus (Tecator). For sulphur determination, the plant material was wet mineralized in concentrated nitric acid and after evaporation mixed with magnesium nitrate and evaporated again. The samples thus prepared were mineralized in a muffle furnace, initially at 300°C (for 2 hours) and then at 450°C (for 3 hours). The remains were dissolved in diluted nitric acid (25%) (Ostrowska et al. 1991). Sulphur was determined in solutions prepared in the above way using the ICP–AES method in an Ultrace JY 238 apparatus.

The results were verified statistically according to a fixed model, in which the factor was soil fertilization or soil. One–way ANOVA was used for the computations and the significance of differences was estimated using LSD Fisher test at significance level $\alpha<0.05$ (Stanisz 1998).

RESULT AND DISCUSSION

Organic materials used for the experiments differed in chemical composition, including nitrogen and sulphur content (Table 1). Peat supplement to sewage sludge diminished the content of most elements in the mixture in comparison with the content of elements in sludge, except the nitrogen content in A (MSSA) sludge mixture, which was a result of a higher level of this nutrient in peat.

The soil material used for the analyses belonged to different groups according to the grain size distribution, but it also significantly differed in chemical properties, including total nitrogen and total sulphur content (Table 2).

The three-year average of maize biomass yields (both aerial parts and roots) from the treatments obtained on weakly loamy sand (SI) were notably (over 20%) lower than yields produced on the other soils: sandy silt loam (SII) and medium silt loam (SIII) (Figure 1). The difference in the biomass yield obtained on sandy silt loam (SII) and medium silt loam (SIII) was not significant.

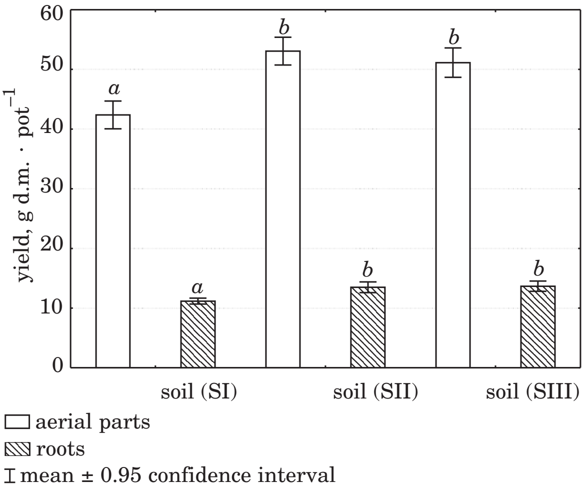

Fig. 1. Mean yields of aerial parts and of roots of maize from fertilization objects for a three-year period.
Means followed by the same letters did not differ significantly at $\alpha < 0.05$ according to Fisher's test

Fertilization with sewage sludge or the mixtures with peat and the treatment with farmyard manure produced markedly greater yields than the treatment where only mineral compounds were used (Table 3). Higher yields from the mineral salt treatment were recorded in the first year of the experiment (Figure 2). In the subsequent years, maize's response to fertiliza-

Table 3

Average (for 3 years) yields of maize aerial parts and roots, dry mass, total nitrogen and sulphur content in maize

Object	Yield of biomass		Nitrogen in the plant		Sulphur in the plant	
	g d.m.·pot^{-1}		g N·kg^{-1} d.m.		g S·kg^{-1} d.m.	
	PAG*	R**	PAG*	R**	PAG*	R**
No fertilisation (0)	22.1 *a*	7.4 *a*	8.1 *a*	6.95 *a*	0.98 *cd*	1.69 *b*
NPK	42.5 *b*	10.1 *b*	19.2 *d*	15.68 *b*	0.65 *a*	0.69 *a*
Farmyard manure (FYM)	48.0 *c*	13.1 *cd*	12.5 *bc*	7.81 *a*	0.92 *bc*	1.70 *b*
Sewage sludge A (SSA)	47.3 *bc*	11.8 *c*	13.1 *c*	7.83 *a*	1.06 *d*	2.99 *d*
Sewage sludge A + peat (MSSA)	49.3 *c*	11.8 *c*	12.7 *bc*	7.88 *a*	0.91 *bc*	1.86 *bc*
Sewage sludge B (SSB)	50.4 *c*	14.1 *d*	12.7 *bc*	7.21 *a*	1.00 *cd*	2.15 *c*
Sewage sludge B + peat (MSSB)	55.6 *d*	15.8 *e*	11.1 *b*	7.19 *a*	0.82 *b*	1.54 *b*

* aerial parts, ** roots
Means followed by the same letters in columns did not differ significantly at $\alpha < 0.05$ according to Fisher's test

tion with organic materials was positive despite the blurring differences in the harvest from soils of the granular structure of weakly loamy sand (SI) and sandy silt loam (SII). In both cases, higher biomass yields were obtained when sewage sludge and peat mixtures were used than when sludge alone was applied. The fertilizer value of municipal sewage sludge was confirmed in numerous studies (CHRISTODOULAKIS, MARGARIS 1996, STĘPIEŃ et al. 2000). Also in the authors' own investigations, fertilization with organic materials, including sewage sludge, produced better results expressed by the amount of maize biomass yields than the treatment with mineral salts. This effect cannot be attributed exclusively to the activity of the applied sewage sludge or its mixtures with peat. Beside the residual fertilizer effect of organic materials, the supplementary mineral fertilization conducted in the second and third year of the research proved to be an important factor affecting plant yielding. This conclusion was supported by the results obtained by EVANYLO (1999), who demonstrated that about 30% of nitrogen is released from sewage sludge in the first year after application as a result of mineralization, in contrast to 15% in the second year and only 7% in the third year. It may be assumed that the factor that determined plant yielding in the treatments where organic substances were used may be the introduction of other components with organic materials, such as sulphur, magnesium or microelements to soil, whose amounts were not balanced. WIATER et al. (2004) observed inferior direct effect of sewage sludge granulate on maize yield than that achieved by mineral fertilization, but the residual effect of the granulate fertilizer was better. CHATHA et al. (2002), DRAB and DEREN-

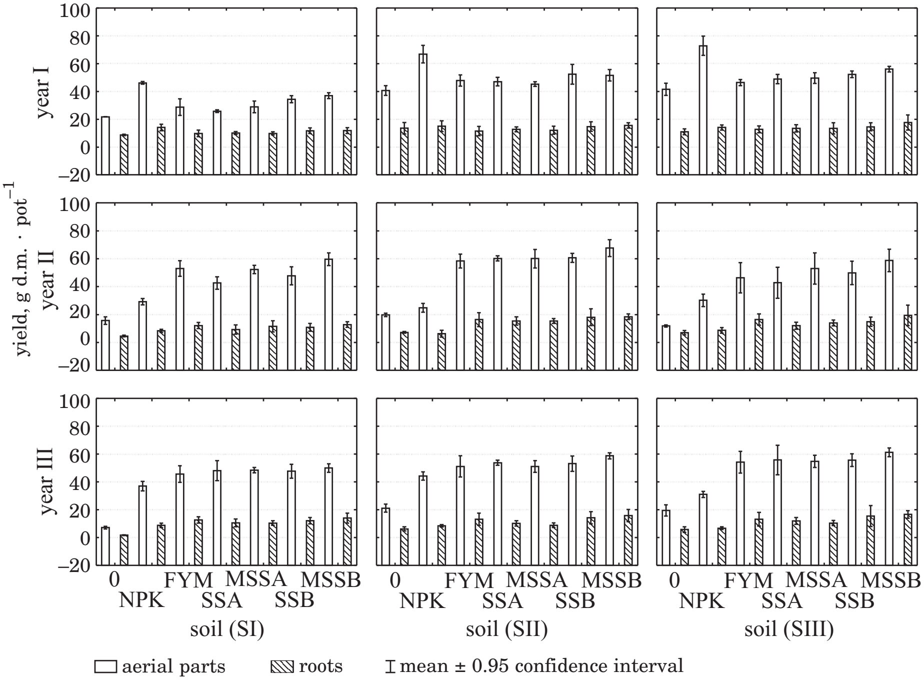

Fig. 2. Yield of aerial parts and roots of maize from the three- years experiment on each soil: 0 – soil without fertilization, NPK – soil with addition of mineral salts, FYM – soil with addition of farmyard manure, SSA – soil with addition of municipal sewage sludge A, MSSA – soil with addition of sewage sludge A mixture with peat, SSB – soil with addition of municipal sewage sludge B, MSSB – soil with addition of sewage sludge B mixture with peat

gowska (2003) and Lavado (2006) demonstrated an advantageous effect of fertilization with sewage sludge on crop yields. Moreover, Vasseur et al. (1998) and Akrivos et al. (2000) revealed that crop yield, irrespective of the type of soil, was conditioned by the quantity of the applied sewage sludge.

The biomass of aerial parts and roots of plants from the mineral salt (NPK) treatment had the highest concentrations of nitrogen (Table 3). The three-year average nitrogen content in this treatment was 19.2 g N·kg^{-1} d.m., being from 46% to 73% higher than the concentration determined in the aerial parts of maize fertilized with organic materials. Contrary to mineral fertilization, more nitrogen contained in farmyard manure or sewage sludge occurs in organic compounds unavailable to plants. The rate of release of mineral nitrogen forms from organic material is conditioned by intensity of biological process, which may limit the effect of this fertilizer component, especially during the initial period (Barbarika et al. 1985, Gondek and Chmiel 2006). Bigger differences in nitrogen content were observed in roots, where it was from 99% to 118% lower in the organic treatments than in the treatment where mineral fertilization with this component was con-

Irrespective of the applied fertilization or soil, more total sulphur was found in maize roots, which resulted from the accumulation of this element in a much smaller yield of this maize part (Table 3, Figure 5). The applied fertilization with organic substances significantly raised sulphur content in maize biomass in comparison with the content found in biomass of plants fertilized with nitrogen, phosphorus and potassium as mineral salts (NPK). The average sulphur content in maize biomass, irrespectively of its part, in

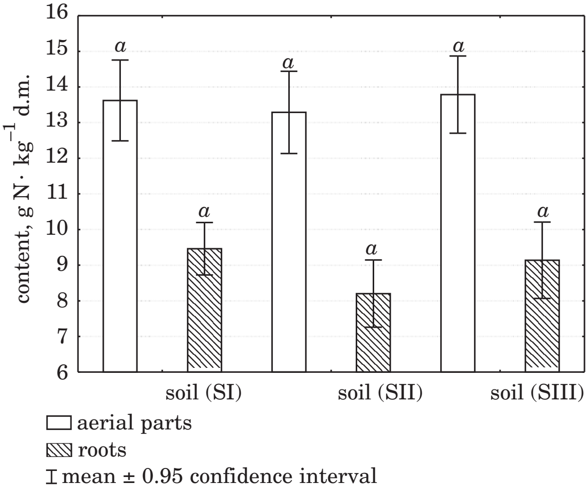

Fig. 3. Average (for 3 years) nitrogen content in maize aerial parts and roots from treatments

Means followed by the same letters did not differ significantly at $\alpha < 0.05$ according to Fisher's test

ducted. No significant differences in nitrogen content in maize biomass were found depending on soil (Figure 3), and the influence of sewage sludge fertilization on nitrogen concentrations in plant biomass was comparable with the effect of fertilization with sewage sludge mixtures and peat (Figure 4). Also in the previous research conducted by Gondek and Filipek-Mazur (2006), irrespective of the fertilization date or cultivated plant, or even its part, the highest nitrogen concentrations were found in plant biomass from mineral salt treatments. Moreover, farmyard manure and sewage sludge used on different dates did not differentiate largely the content of this nutrient in plants. In the authors' own investigations, an identical nitrogen dose was applied to all soils in treatments fertilized with organic materials and mineral salts. Considering the nitrogen amount which may be mobilised from the applied organic substances and its supplementation with the mineral form of this element or increased organic substance dose contributed to improved nitrogen uptake by plants. Jakubus (2006) points to a relatively weak effect of sewage sludge on chemical composition of plants, although the author demonstrated that it depended on the type of sewage sludge.

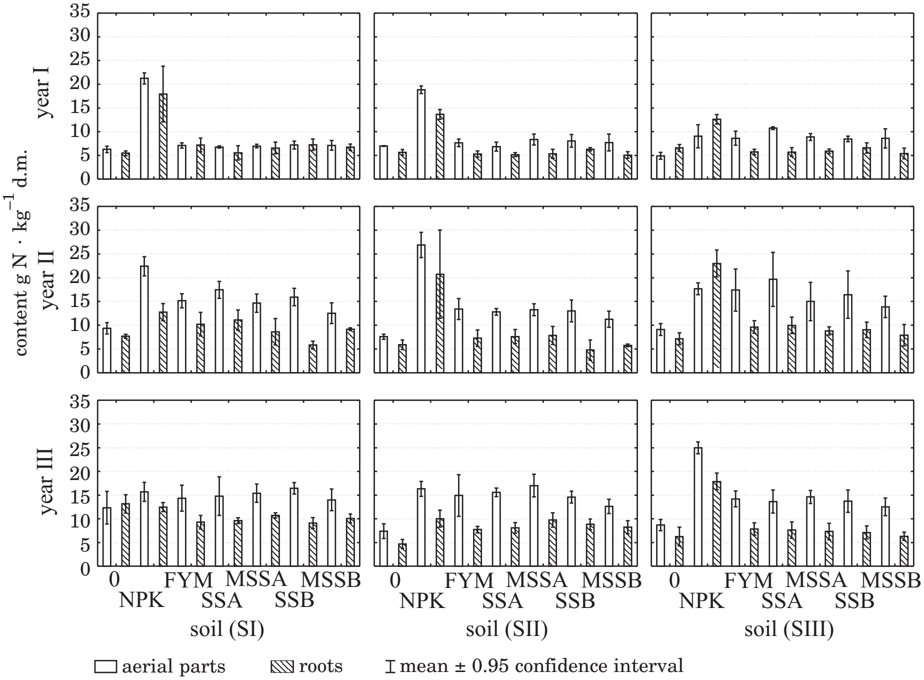

Fig. 4. Nitrogen content in maize aerial parts and roots from three soils in each year of the experiment; cf. Fig. 2 for the key

the treatment without fertilization (0) (at markedly smaller yield) did not differ significantly from its content determined in plant biomass from the treatments fertilized with farmyard manure (FYM) or mixtures of sewage sludge with peat (MSSA, MSSB) – Figure 5. Greater relative variation in sulphur content was detected in maize biomass depending on soil (Figure 6). In light soil (SI), the content was the highest, whereas the mean sulphur content in maize biomass from sandy silt loam (SII) and medium silt loam (SIII) soils did not differ markedly for fertilized treatments and years. These dependences clearly resulted from concentrations of this element in a lower yield of maize cultivated in weakly loamy sand (SI), but not from the content of bioavailable forms of this element in soils. According to KALEMBASA and KUZIEMSKA (2008), fertilization with sewage sludge markedly affects sulphur concentrations in plants, although, as demonstrated by CARDELLI et al. (2006), the rate of organic materials mineralization, including waste materials, may be different, which considerably conditions sulphur transfer into the soil solution and its uptake by plants. Also in the research conducted by WOŁOSZYK (2003), plant fertilization with composts manufactured from sewage sludge, both with and without industrial waste supplement, increased sulphur content in grass mixture. According to this author, fertilization with

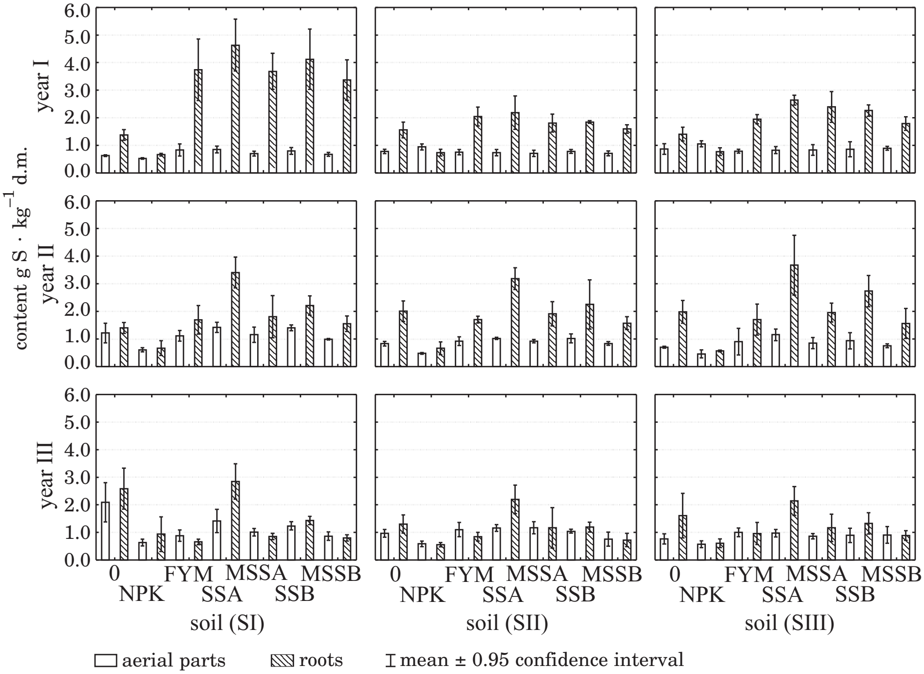

Fig. 5. Sulphur content in maize aerail parts and roots from three soils in each year of the experiment; cf. Fig. 2 for the key

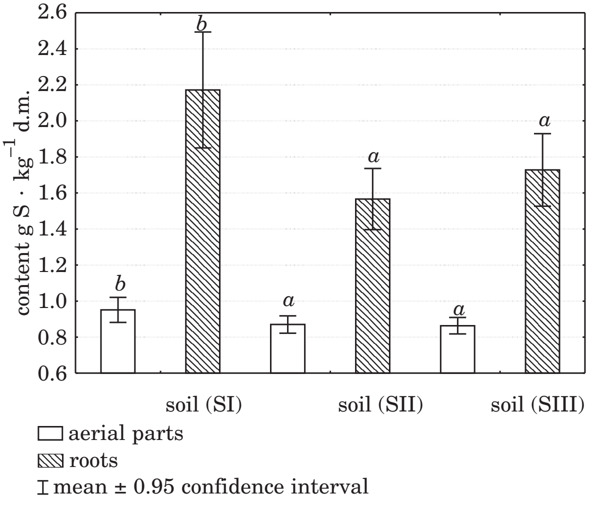

Fig. 6. Average (for 3 years) sulphur content in aerial parts and roots from fertilization objects for the three years of the experiment

Means followed by the same letters did not differ significantly at $\alpha < 0.05$ according to Fisher's test

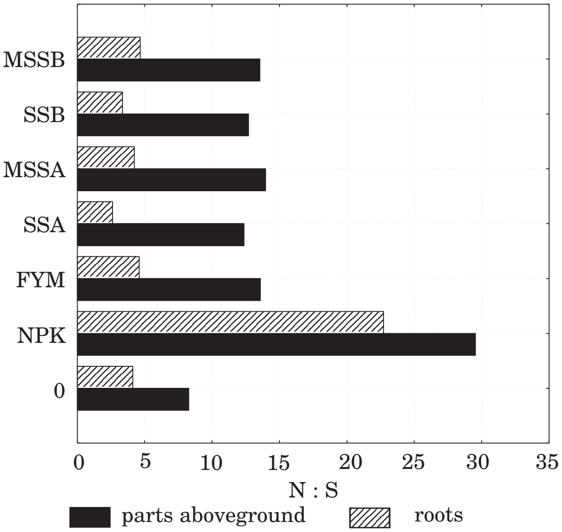

Fig. 7. An average (for 3 years and 3 soils) ratio N : S in aerial parts and roots
cf. Fig. 2 for the key

composts produced from sewage sludge did not have any marked influence on sulphur concentrations in plant biomass in the second year of the fertilizer effect of these materials.

According to Fotyma (2003), the optimal N:S ratio in plants depending on species is 15-10:1. In the conducted research, the values of the N:S ratio in maize aerial parts from the treatments fertilized with organic materials fell within the range of optimal values (means for soils and years) – Figure 7. Much smaller values of this parameter characterized maize root biomass, which resulted from much higher sulphur content in these plant parts and much lower nitrogen concentrations in roots than in aerial parts. The widest N:S ratio was found in the aerial parts and roots of maize receiving mineral fertilizers (NPK), which was determined by the soil depletion of bioavailable sulphur forms (Filipek-Mazur et al. 2006).

CONCLUSIONS

1. Over the three-year period of the research, fertilization with sewage sludge and mixtures of sludge with peat produced a significantly better effect on maize yields than a treatment with mineral salts.

2. In comparison with organic materials and farmyard manure applied to the soil, fertilization with mineral salts markedly increased nitrogen concentrations in maize biomass.

3. The sulphur content grew significantly in maize biomass fertilized with sewage sludge in comparison with concentrations of this element assessed in plants fertilized with farmyard manure.

4. Values of the N:S ratio in maize shoots from treatments fertilized with organic materials were within the range of optimal values. The widest N:S ratio was assessed in aerial parts and roots of maize receiving mineral fertilization (NPK).

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COMPARISON OF THE EFFECT OF LIMING AND MAGNESIUM TREATMENT OF HEAVY METAL CONTAMINATED SOIL ON THE CONTENT OF MAGNESIUM, CALCIUM AND IRON IN BROAD BEANS (*VCIA FABA* L. SSP. *MAIOR*)

Janina Gospodarek[1], Aleksandra Nadgórska-Socha[2]

[1]Chair of Agricultural Environment Protection
University of Agriculture in Krakow
[2]Chair of Ecology, University of Silesia

Abstract

Tests have been conducted to determine the effect of liming and magnesium treatment on the content of magnesium, calcium and iron in broad bean plants growing on soil polluted with such heavy metals as cadmium, lead, nickel, copper and zinc.

In 2005, an experiment was conducted in the village Zagaje Stradowskie (Świętokrzyskie Province) on degraded Chernozem formed from loess, acid in reaction and containing 1.13% of organic carbon. Analyses were performed on aerial parts of cv. White Windsor broad bean (*Vicia faba* L. ssp. *maior)*, cultivated in three series: on limed soil, on soil receiving magnesium fertilizers; on unlimed soil without magnesium fertilization. In each series, the plants were cultivated on the following objects: unpolluted soil with a natural content of heavy metals (control); unpolluted soil with a natural content of heavy metals and mineral fertilization (control+NPK); soil polluted with a cadmium dose 4 $mg \cdot kg^{-1}$ d.m.; soil polluted with a dose of 530 $mg \cdot kg^{-1}$ of lead; soil contaminated with a copper dose 85 $mg \cdot kg^{-1}$ d.m., soil contaminated with a dose of 1000 $mg \cdot kg^{-1}$ of zinc and soil polluted with a nickel dose 110 $mg \cdot kg^{-1}$ d.m. Liming was based on the analysis of hydrolytic acidity of soils from individual objects. The administered dose was established according to 1Hh. Magnesium treatments were identical in all objects. i.e. 20.4 $mg \cdot kg^{-1}$ soil d.m.

Soil contamination with zinc or nickel leads to a considerable decrease in magnesium and calcium level in broad bean aerial parts but rises iron level. Liming rather than ma-

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dr inż. Janina Gospodarek, Chair of Agricultural Environment Protection, University of Agiculture in Krakow, al. Mickiewicza 21, 31-120 Krakow, Poland

gnesium fertilization applied to soil polluted with heavy metals, such as zinc or nickel, contributes to balancing the content of the analyzed macronutrients in broad beans. The content of Ca, Fe and Mg in plants after liming approached the level determined in the control plants.

Key words: heavy metals, magnesium fertilization, liming, accumulation, Mg, Ca, Fe.

PORÓWNANIE ODDZIAŁYWANIA WAPNOWANIA I NAWOŻENIA MAGNEZOWEGO GLEBY SKAŻONEJ METALAMI CIĘŻKIMI NA ZAWARTOŚĆ MAGNEZU, WAPNIA I ŻELAZA W ROŚLINACH BOBU (*VICIA FABA* L. SSP. *MAIOR*)

Abstrakt

Celem badań było określenie wpływu wapnowania i nawożenia magnezowego na zawartość mangezu, wapnia i żelaza w roślinach bobu rosnących w warunkach gleby zanieczyszczonej pojedynczymi metalami ciężkimi: kadmem, ołowiem, niklem, miedzią i cynkiem.

Gleba użyta w doświadczeniu to czarnoziem zdegradowany wytworzony z lessu o odczynie kwaśnym i zawartości węgla organicznego 1,13%. Doświadczenie przeprowadzono w 2005 r., w miejscowości Zagaje Stradowskie (woj. świętokrzyskie). Analizie poddano części nadziemne bobu (*Vicia faba* L. ssp. *maior*) odm. Windsor Biały uprawianego w trzech seriach: na glebie wapnowanej; poddanej nawożeniu magnezowemu; niewapnowanej i nienawożonej magnezem. W każdej serii rośliny uprawiano w następujących obiektach: gleba niezanieczyszczona – o naturalnej zawartości metali ciężkich (kontrola); gleba niezanieczyszczona – o naturalnej zawartości metali ciężkich nawożona mineralnie (kontrola + NPK); gleba zanieczyszczona kadmem w dawce 4 $mg \cdot kg^{-1}$ s.m.; gleba zanieczyszczona ołowiem w dawce 530 $mg \cdot kg^{-1}$ s.m.; gleba zanieczyszczona miedzią w dawce 85 $mg \cdot kg^{-1}$ s.m.; gleba zanieczyszczona cynkiem w dawce 1000 $mg \cdot kg^{-1}$ s.m.; gleba zanieczyszczona niklem w dawce 110 $mg \cdot kg^{-1}$ s.m. Wapnowanie przeprowadzono opierając się na analizie kwasowości hydrolitycznej gleby z poszczególnych obiektów. Zastosowano dawkę według 1 Hh. Na wszystkich obiektach zastosowano jednakowe nawożenie magnezowe: 20,4 $mg\ Mg \cdot kg^{-1}$ s.m. Skażenie gleby cynkiem lub niklem prowadzi do znacznego obniżenia poziomu magnezu i wapnia w częściach nadziemnych bobu, a podwyższa poziom żelaza. Wapnowanie gleby skażonej metalami ciężkimi, takimi jak cynk lub nikiel, bardziej niż nawożenie magnezowe przyczynia się do zrównoważenia zawartości badanych makropierwiastków w roślinie – zawartość Ca, Fe i Mg w roślinach po zwapnowaniu gleby zbliżyła się do poziomu stwierdzonego w roślinach kontrolnych.

Słowa kluczowe: metale ciężkie, wapnowanie, akumulacja, Mg, Ca, Fe.

INTRODUCTION

Liming and magnesium fertilization are usually mentioned as treatments which alleviate the unfavourable effect of heavy metals on plants (HELMISAARI et al. 1999). Calcium oxide mixed with sodium sulphide ($Na_2S/CaO=1:1$) significantly reduces availability of heavy metals, such as Zn, Cu, and Ni (WANG et al. 2008). Liming most effectively reduces the solubility and the plant uptake of Zn and Cd in pea (*Pisum arvense* L.) (KREBS at al. 1998). Liming the soil fertilized with sewage sludge reduced the heavy metal content in plant tissues of *Brassica chinensis*, which were all below the admissible levels for vegetables except Fe (Wong et al. 2001). On the other hand, magnesium in plants is crucial for proper metabolism in cells and in the whole organism. Magnesium deficiency in plants appears most frequently in crops growing on excessively acidified soils (Kabata-Pendias, Pendias 1993).

Heavy metals present in soil may variously affect the macronutrient content in plants. Copper (administered to soil at doses of 4, 40, 400 mg kg^{-1}) increased the content of calcium, sodium, magnesium and potassium, but decreased that of phosphorus in spring barley. The increased zinc content in soil (dosed as mentioned above) was accompanied by a rise in the content of calcium, magnesium, potassium and partly phosphorus and sodium in plants. High doses of zinc resulted in decreased levels of phosphorus and sodium in spring barley (Wyszkowski et al. 2006).

The investigations were conducted to determine the effect of liming and magnesium treatment on the content of magnesium, calcium and iron in broad bean plants growing on soil polluted with single heavy metals: cadmium, lead, nickel, copper or zinc.

MATERIALS AND METHODS

In 2005, a field experiment was conducted in the village Zagaje Stradowskie (Świętokrzyskie Province) on degraded Chernozem soil formed from loess, acid in reaction and containing 1.13% or organic carbon. Analyses were performed on aerial parts of cv. White Windsor broad beans (*Vicia faba* L. ssp. *maior)*, cultivated in three series: on limed soil, on soil receiving magnesium fertilizers; on unlimed soil without magnesium fertilization. In each series, the plants were cultivated on the following objects: unpolluted soil with a natural content of heavy metals (control); unpolluted soil with a natural content of heavy metals and mineral fertilization (control+NPK); soil polluted with a cadmium dose 4 $mg \cdot kg^{-1}$ d.m.; soil polluted with a dose of 530 $mg \cdot kg^{-1}$of lead; soil contaminated with a copper dose 85 $mg \cdot kg^{-1}$ d.m., soil contaminated with a dose of 1000 $mg \cdot kg^{-1}$of zinc and soil polluted with a nickel dose 110 $mg \cdot kg^{-1}$ d.m. The following lime doses were administered (in mg $CaO \cdot kg^{-1}$ soil d.m.): control – 619, control + NPK – 672, soil polluted with cadmium – 630, soil polluted with lead – 596, soil polluted with copper – 798, soil polluted with zinc – 1.142, soil polluted with nickel – 818. Magnesium treatments were identical for all objects (20.4 mg $Mg \cdot kg^{-1}$ soil d.m.). Magnesium was supplemented to soil as a water solution of $MgSO_4 \cdot 7H_2O$. The method for heavy metal application, the basic fertilization, soil pH in each object as well as the methods used for soil chemical analyses have been presented in another paper (Gospodarek, Nadgórska-Socha 2007). Liming was conduced on the basis of hydrolytical acidity analysis of soils from individual objects. The administered dose was established according to 1Hh. Plant material samples for chemical analyses were collected at the seed milk maturity phase. The chemical analysis of the plant material involved determinations of iron, magnesium and calcium. Plant material was washed in tap and distilled water, dried to constant weight at 105°C, ground to fine powder, then mineralized and dissolved in 10% HNO_3. After filtration, the Mg, Ca, Fe content was measured using Flame Atomic Absorption Spectrometry (FAAS) (Ostrowska et al. 1991, Azcue, Murdoch 1994). The accuracy of the analytical procedure was controlled by using samples of the reference material in each series of analysis (Certified Reference material CTA-OTL-1 Oriental Tobacco Leaves). The data were processed using Statistica software to compute significant statistical differences between samples ($p<0.05$) according to Tukey's multiple range test.

RESULTS AND DISCUSSION

Liming caused an increase in the soil pH by *ca* 0.6-1 unit, whereas magnesium treatment did not result in any major changes in the soil pH (Gospodarek, Nadgórska-Socha 2008). Soil contamination with zinc and nickel led to a significant decrease in magnesium content in broad bean aerial parts (Figure 1). Magnesium concentration in broad beans growing on copper contaminated soil was also slightly lower than in the control. In zinc contaminated soil, liming caused *ca* 13-fold increase in magnesium content, whereas magnesium fertilization led to *ca* 6-fold increase in this element. Neither liming nor magnesium treatment of nickel polluted soil had any significant influence on magnesium content. On the other hand, on soil polluted with lead or cadmium, similarly to the control soils, magnesium treatment contributed to a notable increase in magnesium content in broad bean aerial parts, whereas liming did not cause any significant changes (in the soil contaminated with Pb) or contributed to a decline in this element level in broad bean aerial parts (in control plants and in plants growing on cadmium contaminated soil). In research conducted by Wyszkowski (2002), soil contamination by cadmium (10, 20, 30 and 40 mg $Cd \cdot kg^{-1}$) and magnesium fertilization (50 and 100 mg $Mg \cdot kg^{-1}$ of soil) did not cause much variation in the distribution of macronutrients in aerial organs and roots of yellow lupine.

In the case of copper polluted soil, magnesium treatment only slightly raised the plant magnesium content, while liming caused an increase in magnesium concentration in broad bean aerial parts by ca 25%. In the authors' previous research (Gospodarek, Nadgórska-Socha 2008), which focused on the effect of different lime doses on magnesium content in broad bean aerial parts, a decline in this element was also observed in plants harvested

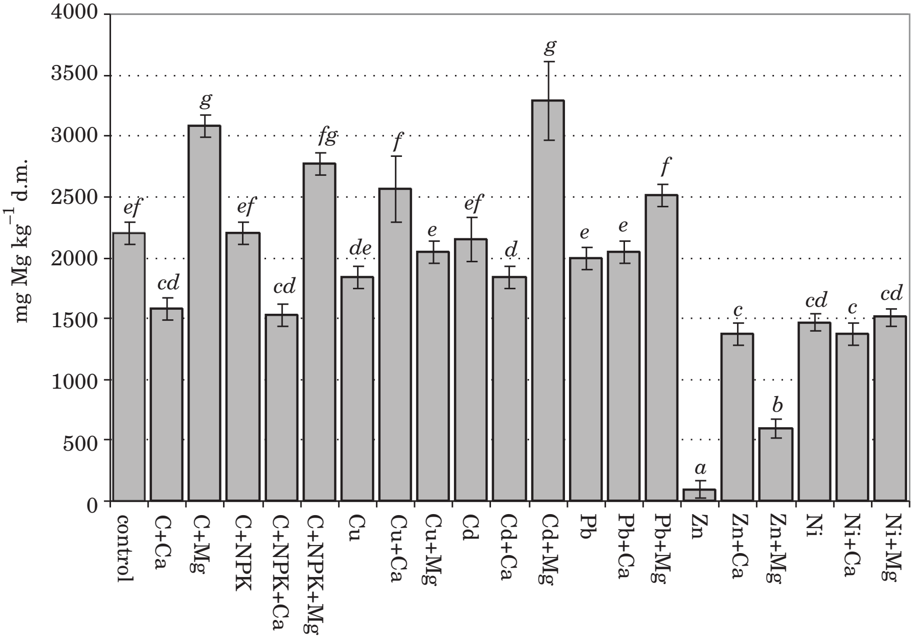

Fig. 1. Magnesium content in aerial parts of broad bean (*Vicia faba* L. ssp. *maior*) cultivated in unpolluted soil (control, NPK) and in soil contaminated with single heavy metals and after application of liming or magnesium treatment. Values marked with different letters are statistically different at $p < 0.05$

from plots with cadmium contaminated soil as a result of soil liming. On the other hand, liming of copper contaminated soil (using both the higher and lower doses) caused a slight decrease in Mg content. In nickel polluted soil, a double dose of lime diminished magnesium content in broad bean aboveground parts by about 33% in comparison with the object contaminated with this element but not limed. In contrast, in soil contaminated with zinc, liming (both when the lower and higher CaO doses were administered) led to an increase in Mg content reaching almost the level similar to that determined in plants harvested from unpolluted plots. However, liming did not affect magnesium concentrations in aerial parts of broad bean cultivated on lead polluted soil.

Soil pollution with zinc and nickel caused a very high decline (15-fold and 5-fold, respectively) in calcium content in broad bean aerial parts (Figure 2). Liming of soil contaminated with zinc caused *ca* 9-fold increase in the content of this element, whereas magnesium fertilization doubled the Ca level. In plants growing on nickel contaminated soil, liming approximately doubled the Ca content in aerial parts while magnesium treatment did not cause any notable changes in the content of this element. Similar results caused by liming on the above heavy metals were discussed in the authors' previous works (Gospodarek, Nadgórska-Socha 2008). Soil contami-

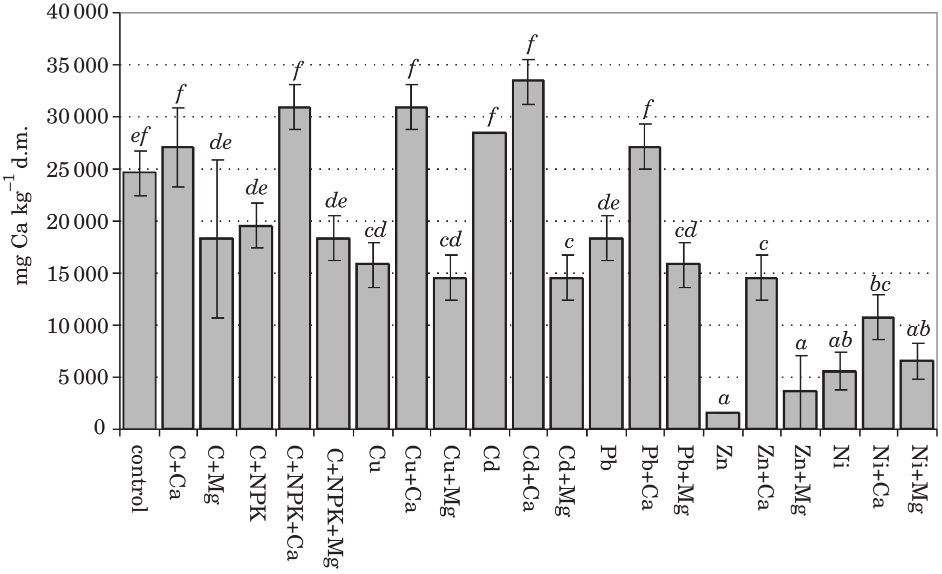

Fig. 2. Calcium content in aerial parts of broad bean (*Vicia faba* L. ssp. *maior*) cultivated in unpolluted soil (control, NPK) and in soil contaminated with single heavy metals and after application of liming or magnesium treatment. Values marked with different letters are statistically different at $p < 0.05$

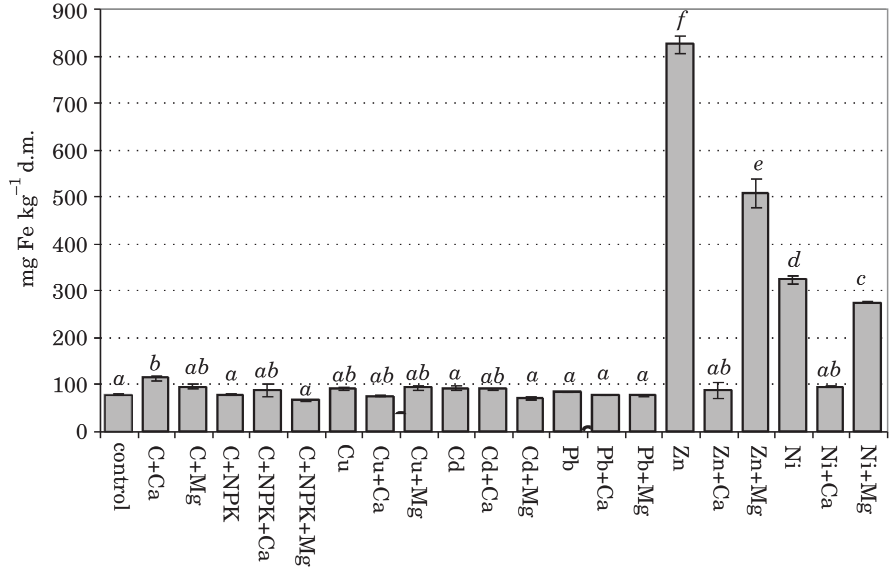

Fig. 3. Iron content in aerial parts of broad bean (*Vicia faba* L. ssp. *maior*) cultivated in unpolluted soil (control, NPK) and in soil contaminated with single heavy metals and after application of liming or magnesium treatment. Values marked with different letters are statistically different at $p < 0.05$

nation with copper or lead only slightly decreased calcium level in broad bean plants. In both cases, liming significantly raised this element in broad bean plants, whereas magnesium fertilization did not markedly affect this characteristic. On the other hand, in the case of broad bean plants grown on cadmium contaminated soil, the calcium level was even slightly higher than in the control plants. Liming caused further increase in the Ca level, while magnesium fertilization contributed to a significant (two-fold) decrease in this element in aerial parts of the plants.

Soil contamination with copper, lead and cadmium did not markedly influence the iron content in broad bean aerial parts (Figure 3). This element level ranged from 67 to 114 mg·kg^{-1} dry mass. According to literature (Kabata-Pendias, Pendias 1993), the iron level in legumes ranges widely (75--400 mg·kg^{-1} d.m.). In the case of these heavy metals, no significant changes in Fe concentrations in broad bean aerial parts were observed, either caused by liming or by magnesium fertilization. However, soil pollution with nickel and zinc caused a considerable (*ca* three-fold for Ni and eight-fold for Zn) increase in the iron content. It might have been associated with a decline in the soil pH in objects polluted with these elements (Gospodarek, Nadgórska-Socha 2007). Liming of the above soils caused a decrease in the iron content to the same level as in the control plants. Magnesium fertilization also contributed to a depression in this element, but to a smaller degree than liming. On the other hand, in the authors' previous research (Gospodarek, Nadgórska-Socha 2008) conducted on soil contaminated with nickel or zinc, only a dose of magnesium computed according to 2 Hh lowered the Fe level to the same as noticed in the control plants. The reports on the iron content in plants after liming are varied (Scheffer et. al 1978, Kotowska 1992), for example meadow plants absorbed iron best in the objects where liming was conducted (Maciejewska, Kotowska 1998). It has also been stated that calcium bioavailability improves plant resistance to deficiency or excess of iron (Kabata-Pendias, Pendias 1993).

CONCLUSIONS

1. Soil contamination with zinc or nickel leads to a considerable decrease in magnesium and calcium in broad bean aerial parts but rises the iron level.

2. Liming rather than magnesium fertilization of soil polluted with heavy metals, such as zinc or nickel, contributes more to balancing the content of the analyzed macronutrients in plants. The content of Ca, Fe and Mg in plants after liming approached the level determined in the control plants.

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WATER CHEMISTRY OF LAKE GIŁWA

Jolanta Grochowska, Renata Tandyrak

Chair of Environmental Protection Engineering
University of Warmia and Mazury in Olsztyn

Abstract

The study was carried on Lake Giłwa (100.8 ha, 9.4 m), in the drainage basin of the Giłwa and Pasłęka rivers. The data obtained in the study allowed the authors to classify Lake Giłwa as a water body belonging to the third stability degree according to PATALAS (1960). As evidenced in the study, Lake Giłwa is a highly eutrophic reservoir. The lake waters were characterized by a high content of nutrients, up to 1.40 mg $P \cdot dm^{-3}$ and 12.47 mg $N \cdot dm^{-3}$. The high fertility of the lake was also exhibited by the values of BOD_5 reaching 7 mg $O_2 \cdot dm^{-3}$, chlorophyll *a* content (73 $mg \cdot m^{-3}$) and low transparency – 0.7 m. In the peak of the summer, the stagnation oxygen profile is represented by a clinograde curve typical for eutrophic lakes, while carbon dioxide distribution in the water column is shown by a „reverse" clinograde curve, also typical for fertile reservoirs.

The study has revealed that the water in Lake Giłwa is well buffered, as shown by the alkalinity values, 2.5-5.0 mval dm^{-3}. Total hardness of the reservoir water varied from 157.1 to 278.8 mg $CaCO_3 \cdot dm^{-3}$, which is typical of hard water. The hardness was conditioned mainly by the calcium content. With the River Giłwa, the lake receives wastewater from the wastewater treatment plant in Gietrzwałd, which is manifested, for example, by the high values of electrolytic conductivity (321-476 $\mu S \cdot cm^{-1}$), indicating the degree of mineral pollution of the lake. Despite the wastewater input, the amount of chlorides is rather low, 20 mg $Cl \cdot dm^{-3}$ at the most.

Key words: lake, nutrients, preliminary production, Secchi disc visibility, eutrophication.

CHEMIZM WÓD JEZIORA GIŁWA

Abstrakt

Badaniami objęto jezioro Giłwa (100,8 ha, 9,4 m) położone w dorzeczu Giłwy-Pasłęki. Pod względem dynamiki wód jest to zbiornik o III stopniu statyczności wg PATALASA (1960). Badania chemiczne wód wykazały, iż jezioro Giłwa jest zbiornikiem silnie zeutrofizowanym. W jego wodach stwierdzono bardzo wysoką zawartość związków biogenicznych – 1,40 mg

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Jolanta Grochowska, PhD, Eng., Junior Professor, Chair of Environmental Protection Engineering, University of Warmia and Mazury, Prawocheńskiego 1, 10-957 Olsztyn, Poland, e-mail: jgroch@uwm.edu.pl

$P \cdot dm^{-3}$ i 12,47 mg $N \cdot dm^{-3}$. O dużej żyzności jeziora świadczyły także wartości BZT_5, dochodzące do 7,0 mg $O_2 \cdot dm^{-3}$, ilość chlorofilu *a* (ok. 73 $mg \cdot m^{-3}$) i niska przezroczystość wody – 0,7 m. W szczytowym okresie lata zawartość tlenu w wodzie obrazowała krzywa klinogradowa, typowa dla jezior eutroficznych, zaś rozkład dwutlenku węgla w słupie wody miał kształt odwrotnej klinogrady, co również jest typowe dla jezior żyznych.

Badania wykazały, że wody jeziora Giłwa są dobrze zbuforowane, o czym świadczyły wartości alkaliczności od 2,5 do 5,0 mval dm^{-3}. Twardość ogólna wód tego akwenu zmieniała się od 157,1 do 278,8 mg $CaCO_3 \cdot dm^{-3}$, co pozwala określić jego wody jako twarde. O twardości wód decydowała głównie zawartość wapnia. Za pośrednictwem rzeki Giłwy do jeziora doprowadzane są ścieki z oczyszczalni ścieków w Gietrzwałdzie, co uwidacznia się m.in. w wysokich wartościach przewodności elektrolitycznej (321-476 $\mu S \cdot cm^{-1}$), wskazującej na stopień zanieczyszczenia wód związkami mineralnymi. Pomimo dopływu ścieków, w wodach analizowanego zbiornika stwierdzono niewielką ilość chlorków – do 20 mg $Cl \cdot dm^{-3}$.

Słowa kluczowe: jezioro, związki biogenne, produkcja pierwotna, widzialność krążka Secchiego, eutrofizacja.

INTRODUCTION

Lake ecosystems undergo many complex chemical, physical and biological transformations (KUBIAK, TÓRZ 2005). Directions of these transformations are determined by water composition, which in turn is shaped by several factors. Among the most important ones are the geological structure and type of land use in a watershed, capacity of the soil sorption complex, weathering and solubility of minerals present in a watershed, atmospheric conditions, mixing of waters of different composition, and types of aquatic organisms (LETCHER et al. 2002, SCHOONOVER, LOCKABY 2006).

Although the chemical composition of water and trophic condition of most lakes located in Olsztyńskie Lakeland (Pojezierze Olsztyńskie) have been thoroughly analysed, there are still some lakes that have not been examined. Lake Giłwa in Rentyny is an example of a lake that has not been carefully studied until today. The literature data regarding this water body include morphometric properties and the temperature and DO measurements of September 1962. Therefore, it has been decided, for scientific as well as practical purposes, to elaborate on the collected material concerning the water chemistry of Lake Giłwa.

The main goal of this paper is to characterize the hydrochemical properties of Lake Giłwa and its trophic condition.

MATERIAL AND METHOD

Lake Giłwa is located approximately 20 km west of Olsztyn, in the Giłwa-Pasłęka Rivers drainage basin. The geographic coordinates are 53°46′2″ N and

20°14′2″ E. To the north the lake is adjacent to the village Rentyny. The lake's axis runs from the north-west to the south-east. The surface area is 100.8 ha and the max. depth is 9.4 m (Table 1, Figure 1). Detailed morphometric parameters are given in Table 1.

Table 1

Detailed morphometric data and lake parameters
(after Institute of Inland Fisheries, Olsztyn, 1964)

Parameter	Values
Water table surface area (ha)	100.8
Maximum depth (m)	9.4
Mean depth (m)	3.7
Relative depth	0.0094
Depth index	0.39
Volume (thousand m^3)	3722.2
Maximum length (km)	2.2
Maximum width (km)	0.6
Elongation	3.7
Shoreline length of the lake bowl (km)	6.8
Shoreline development	1.95

The watershed draining directly to the lake of 1.66 km^2 surface area is dominated by forests (72.9%). The lake is intensively used for recreation; shores are occupied by recreation centres, camping fields, summer houses and bathing beaches.

Lake Giłwa is a flow-through reservoir. The Giłwa River enters the lake in the southern and flows out in the north-western part of the lake. The river feeds the lake with the treated wastewater discharged from the wastewater treatment plant in Gietrzwałd.

The analyses of the physicochemical properties of the water in Lake Giłwa were performed three times, on the following dates: November 27, 2007, April 29 and September 3, 2008. Water for analyses was taken from the deepest site in the lake, determined with the help of a bathymetric chart and GPS. Water samples were taken from 1 m depth under the water table and 1 m above the bottom. Simultaneously, temperature and DO were measured at every meter of the water column depth. Water transparency was measured with Secchi disc. Chemical analyses were done in accordance with the methods by HERMANOWICZ et al. (1999).

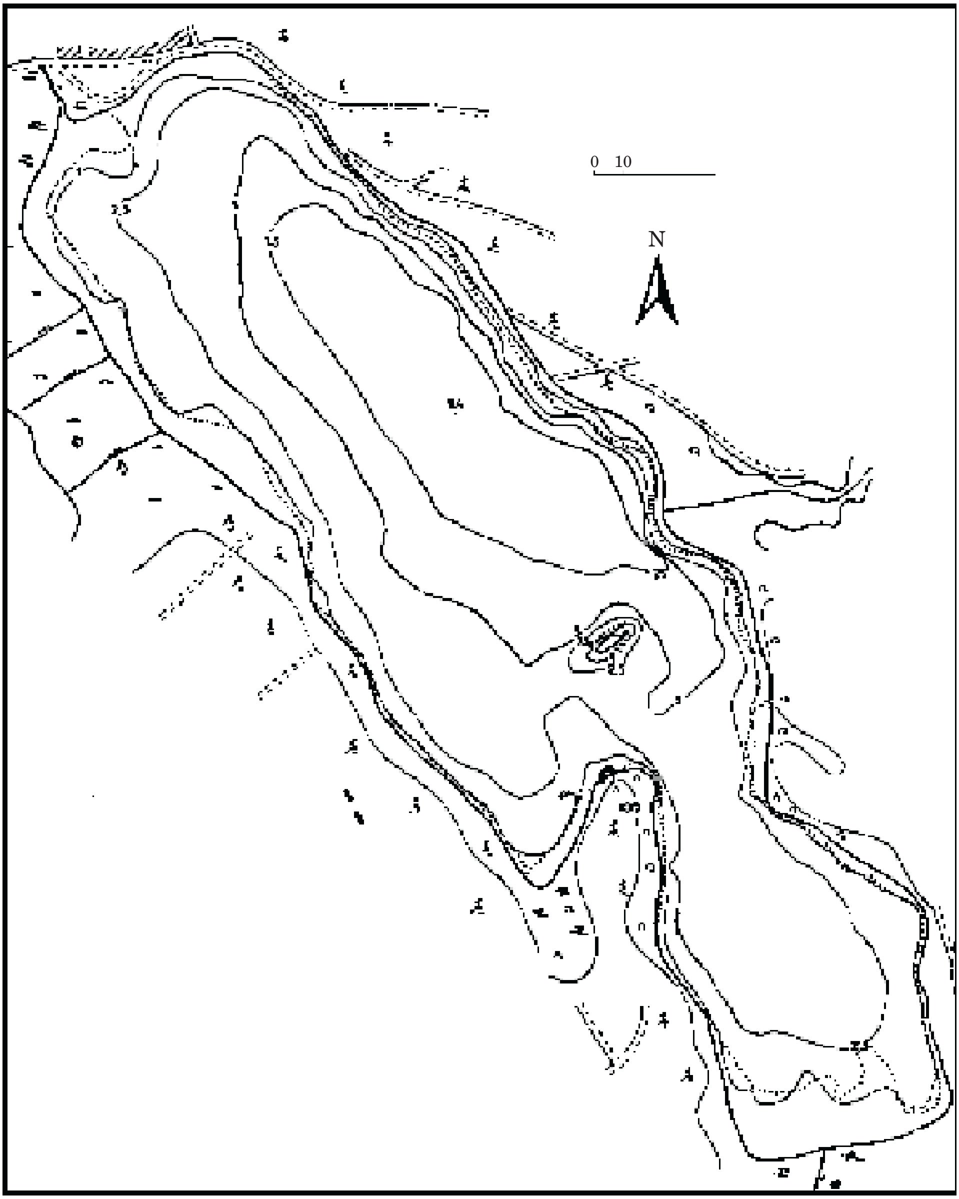

Fig. 1. Bathymetric map of Lake Giłwa

RESULTS AND DISCUSSION

Lake Giłwa is not very deep (9.4 m) but relatively large (100.8 ha). To the west, south and partly the east, the lake is surrounded by a forest, which considerably limits the inflow of water to the lake. Moreover, it is a flow-through reservoir, although this lake's characteristic is only weakly evident. Therefore, the intensity of water circulation is determined mostly by the lake's morphometric properties and exposure to wind. The first analysis (November) revealed that the whole water mass circulated at the temperature of 3.8°C. At the end of April, the water column was thermally stratified with a 6.0°C temperature difference between the surface and the bottom (Table 2). In early September, the lake stratified into a 6-m thick epilimnion and a metalimnion of the $3^{o}C \cdot m^{-1}$ gradient (Table 2). Based on the measurements taken on September 4, 1962 by OLSZEWSKI et al. (1978), the thermal variability in the water column was assessed as small, 15.7--14.5°C. The theoretical water mixing depth in Lake Giłwa, calculated after PATALAS (1960) from the empirical equation E = 4.4 vD, is 5.2 m. The study shows that it is 0.8 m lower than the actual thickness of the epilimnion observed at the end of the summer. Taking into account the criteria given by PATALAS (1960), Lake Giłwa can be classified as belonging to the 3rd static degree, whereas OLSZEWSKI et al. (1978) described the lake as having the ability to create weak strata.

Table 2

Thermal and oxygen profiles in lake Giłwa

Depth (m)	Autumn		Spring		Summer	
	temperture	oxygen	temperature	oxygen	temperature	oxygen
	(°C)	(mg $O_2 \cdot dm^{-3}$)	(°C)	(mg $O_2 \cdot dm^{-3}$)	(°C)	(mg $O_2 \cdot dm^{-3}$)
0	3.8	9.6	13.6	18.2	18.7	10.7
1	3.8	9.8	13.6	16.2	18.7	10.2
2	3.8	9.8	13.0	15.0	18.5	9.6
3	3.8	9.8	11.0	14.4	18.4	9.4
4	3.8	9.8	10.4	12.6	18.2	8.0
5	3.8	9.8	8.8	10.1	17.8	4.8
6	3.8	9.8	8.8	10.6	17.2	2.2
7	3.8	9.8	8.4	6.9	14.2	0.0
8	3.8	9.8	8.0	6.4	11.5	0.0
9	3.8	9.6	7.6	4.8	9.3	0.0

Oxygenation of the lake water depended on the season. Oxygen content in the autumn was similar in the whole water column and equalled 9.8 mg

$O_2 \cdot dm^{-3}$ (74.1% saturation) – Table 2. At the end of April, oxygen content in the surface water layer was high, 16.2 mg $O_2 \cdot dm^{-3}$ (173.8% saturation) with 4.8 mg $O_2 \cdot dm^{-3}$ (39.9% saturation) near the bottom (Table 2). In the peak of the summer stagnation, oxygen conditions deteriorated. High concentrations of the gas were measured only in the 4-m thick layer (from 113.5 to 84.0% saturation), decreasing in the deeper layers to reach the zero value at 7 m depth (Table 2). Good oxygen conditions from April until September must have been due to the intensive primary production as confirmed by the deficit of free carbon dioxide and the high reaction (9.02 pH in April, 8.30 pH in September) – Table 3. Simultaneously, organic matter decomposed in the near-bottom water layers due to the continuous thermal stratification, consuming all oxygen and causing its total deficit near the bottom. The intensive mineralization of organic compounds occurred in parallel to an

Table 3

Selected chemical parameters of water of Lake Giłwa

Parameter		Date		
		21 Nov.	29 Apr.	03. Sept.
Reaction (pH)	*P*	8.12	9.02	8.30
	D	8.00	8.15	7.37
Carbon dioxide ($mg\ CO_2 \cdot dm^{-3}$)	*P*	8.8	0.0	0.0
	D	11.0	11.0	29.0
Conductivity ($\mu S \cdot cm^{-1}$)	*P*	368	417	321
	D	370	357	476
Chlorides ($mg\ Cl \cdot dm^{-3}$)	*P*	15.0	16.0	16.0
	D	15.0	20.0	19.0
Calcium ($mg\ Ca \cdot dm^{-3}$)	*P*	54.98	57.83	49.98
	D	54.98	65.69	73.54
Magnesium ($mg\ Mg \cdot dm^{-3}$)	*P*	14.0	7.6	7.2
	D	14.0	10.0	21.2
Alkalinity ($mval \cdot dm^{-3}$)	*P*	3.1	3.1	2.7
	D	3.1	3.5	5.0
Total hardness ($mval \cdot dm^{-3}$)	*P*	4.00	3.57	3.14
	D	4.00	4.18	5.57
Oxidization ($mg\ O_2 \cdot dm^{-3}$)	*P*	11.2	12.8	28.8
	D	12.0	17.6	24.0
BOD_5 ($mg\ O_2 \cdot dm^{-3}$)	*P*	2.1	7.4	6.6
	D	3.2	1.9	4.7

P – surface layer
D – bottom layer

increase in free carbon dioxide (to 29.0 mg $CO_2 \cdot dm^{-3}$ in September) – Table 3. According to ROGALSKI et al. (2005), carbon dioxide is the main product of respiration and matter decomposition processes occurring in the bottom mud. The described distribution of oxygen in the peak of the summer stagnation is illustrated by the so-called clinograde curve, typical for eutrophic lakes.

One of the indicators of excessive water fertility is the low visibility of Secchi disc. Water transparency in Lake Giłwa ranged from 0.70 to 2.50 m (Figure 2) and oscillated during the vegetation period around 0.8 m. Apparently, the excessive biomass of algae was the limiting factor for sunlight penetration in the lake, as shown by the highest amount of chlorophyll *a* (indicator of the primary production, equal 73.50 $mg \cdot m^{-3}$), which occurred simultaneously with the lowest water transparency (0.70 m) – Figure 2.

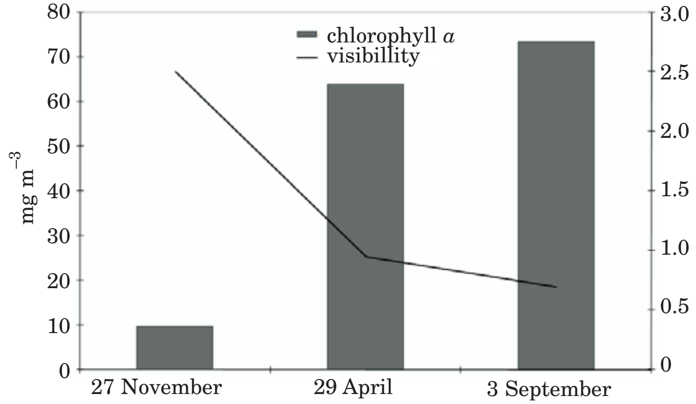

Fig. 2. Chlorophyll *a* content and visibility of Secchi disc in water of Lake Giłwa

The excessive primary production in Lake Giłwa was most probably caused by the high concentration of nutrients, which ranged from 2.30 to 12.47 mg $N \cdot dm^{-3}$ and from 0.13 to 1.40 mg $P \cdot dm^{-3}$ (Figures 3, 4). Such values are typical for highly fertile lakes. The pools of nitrogen and phosphorus were dominated by organic forms (Figures 3, 4), which confirms the high fertility and productivity of the lake (GROCHOWSKA, TANDYRAK 2007). Following the lake classification by ZDANOWSKI (1982), Lake Giłwa can be classified as polytrophic, i.e. 4th trophic condition degree, in terms of the spring content of total P. According to HILLBRICHT-ILKOWSKA and WIŚNIEWSKI (1993), who included water transparency, total P and chlorophyll *a* into their classification, Giłwa is the heavily eutrophic lake.

Assessment of the production processes in a lake is possible through BOD_5 determination. In the surface waters of Lake Giłwa, the values of that parameter were high, approx. 7 mg $O_2 \cdot dm^{-3}$, during the vegetation period, demonstrating fairly advanced eutrophication. At the same time, the permanganate value (BOD_5-Mn), which reveals the reservoir's abundance in

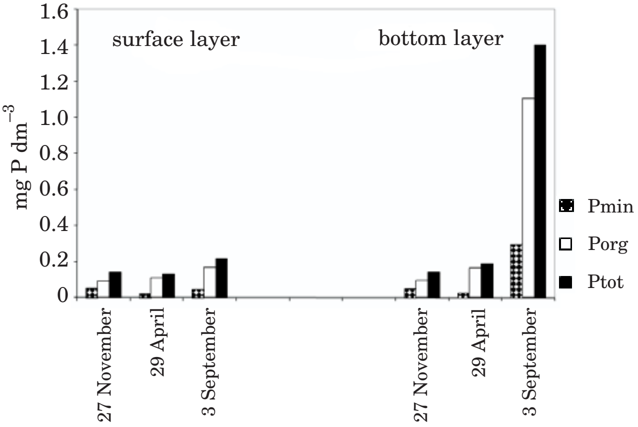

Fig. 3. Changes of phosphorus compounds content in waters of lake Giłwa

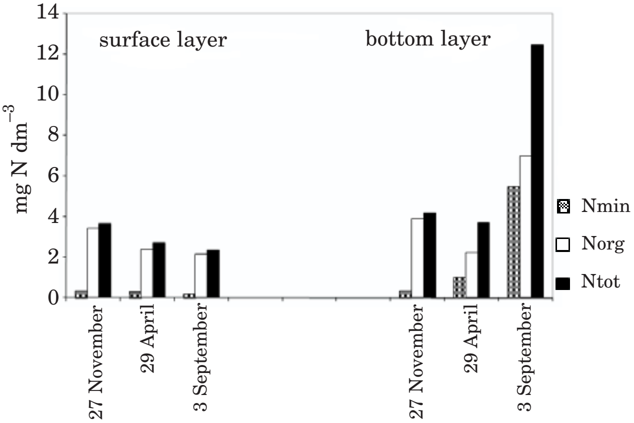

Fig. 4. Changes in the content of nitrogen compounds in water of Lake Giłwa

allochthonous organic matter, was determined in Lake Giłwa waters. The values ranged from 11.2 to 28.8 mg $O_2 \cdot dm^{-3}$ (Table 3). The ratio between the permanganate value and the BOD_5 was all the time higher than 1.0, ranging from 1.7 to 5.3. Such a ratio indicates the dominance of organic matter resistant to degradation over the easily degradable fraction, which can be attributed to the afforested surroundings of the lake and the influence of the Giłwa River, which received treated sewage. JANUSZKIEWICZ (1969) argues that afforested watersheds export to the water large amounts of humic compounds resistant to biological decomposition.

OLSZEWSKI and PASCHALSKI (1959), who examined the alkalinity of the Mazurian Lakes, distinguished three groups. The alkalinity of Lake Giłwa (2.7--5.0 mval$\cdot$dm^{-3}; Table 3) would classify this water body into the 3rd alkalinity group, which means that its water is well buffered. Total hardness of the water in Lake Giłwa ranged from 3.14 to 5.57 mval·dm^{-3} (157.1-278.8 mg $CaCO_3$·dm^{-3}) – Table 3. In accordance with the classification by Dojlido (1995), the water in Lake Giłwa is hard. The hardness was mainly determined by the calcium content varying from 49.98 to 73.54 mg Ca·dm^{-3} (Table 3). Despite the forest cover of much of the watershed, the concentrations of calcium measured in the lake were high, which is assumingly due to the wastewater import by the Giłwa River. Kowalski (1997) claims that calcium content in the surface waters is determined by the processes initiated by the presence of organic pollutants imported with wastewater. In the surface water of Lake Giłwa, the least of calcium was observed during the summer stagnation, in parallel with the maximum chlorophyll a concentration and the lowest water transparency. Such a relationship must be connected to biological decalcification during intensive photosynthesis, which is when lack of free carbon dioxide causes degradation of calcium hydrocarbonate and free CO_2 is released (Hĺkanson et al. 2005). Neutral calcium carbonate thus created settles down and increases calcium content near the bottom. This explanation seems to be confirmed by the present study. The near-bottom water in Giłwa was calcium-rich (Table 3).

Electrolytic conductivity indicates the degree of water pollution with mineral compounds. Marszelewski (2005) analysed the electrolytic conductivity of lakes in North Poland and selected a group of eutrophic lakes in which the values of this parameter were from 200 to 400 µS·cm^{-1}. The conductivity of water in Lake Giłwa ranged from 321 to 476 µS·cm^{-1} (Table 3), corresponding to the range typical for the lakes of considerable fertility. Marszelewski (2005) report that in heavily eutrophicated lakes electrolytic conductivity in summer differs along the water column, with the values increasing towards the bottom. In Lake Giłwa, the conductivity increased with the depth (Table 3). The high electrolytic conductivity of the water in Lake Giłwa is probably related to the wastewater import.

Chloride ions dissolved in water originate from the ground or arrive with contaminants. According to the classification by Olszewski and Paschalski (1959), water receiving no sewage contains to 15 mg Cl·dm^{-3}. Despite being located in an area lacking a sewage collection system, and receiving treated wastewater from the Giłwa River, Lake Giłwa was determined to contain 15-20 mg Cl·dm^{-3} (Table 3), which is only sightly in excess of the above threshold value.

CONCLUSIONS

1. Lake Giłwa is a 3rd static degree reservoir (Patalas 1960).

2. The summer oxygen distribution in the water column follows a clinograde curve, which indicates high fertility of the lake.

3. Lake Giłwa is nutrient rich. The concentrations of nutrients are, for example, 1.4 mg P dm^{-3} and 12.47 mg N dm^{-3}.

4. According to the criteria given be ZDANOWSKI (1982), Lake Giłwa is polytrophic, i.e. 4th trophic state degree, whereas from the guidelines suggested by HILLBRICHT-ILKOWSKA and WIŚNIEWSKI it can be concluded that it is a heavily eutrophic lake.

5. The water in Lake Giłwa is well buffered, hard, and considerably rich in calcium.

6. In the water in Lake Giłwa allochthonous organic matter dominates (the ratio between the permanganate value and BOD$_5$ higher than 1).

7. The high fertility of the water in Lake Giłwa is confirmed by the electrolytic conductivity reaching 476 $\mu S \cdot cm^{-1}$.

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USE OF O-PHOSPHOSERINE (OPS) FOR THE SEPARATION OF PEPTIDES ON IMMOBILIZED COPPER IONS

Monika Karaś

Chair of Biochemistry and Food Chemistry
University of Life Sciences in Lublin

Abstract

Recent research into the structure and properties of proteins and peptides as physiologically active diet components has spurred a new interest in the isolation and investigation of bioactive peptides of animal, plant and microbiological origin. The isolation and separation of protein and peptide mixtures requires advanced procedures. It usually involves a multi-stage separation process on chromatographic columns with various packing. Immobilised Metal Ion Affinity Chromatography (IMAC) is frequently used in the complex process of obtaining peptide fractions. Affinity Chromatography (IMAC) relies on the specific interactions between amino acids, their reactive groups in proteins and peptides and „transitory" metal ions, in particular Cu^{2+}. Those ions are immobilised by the chelating compound on the bed, forming specific adsorbents which bind proteins and peptides.

The aim of this study was to determine whether o-phosphoserine (OPS) can be used for the immobilization of copper ions on Sephadex G25 during the separation of peptides and proteins isolated from string beans.

Frozen pods of dwarf, green-podded string bean cv. *Fana* were used in the study. Peptide were extracted from well-homogenized string bean pods with tris-HCl buffer (pH 7.5), from which high molecular weight proteins were isolated with methanol, acetone, 20% trichloroacetic acid and the Magnafloc M-22S cation flocculant. The protein and peptide content of the separated fractions was determined. The peptide content depended on the type of extract from which high molecular weight proteins were isolated. The results obtained by using OPS as a chelating agent in the separation of string bean can be recommended for analysis of plant peptides.

Key words: peptides, IMAC, metal ions, o-phosphoserine.

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dr Monika Karaś Chair of Biochemistry and Food Chemistry, University of Life Sciences in Lublin, ul. Skromna 8, 20-704 Lublin, Poland, e-mail: monika.karas@up.lublin.pl

WYKORZYSTANIE OPS W PROCESIE ROZDZIAŁU PEPTYDÓW NA UNIERUCHOMIONYCH JONACH MIEDZI

Abstrakt

Rozwój nauki o strukturze oraz właściwościach białek i peptydów jako fizjologicznie aktywnych składnikach diety przyczynił się do wzrostu zainteresowania izolowaniem i badaniem bioaktywnych peptydów pochodzenia zwierzęcego, roślinnego i mikrobiologicznego.

Izolowanie i rozdział mieszanin białek i peptydów wymaga zaawansowanej procedury. Stosuje się zazwyczaj kilkustopniowy rozdział na kolumnach chromatograficznych z różnym wypełnieniem. W tak skomplikowanym procesie otrzymywania frakcji peptydowych szerokie zastosowanie znalazła chromatografia powinowactwa na unieruchomionych jonach metali IMAC (Immobilized Metal Ion Affinity Chromatography). Chromatografia powinowactwa wykorzystuje specyficzne oddziaływania między aminokwasami oraz ich reaktywnymi ugrupowaniami w białkach i peptydach a jonami metali „przejściowych”, szczególnie zaś z Cu^{2+}. Jony te są immobilizowane przez związek chelatujący na złożu, i w ten sposób stanowią specyficzne adsorbenty wiążące białka lub peptydy.

Celem pracy było zbadanie przydatności OPS (o-fosfoseryny) jako czynnika unieruchamiającego jony miedzi na złożu w procesie rozdziału peptydów i białek wyizolowanych z fasoli szparagowej metodą IMAC. Materiałem do badań były mrożone strąki fasoli szparagowej karłowatej zielonostrąkowej, odmiany *Fana*. Peptydy i białka izolowano z fasoli szparagowej buforem Tris-HCl, z otrzymanego ekstraktu białka wysokocząsteczkowe wydzielono: metanolem, acetonem, 20% kwasem trichlorooctowym i kationowym flokulantem Magnafloc M-22S. W otrzymanych frakcjach oznaczono zawartość białka i peptydów. Peptydy obecne w fasoli szparagowej charakteryzowały się zbliżonym powinowactwem do jonów miedzi. Wykazano, że rozdział peptydów zależy w małym stopniu od właściwości czynnika zastosowanego podczas usuwania białek z ekstraktu. Przebieg rozdziału z wykorzystaniem OPS jako czynnika chelatującego w technice IMAC z powodzeniem może być stosowany do rozdziału peptydów z ekstraktów roślinnych.

Słowa kluczowe: peptydy, IMAC, jony metali, o-fosfoseryna.

INTRODUCTION

Recent research into the structure and properties of proteins and peptides as physiologically active diet components has spurred a new interest in the isolation and investigation of bioactive peptides of animal, plant and microbiological origin. The isolation and separation of protein and peptide mixtures requires advanced procedures. It usually involves a multi-stage separation process on chromatographic columns with various packing. Immobilised Metal Ion Affinity Chromatography (IMAC) is frequently used in the complex process of obtaining peptide fractions. Affinity Chromatography (IMAC) relies on the specific interactions between amino acids, their reactive groups in proteins and peptides and „transitory” metal ions, in particular Cu^{2+}. Those ions are immobilised by the chelating compound on the bed, forming specific adsorbents which bind proteins and peptides. Immobilised metal ions have been successfully applied in separation of the products of proteolysis of leguminous plants seeds proteins (BARANIAK, KRZEPIŁKO 2005).

The fresh vegetables and aquatic plants are rich in metal ions, which can be removed by the chelating compound on the bed (without immobilized metal ions) (BOSIACKI, TYKSIŃSKI 2009, SENZE 2009).

The aim of this study was to determine whether o-phosphoserine (OPS) can be used for the immobilization of copper ions on Sephadex G25 during the separation of peptides and proteins isolated from string beans.

MATERIALS AND METHODS

Isolation of peptides from plant material

Frozen pods of dwarf, green-podded string bean cv. *Fana* were used in the study.

Peptides were extracted from well-homogenized string bean pods with Tris –HCl buffer (pH 7.5). Homogenate of 1 g sample of frozen string beans was stirred with a magnetic stirrer with 10 cm^3 of tris–HCl buffer (pH 7.5) for 2 hours at room temperature. The solid fraction was separated by centrifugation at 4,000 rpm for 15 min. The collected extract was lyophilized and used for further determinations. The fractions albumins and globulins dissolved lyophilizes were precipitated by acetone, methanol, 20% TCA and cationic flocculant Magnafloc M-22S. The extracts were separated by immobilized copper ion affinity chromatography.

Gel preparation for peptide separation by IMAC

Sephadex G-25 medium was mixed with a solution containing 0.0375 g $NaBH_4$, 10 cm^3 2 M NaOH and 1 cm^3 of epichlorohydrin. The suspension was mixed slowly for 2 hours at room temperature. Meanwhile, 10 cm^3 of 2 M NaOH and 5 cm^3 of epichlorohydrin solutions were gradually added. The mixture was left overnight to complete the reaction. The gel was washed on a Büchner funnel and dried. The dried gel was mixed with 25 cm^3 of a solution containing 5.3 g Na_2CO_3, 2.5 g *o*-phosphoserine (OPS) and 0.03 g $NaBH_4$. The suspension was left overnight at 60°C and stirred slowly from time to time. After 24 hours, the gel was washed with distilled water followed by a diluted acetic acid and distilled water again, to neutralize the pH. 50 cm^3 of a solution containing copper ions at a concentration of 1 $mg \cdot cm^{-3}$ was added to the gel. Next the bed was transferred to a 1.5 cm diameter and 12 cm long column. The column was equilibrated with 40 cm^3 of a 0.05 M solution of Tris-HCl buffer, pH 7.5. A 2 cm^3 sample was applied to a glass column packed with gel, and fractions 1-36 were eluted with a pH gradient of Tris-HCl buffer: 7.5, 5.5, 4.5 and back to 7.5 (adjusted with EDTA--fraction 37-70). 4 ml fractions were collected at a flow rate of 40 $cm^3 \cdot hour^{-1}$.

Methods for determinations

Protein concentration was determined by using BRADFORD's method (1976) with bovine serum albumin (BSA) as a standard (595 nm). Peptide content was determined spectrophotometrically with trinitrobenzesulfonic acid (TNBS), according to HABEEB's method (1966) modified by ADLER-NISSEN (1979). Leucylglycine was used as standard (340 nm).

RESULTS AND DISCUSSION

The process of affinity chromatography involving chelate-bound metal ions is conditioned by many factors (UEDA et al. 2003). The type of the applied support, activating factor and metal ion chelating compound also play an important role in the process (ZACHARIOU, HEARN 2000). The possibility of reversible protein-metal ion binding as well as maintaining their permanent bonds with chelating compounds are also important. The effectiveness of different metal ions and chelating factors can be tested reliably only with the use of the same material which is separated under identicalal elution conditions.

Peptide affinity for metal ions is determined by peptide structure, type of metal ions, type of chelating compound, pH, type of solvent, presence of salt and competitive ligands (CHAGA 2001, PORATH, OLIN 1983).

In IMAC, the most popular ligands are iminodiacetic acid (IDA), nitrilotriacetic acid (NTA), carboxymethylated aspartic acid (CM-Asp) and tri(carboxymethyl)ethylene diamine (TED). The effectiveness of OPS as a chelating agent was tested in the present study. This compound has not been used for the isolation of vegetable proteins and peptides to date.

Orthophosphoserine was tested in a model protein study and in the separation of human serum proteins by ZACHARIOU and HEARN (2000). O-phosphoserine is a constituent of biological membranes and it participates in ion transport. It forms complexes with metals and, subject to the degree of dissociation, it may be a tridonor compound similarly to iminodiacetic acid – binding metal ions through oxygen atoms in phosphate and carboxyl groups and nitrogen atoms in the amino group. According to the cited authors, OPS has higher affinity than IDA for iron and aluminium ions and lower affinity for copper ions. The binding capacity of the Cu ion-OPS chelate was comparable to that of chelates formed by hard metal ions (iron and aluminium) and OPS. The effectiveness of copper ions chelated with OPS on the same bed was compared in this study. It was found that OPS, similarly as IDA, forms chelates with the same structure, using three donor sites for this purpose. The effect of chelates other than IDA on the separation process has been also investigated by other authors. In order to compare the absorption of reference proteins, lysozyme, ovoalbumin, beef albumin, conalbumin and wheat germ agglutinin, on columns packed with Sepharose 6B with copper and nickel ions immobilized with IDA and tris(2-aminoethyl)amine, SHARMA, AGARWAL (2001) described them using four different isotherm models.

Langmuir-Freundlich model was found to be most suitable for explaining cooperativity and quantitative differences in protein binding in all tested systems. The analyzed proteins showed the highest affinity for copper ions chelated with IDA, and their affinity for the other systems varied. CHAOUK and HEARN (1999) reported good results of the separation of human serum proteins in an alkaline environment as Cu ions were mobilized on Sepharose CL-4B with a new tridonor compound, N-2 sodium pyridyl-methylamine acetate. Columns containing TED-Novarose, with chelate-agarose, have been used for the separation of beef calmodulin (CHAGA et al. 1996). Nitrilotriacetic acid has been used to immobilize Ni ions on agarose in the process of purifying proteins obtained by genetic recombination (GLYNOU et al. 2003). Carboxymethyl asparagine has been used for immobilizing cobalt ions on Superflow 6 in order to purify dehydrogenase from chicken breast muscles (CHAGA et al. 1999).

The ligands used in IMAC form tri-(IDA), four-(NTA, CM-Asp), five-donor (TED) complexes (chelates) with metal ions. The number of coordinate bonds between metal ions and chelating compounds determines the general affinity of chelates for proteins and the overall stability of the complex. The higher the donor state, the greater the stability of chelates and the weaker the binding of proteins. In aqueous solutions, chelates undergo strong solvation and all free coordination positions of metals are occupied by water molecules and by the hydroxyl or amino groups of buffer compounds. During separation, these groups are easily replaced by protein, peptide or amino acid molecules.

IDA is commonly used in IMAC. IDA-metal chelates have the highest protein-binding capacity. This ligand forms the most stable complexes with Cu^{2+}, followed by Ni^{2+}, Zn^{2+} and Co^{2+}. Another chelating compound, TED, has been tested with respect to Ni^{2+} and Fe^{3+} ions during the separation of serum proteins (PORATH, OLIN 1983). TED-metal chelates show much lower protein-binding capacity. These compounds are often used for removing metal ions from metalloenzymes. The adsorptive properties of NTA place it between IDA and TED. NTA is particularly effective in Ni^{2+} ion binding.

Chromatograms developed for protein concentrations determined by measuring absorbance at a wavelength of 280 nm are presented in Figures 1-4. When the ions were immobilized with OPS, the maximum absorbance was observed in fractions 3 or 4, and protein concentrations following the use of copper ions reached 1.365 $mg \cdot cm^{-3}$ for acetone, 2.858 $mg \cdot cm^{-3}$ for the flocculant, 1.598 $mg \cdot cm^{-3}$ for methanol and 0.914 $mg \cdot cm^{-3}$ for 20% TCA. Depending on the precipitating agent used (methanol, 20% TCA and flocculant), additional peaks were detected for fractions 3-7 and 12-13 (Table 1).

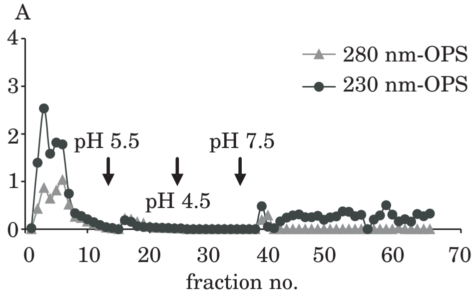

Fig. 1. Elution profiles of the non-bound peptides from IMAC chromatography of extracts from string bean on Cu(II)-OPS-Sephadex
(precipitated with methanol after separation in columns)

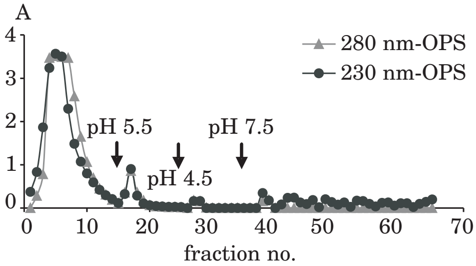

Fig. 2. Elution profiles of the non-bound peptides from IMAC chromatography of extracts from string bean on Cu(II)-OPS-Sephadex
(precipitated with acetone after separation in columns)

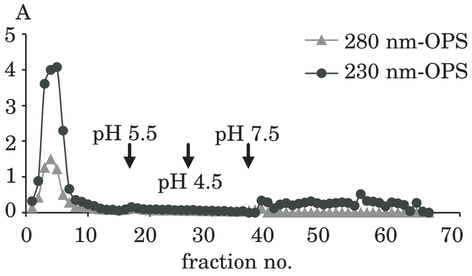

Fig. 3. Elution profiles of the non-bound peptides from IMAC chromatography of extracts from string bean on Cu(II)-OPS-Sephadex
(precipitated with 20% TCA after separation in columns)

Chromatograms obtained for peptide levels determined by measuring absorbance at a wavelength of 230 nm are presented in Figures 1-4. As the copper ions were immobilized with OPS, the maximum absorbance was observed in fraction 6, and peptide content was as follows: 0.0261 $\mathrm{mg \cdot cm^{-3}}$ for acetone, 0.024 $\mathrm{mg \cdot cm^{-3}}$ for the flocculant, 0.0258 $\mathrm{mg \cdot cm^{-3}}$ for methanol and 0.0079 $\mathrm{mg \cdot cm^{-3}}$ for 20% TCA. Smaller peaks were noted for further

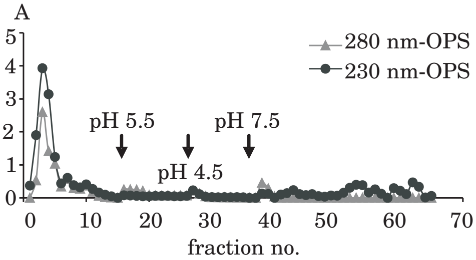

Fig. 4. Elution profiles of the non-bound peptides from IMAC chromatography of extracts from string bean on Cu(II)-OPS-Sephadex
(precipitated with flocculant after separation in columns)

fractions, e.g. 11-15, 18-20, 27-30 or above 36, depending on the precipitating agent used (Table 2). A similar dependency was observed by VANCAN et al. (2002) who tested the affinity of human immunoglobulin IgG for Cu, Ni and Zn ions immobilized with IDA on Sepharose-6B activated with epichlorohydrin. Following the application of a phosphate buffer in the pH gradient in the elution process, as much as 81.4% of protein was removed from the column with copper ions solely in the regeneration process with the use of 50 mM EDTA. When separating raw extract (10% purity) of somatotropin (STH), the human growth hormone, LIESIENÉ et al. (1997) obtained a fraction image identical to that reported in this study after separation in columns with immobilised copper ions on a cellulose bed (Chelat-Carnosel). The highest protein content (measured at A_{280}) was detected in the first few frac-

Table 1

Protein content of extracts precipitated with various agents after separation in OPS columns with immobilised copper ions ($mg \cdot cm^{-3}$)

Precipitated agent	Acetone		Methanol				20% TCA	Flocculant	
Fraction	3-7	12-13	3-7	12-13	27-30	38-39	3-7	3-7	12-13
Cu^{2+}	6.137		6.503				1.459	7.683	0.407

Table 2

Peptide content of extracts precipitated with various agents after separation in OPS columns with immobilised copper ions ($mg \cdot cm^{-3}$)

Precipitated agent	Acetone				Methanol		
Fraction	3-9	11-15	18-20	38-41	3-9	11-15	38-41
Cu^{2+}	0.127	0.048	0.012	0.009	0.118	0.020	0.019
Precipitated agent	20% TCA				flocculant		
Fraction	3-9	11-15	38-41	3-9	11-15	27-30	38-41
Cu^{2+}	0.041	0.007	0.006	0.176	0.015	0.007	0.014

tions eluted with phosphate buffer, pH 7.8 with 0.5M sodium chloride. When the gradually decreasing pH gradient of acetic buffer (pH 6.6 to 4.3) was applied, only a few small peaks with absorbance below 0.1 were detected. In the process of isolating green fluorescent protein (GFPuv) (genetically unmodified), the highest efficiency was reported in respect of copper ions, followed by nickel ions, while the lowest results were obtained for zinc and cobalt ions (Li et al. 2001). Varlamov et al. (1995) recommended the use of IDA-Sepharose with immobilized copper ions at the final purification stage of beta-dopamine hydroxylase from beef bone marrow adrenaline, due to more effective interactions with these ions, compared with copper, zinc and nickel ions.

The cited authors and the authors of this study tested copper ions which form coordinate bonds with electrodonor atoms, prefer bonds with nitrogen, but also interact with oxygen and sulfur. Their efficiency should be determined by the availability of amino acids such as histidine, cysteine or tryptophan. The results of this study suggest that the process of peptide separation is also dependent, although to a lower degree, on the properties of the agent used for protein removal from the extract. In all the cases, the highest peptide content was recorded in fractions 3-9, and additional absorbance maxima (with a substantially lower peptide content) were observed in further fractions (Table 2). A similar fraction image was reported by George et al. (1997), who used copper ions immobilised on Sephadex G-25 in the separation of chick pea protein hydrolysates. Okhubo et al. (1980) observed three maximum absorbance values during the separation of rat liver nucleoside diphosphatase, initially purified in a column packed with DEAE cellulose and Sephadex G-200, on a column with chelated copper ions to epoxy-activated Sepharose 6B. Following the separation of soy beta-conglycinin hydrolysate, obtained through the application of protease S (with *Bacillus* sp.), in a column packed with Sephadex G-25, Chen et al. (1995) noted two maximum values when absorbance was measured at a wavelength of 280 nm and found six peptides in the resulting content. Saito et al. (1991) demonstrated that the yield of caseinoglycopeptides isolated from sweet cheese whey depended on the method of whey protein precipitation.

CONCLUSION

1. Affinity chromatography with the use of metal ions immobilized to OPS-Sephadex G-25 may be successfully used for the separation of peptides isolated from string beans.

2. The results obtained by using OPS as a chelating agent in the separation of string bean peptides were comparable with those reported for IDA, which indicates that OPS can be recommended for analysis of plant peptides.

3. Agents used for removing high molecular weight compounds (soluble at pH 7.5) from extracts of proteins and peptides present in string beans considerably affect the qualitative and quantitative composition of the resulting filtrate.

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THE EFFECT OF SOIL CONTAMINATION WITH DIESEL OIL AND PETROL ON THE NITRIFICATION PROCESS

Jan Kucharski, Monika Tomkiel, Edyta Boros, Jadwiga Wyszkowska

Chair of Microbiology
University of Warmia and Mazury in Olsztyn

Abstract

The effect of soil contamination with diesel oil and petrol on the nitrification process was investigated in a laboratory experiment. Samples of typical brown soil developed from loamy sand, of pH of 6.6 in 1M KCl, Hh –11.38 mmol$^+$ kg^{-1} soil, S – 77.67 mmol$^+$ kg^{-1} soil and C_{org} – 8.50 g kg^{-1} were analyzed. The experiment was performed in three replications, and for each test 100 g air-dry soil sample was placed in 150 cm^3 beakers. Soil samples were contaminated with diesel oil and petrol with the addition of rapeseed oil and ethanol. The source of nitrogen was ammonium sulfate in the amount of 0 and 250 mg N per kg^{-1} soil. The content of N-NO_3^- and N-NH_4^+ was determined on experimental days 14, 28 and 42. Soil moisture was kept constant at 50% capillary water capacity throughout the experiment.

Fertilizer nitrogen was subject to strong immobilization in soil contaminated with diesel oil and petrol. Both pollutants strongly inhibited the nitrification process. Diesel oil had a much stronger inhibitory effect on nitrification than petrol. Rapeseed oil also proved to be a powerful inhibiting factor. On experimental day 42, diesel oil reduced ammonium cation oxidation by 99%, and petrol – by 88%.

Key words: diesel oil, petrol, soil, nitrification, N-NH_4^+, N-NO_3^-.

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prof. dr hab. Jan Kucharski, Chair of Microbiology, University of Warmia and Mazury in Olsztyn, pl. Łódzki, 10-727 Olsztyn, Poland, phone: 089 523-49-38, e-mail: jan.kucharski@uwm.edu.pl.

PROCES NITRYFIKACJI W GLEBIE ZANIECZYSZCZONEJ OLEJEM NAPĘDOWYM I BENZYNĄ

Abstrakt

W doświadczeniu laboratoryjnym badano wpływ zanieczyszczenia gleby olejem napędowym i benzyną na przebieg procesu nitryfikacji. Do badań wykorzystano próbki gleby brunatnej właściwej wytworzonej z piasku gliniastego o pH w 1M KCl 6,6, Hh – 11,38 mmol$^+$ kg^{-1} gleby, S – 77,67 mmol$^+$ kg^{-1} gleby, C_{org} – 8,50 g kg^{-1}. Badania wykonano w trzech powtórzeniach, umieszczając w zlewkach o pojemności 150 cm^3 po 100 g powietrznie suchej gleby. Próbki glebowe zanieczyszczono olejem napędowym, benzyną i domieszką oleju rzepakowego i etanolu. Jako źródło azotu zastosowano siarczan amonu w ilości 0 i 250 mg N kg^{-1} gleby. Zawartość N-NO_3^- i N-NH_4^+ oznaczono w 14., 28. i 42. dniu założenia doświadczenia. Przez cały okres trwania badań utrzymywano stałą wilgotność gleby na poziomie 50% kapilarnej pojemności wodnej.

Stwierdzono, że w glebie zanieczyszczonej olejem napędowym i benzyną zachodziła silna immobilizacja azotu nawozowego. Obydwa zanieczyszczenia silnie hamowały proces nitryfikacji. Zdecydowanie większe zakłócenia w przebiegu procesu nitryfikacji powodował olej napędowy niż benzyna. Również silnym inhibitorem nitryfikacji okazał się olej rzepakowy. W 42. dniu trwania doświadczenia olej napędowy zmniejszał utlenianie kationu amonowego o 99%, a benzyna o 88%.

Słowa kluczowe: olej napędowy, benzyna, gleba, nitryfikacja, N-NH_4^+, N-NO_3^-.

INTRODUCTION

The nitrification process plays a key role in the nitrogen cycle, and nitrifying bacteria are particularly sensitive to environmental conditions. Soil quality can be evaluated in view of the counts of nitrifying bacteria and the intensity of the nitrification process (Castaldi et al. 2009, Dincer, Kargi 2000, Kucharski 2000, Simek 2000). Soil type also affects the process as soils with a high sorptive capacity can inhibit the toxic effect of polluting substances, thus enhancing the intensity of nitrification (Myśków et al. 1996, Wyszkowska, Kucharski 2001).

Increased concentrations of oil-derivative compounds in soil modify the soil's physical and chemical properties, its structure, as well as the composition and populations of soil microbes (Kucharski et al. 2004, Wyszkowska, Kucharski 2001). According to Barabasz (1992), nitrogen mineralization plays an important role in supplying plants with nutrients, but environmental pollution caused by oil-derivative products which reach the soil as a result of extraction and further processing may lead to adverse changes in the process of nitrogen transformation (Amadi et al. 1996, Jorgensen et al. 2000).

The supply of foreign substances to the soil environment may alter the soil's biochemical properties. This study attempted to determine the effect of soil contamination with diesel oil and petrol on the content of mineral nitrogen.

MATERIAL AND METHODS

The effect of soil contamination with diesel oil and petrol on the nitrification process was determined in the study. Samples were collected from the humus horizon of soil classified under natural conditions as typical brown soil developed from loamy sand, of pH of 6.6 in 1M KCl, hydrolytic acidity (Hh) of 11.38 mmol$^+$ kg^{-1}, total exchangeable alkaline cations (S) of 77.67 mmol$^+$ kg^{-1} and organic carbon content (C_{org}) of 8.50 g kg^{-1}.

The variable experimental factors were:

1. Type of pollutant in cm$^3 \cdot$kg^{-1} d.m. soil:
   - 0 (control),
   - DO (diesel oil – 10 cm^3),
   - P (petrol – 10 cm^3),
   - R (rapeseed oil – 1 cm^3),
   - E (ethanol – 1 cm^3),
   - DOR (DO 9 cm^3 + R 1 cm^3),
   - PE (P 9 cm^3 + E 1 cm^3).
2. Ammonium sulfate dose in mg N kg^{-1} soil: 0 and 250.
3. Time of analysis – days 0, 14, 28, 42.

The experiment was conducted in 3 replications. For each test, 100 g of air-dried soil was placed in 150 cm^3 beakers. Soil samples were contaminated (variable 1) and fertilized with ammonium sulfate (variable 2). All components were thoroughly mixed with the soil. The moisture content of soil was brought to 50% capillary water capacity. The beakers were incubated at a temperature of 25°C, and N-NH_4^+ and N-NO_3^- levels were determined in the soil on different days of the experiment (variable 3). The detailed procedure of extracting and determining mineral nitrogen is presented by KUCHARSKI et al. (2009). The quantity of nitrified nitrogen and % inhibition of the nitrification process were determined based on the results (WYSZKOWSKA 2002).

The results were processed statistically with the use of Duncan's multiple range test and a three-factorial analysis of variance. A statistical analysis was performed in the Statistica application (StatSoft, Inc. 2006).

RESULTS AND DISCUSSION

The results of the experiment indicate that soil contamination with oil-derivative products modified the nitrification process. Diesel oil and petrol with the addition of rapeseed oil and ethanol had a varied effect on N-NH_4^+ and N-NO_3^- concentrations (Tables 1 and 2).

Table 1

The effect of soil contamination with oil-derivative substances, rapeseed oil and ethanol on N-NH_4^+ levels (mg kg^{-1} soil)

Type of pollutant *	Time of analysis (days)			
	0	14	28	42
0 mg N kg^{-1} d.m. soil				
0	28.60 ± 0.46	24.63 ± 0.45	22.57 ± 0.59	4.08 ± 0.37
DO	28.83 ± 0.15	23.03 ± 0.23	21.20 ± 1.00	5.52 ± 0.37
P	65.60 ± 0.17	40.90 ± 0.72	21.60 ± 0.70	19.20 ± 1.86
R	28.47 ± 0.23	27.10 ± 0.36	22.23± 0.35	11.76 ± 0.37
E	23.83 ± 0.06	23.20 ± 0.44	20.70 ± 0.44	3.24 ± 0.39
DOR	23.97 ± 0.60	22.17 ± 0.55	20.33 ± 0.40	11.28 ± 0.37
PE	64.27 ± 0.25	37.37 ± 0.49	22.57 ± 0.06	15.60 ± 1.86
250 mg N kg^{-1} d.m. soil				
0	217.67 ± 0.58	155.33 ± 3.06	30.77 ± 0.81	5.40 ± 0.39
DO	221.00 ±1.73	211.00 ± 7.21	37.83 ± 0.75	20.64 ± 0.37
P	236.00 ± 2.65	154.33 ± 3.21	103.00 ± 1.73	24.72 ± 2.71
R	218.33 ± 2.89	34.87 ± 0.55	20.90 ± 0.36	4.56 ± 0.37
E	227.33 ± 2.52	190.67 ± 2.08	52.77 ± 0.35	7.56 ± 0.39
DOR	234.67 ± 2.52	34.03 ± 0.32	23.70 ± 0.17	11.40 ± 0.59
PE	236.00 ± 3.61	146.33 ± 3.51	94.60 ± 0.26	23.04 ± 2.28
$LSD_{0.01}$**	*a* – 1.29, *b* – 0.69, *c* – 0.97, $a \cdot b$ – 1.82, $a \cdot c$ – 2.57, $b \cdot c$ – 1.37, $a \cdot b \cdot c$ – 3.63			

*DO – diesel oil, P – petrol, R – rapeseed oil, E – ethanol

**LSD $_{0.01}$ for: *a* – type of oil-derivative substance and the addition of rapeseed oil and ethanol, *b* – nitrogen source, *c* – time of analysis.

Ammonia nitrogen levels decreased on successive days of the experiment. The highest N-NH_4^+ concentrations were noted on day 1, and the lowest – on day 42. Petrol contamination had a more profound effect on the content of ammonia nitrogen than diesel oil pollution. In a treatment with petrol without ammonium sulfate, high levels of NH_4^+ (40.90 mg kg^{-1}) were noted over 14 days, whereas in treatments with ammonium sulfate – over 28 days (103 mg N-NH_4^+ kg^{-1} d.m. soil). A reverse tendency was observed in respect of the nitrate nitrogen content of soil. The lowest quantities were determined in soil analyzed on the first day, and the highest – on the last (42nd) day of the experiment. Nitrate nitrogen concentrations were higher in soil contaminated with petrol than with diesel oil throughout the entire experiment.

Table 2

The effect of soil contamination with oil-derivative substances, rapeseed oil and ethanol on $N\text{-}NO_3^+$ levels (mg kg^{-1} soil)

Type of pollutant *	Time of analysis (days)			
	0	14	28	42
0 mg N kg^{-1} d.m. soil				
0	7.39 ± 0.03	9.29 ± 0.07	21.53 ± 0.15	26.13 ± 1.07
DO	7.10 ± 0.10	9.11 ± 0.03	0.00 ± 0.00	0.83 ± 0.00
P	7.28 ± 0.06	9.16 ± 0.02	11.37 ± 0.35	24.06 ± 1.07
R	6.61 ± 0.02	8.83 ± 0.08	10.23 ± 0.12	2.13 ± 0.17
E	6.52 ± 0.11	8.36 ± 0.04	9.57 ± 0.04	19.59 ± 1.13
DOR	7.32 ± 0.04	9.27 ± 0.05	0.00 ± 0.00	1.86 ± 0.23
PE	7.32 ± 0.03	8.77 ± 0.04	10.27 ± 0.21	20.28 ± 0.84
250 mg N kg^{-1} d.m. soil				
0	8.47 ± 0.37	41.57 ± 0.15	146.00 ± 1.73	240.97 ± 2.03
DO	7.33 ± 0.03	14.87 ± 0.47	0.00 ± 0.00	3.03 ± 0.21
P	7.54 ± 0.26	18.33 ± 0.21	47.53 ± 0.32	29.91 ± 1.13
R	6.83 ± 0.15	14.37 ± 0.15	43.47 ± 0.06	89.72 ± 1.13
E	6.67 ± 0.09	26.27 ± 0.15	125.67 ± 4.93	212.44 ± 1.84
DOR	7.96 ± 0.05	11.53 ± 0.32	0.00 ± 0.00	2.27 ± 0.23
PE	7.58 ± 0.23	17.43 ± 0.31	34.73 ± 0.45	24.06 ± 1.07
$LSD_{0.01}$**	*a* – 0.71, *b* – 0.38, *c* – 0.53, *a* · *b* – 0.99, *a* · *c* – 1.41, *b* · *c* – 0.75, *a* · *b* · *c* – 1.99			

*DO – diesel oil, P – petrol, R – rapeseed oil, E – ethanol
**$LSD_{0.01}$ for: *a* – type of oil-derivative substance and the addition of rapeseed oil and ethanol, *b* – nitrogen source, *c* – time of analysis.

Soil contamination with diesel oil and petrol had an adverse effect on nitrified ammonium sulfate levels (Table 3). The addition of rapeseed oil and ethanol contributed to the above trend. Diesel oil administered separately and with the addition of rapeseed oil proved to be a stronger inhibitor. In these treatments, negligent quantities of nitrate nitrogen were noted on day 42.

Diesel oil and petrol strongly inhibited the nitrification process (Table 4). Diesel oil had a much stronger inhibitory effect on nitrification than petrol. The addition of rapeseed oil as well as ethanol strongly contributed to the adverse effect of oil derivatives on the nitrification process. On day 42, diesel oil reduced cation oxidation by 99%, and petrol – by 88% in treatments with ammonium sulfate. The tested substances inhibited nitrification in the following order: diesel oil > diesel oil + rapeseed oil > rapeseed oil > petrol + ethanol > petrol > ethanol.

Table 3

The effect of soil contamination with oil-derivative substances, rapeseed oil and ethanol on the levels of nitrified nitrogen in soil fertilized with ammonium sulfate (%)

Type of pollutant *	Time of analysis (days)		
	14	28	42
0	12.91 ± 0.08	49.79 ± 0.75	85.94 ± 0.81
DO	2.30 ± 0.20	0.00 ± 0.00	0.88 ± 0.09
P	3.67 ± 0.08	14.47 ± 0.10	2.34 ± 0.62
R	2.21 ± 0.08	13.29 ± 0.02	35.04 ± 0.49
E	7.16 ± 0.07	46.44 ± 1.98	77.14 ± 0.87
DOR	0.91 ± 0.11	0.00 ± 0.00	0.17 ± 0.00
PE	3.47 ± 0.11	9.79 ± 0.10	1.51 ± 0.62
$LSD_{0.01}$**	a – 0.56, b – 0.42, $a \cdot b$ – 1.12		

*DO – diesel oil, P – petrol, R – rapeseed oil, E – ethanol
**$LSD_{0.01}$ for: *a* – type of oil-derivative substance and the addition of rapeseed oil and ethanol, *b* – nitrogen source, *c* – time of analysis.

The results of the study indicate that diesel oil was a more powerful inhibitor of the nitrification process than petrol. The adverse effect of oil-derivative substances on nitrification was noted by KUCHARSKI et al. (2004). The above authors found that leaded and unleaded petrol administered in the amount of 6 cm^3 kg^{-1} d.m. soil had a stronger inhibitory effect on nitrification than diesel oil. An opposite effect was noted in this study, most probably due to higher doses (10 cm^3 kg^{-1} d.m. soil) of the tested oil-derivative substances than those applied in the above cited experiment.

The studies carried out by AMADI et al. (1996), NIEWOLAK and KOZIEŁŁO (1998) validated the negative effect of oil-derivative substances on the nitrification process. The above authors observed that nitrifying bacteria were highly sensitive to soil contamination with crude oil. The adverse effect of pollution with oil-derivative substances on the soil's biological activity was also noted by XU et al. (2000). The results of the above study pointed to the high sensitivity of bacteria of the genera *Nitrosomonas* and *Nitrobacter* to the tested substances.

KUCHARSKI et al. (2004), NIEWOLAK and KOZIEŁŁO (1998) argued that the dynamics of the nitrification process increases over time as hydrocarbon levels decrease due to evaporation and mineralization. Another crucial factor is the selection of microbes resistant to the toxic effect of soil pollutants. The findings of SCHIE and YOUNG (1998) indicate that low-biodegradable fuel compounds may be metabolized by microorganisms.

Table 4

The nitrification-inhibiting effect of oil-derivative substances, rapeseed oil and ethanol (%)

Type of pollutant *	Time of analysis (days)		
	14	28	42
0 mg N kg^{-1} d.m. soil			
0	1.90 ± 0.42	100.00 ± 0.00	96.84 ± 0.13
DO	1.40 ± 0.56	47.21 ± 1.77	7.91 ± 0.33
P	4.95 ± 0.57	52.48 ± 0.36	91.84 ± 0.61
R	10.04 ± 0.56	55.54 ± 0.50	24.89 ± 5.35
E	0.21 ± 0.18	100.00 ± 0.00	92.68 ± 0.73
DOR	5.63 ± 0.23	52.32 ± 1.01	22.22 ± 5.23
PE			
0	64.23 ± 1.25	100.00 ± 0.00	98.74 ± 0.10
DO	55.89 ± 0.38	67.44 ± 0.30	87.59 ± 0.37
P	65.44 ± 0.37	70.23 ± 0.38	62.76 ± 0.61
R	36.81 ± 0.23	13.90 ± 4.06	11.83 ± 1.18
E	72.25 ± 0.78	100.00 ± 0.00	99.06 ± 0.09
DOR	58.06 ± 0.82	76.21 ± 0.40	90.02 ± 0.39
PE	a – 1.68, b – 0.97, c – 1.37, $a \cdot b$ – 2.37, $a \cdot c$ – 3.36, $b \cdot c$ – 1.94, $a \cdot b \cdot c$ – 4.75		

*DO – diesel oil, P – petrol, R – rapeseed oil, E – ethanol
**LSD $_{0.01}$ for: a – type of oil-derivative substance and the addition of rapeseed oil and ethanol, b – nitrogen source, c – time of analysis.

CONCLUSIONS

1. Soil contamination with diesel oil and petrol has a negative effect on the nitrification process.
2. Diesel oil is a stronger inhibitor of the nitrification process than petrol.
3. The addition of rapeseed oil to diesel oil and the addition of ethanol to petrol reinforces the adverse effect of the studied oil-derivative substances on the dynamics of ammonium nitrogen nitrification in soil.

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EFFECT OF ADDITION OF Ca, Cu, Fe, Zn AND LACTIC ACID TO DRINKING WATER ON CONTENT OF THESE ELEMENTS IN MUSCLES OF SLAUGHTER TURKEYS

Bogusław Makarski[1], **Ewa Makarska**[2]

[1]**Chair of Biochemistry and Toxicology**
[2]**Chair of Chemistry**
University of Life Sciences in Lublin

Abstract

The study was conducted on 300 turkey cocks type BUT-9, grown under optimal conditions for this kind of poultry. The feed was a typical commercial full-dose mix for turkeys. The birds were divided into 5 experimental groups. From the 3rd week of their life, they were given preparations with their drinking water. Group I was the control group, while drinking water for the test groups was enriched as follows: lactic acid (0.4%) for group II, $CuSO_4$ in the amount of 30 mg $Cu \cdot dm^{-3}$ H_2O for group III, $CuSO_4$ (30 mg $Cu \cdot dm^{-3}$) and 0.4% of lactic acid for group IV and $CuSO_4$ in the amount of 50 mg $Cu \cdot dm^{-3}$ for group V. Application of the preparations was terminated after 3 days, when symptoms of poisoning were observed in turkey cocks of groups II and IV, alongside increased mortality rate. Samples of drinking water were taken for analyses, and from each group 10 birds were selected for slaughter to take tissue samples (breast and leg muscles).

The objective of the experiments was to determine the concentration of Ca, Mg, Zn, Cu and Fe in the consumable tissues of turkey cocks.

The study showed that the highest rate of mortality of turkey cocks was observed in the group that was given lactic acid with their drinking water, and these turkeys had increased levels of Ca, Cu, Zn and Fe. In none of the groups, the content of Zn in the breast muscle exceeded 20 mg kg^{-1}, while the concentration of copper in all experimental groups (except the control) was above the level of 10 mg kg^{-1}. Increased doses of copper caused an increase in the concentration of magnesium in the leg muscles, while a combination of copper supplementation with lactic acid resulted in increased accumulation of Cu in both the breast and leg muscles.

Key words: turkeys, zinc, copper, lactic acid, breast and leg muscles.

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prof. dr hab. Bogusław Makarski, Chair of Biochemistry and Toxicology, University of Life Sciences in Lublin, e-mail: boguslaw.makarski@up.lublin.pl

WPŁYW DODATKU Ca, Cu, Fe I Zn ORAZ KWASU MLEKOWEGO DO WODY PITNEJ NA ZAWARTOŚĆ TYCH PIERWIASTKÓW W MIĘŚNIACH INDORÓW RZEŹNYCH

Abstrakt

Badania przeprowadzono na 300 indorach typu BUT-9, odchowywanych w optymalnych warunkach przewidzianych dla tego typu ptaków. Paszą była typowa komercyjna mieszanka pełnoporcjowa dla indyków. Ptaki podzielone na 5 grup doświadczalnych, od 3. tygodnia życia otrzymywały preparaty do wody pitnej. Grupa I była grupą kontrolną, grupa II otrzymywała do picia wodę z kwasem mlekowym (0,4%), grupa III otrzymywała do wody pitnej dodatek $CuSO_4$ w ilości 30 mg $Cu \cdot dm^{-3}$ H_2O, grupa IV – dodatek $CuSO_4$ (30 mg $Cu \cdot dm^{-3}$) i 0,4% kwasu mlekowego, grupa V – dodatek $CuSO_4$ w ilości 50 mg $Cu \cdot dm^{-3}$. Podawanie preparatów zakończono po 3 dniach, ponieważ w grupach II i IV zaobserwowano u ptaków objawy zatrucia i zwiększoną liczbę padnięć. Pobrano próbki wody pitnej do analiz oraz wybrano z każdej grupy po 10 ptaków do uboju, w celu pobrania prób z tkanek (mięsień piersiowy i mięsień udowy).

Celem badań było określenie kumulacji Ca, Mg, Zn, Cu i Fe w tkankach konsumpcyjnych indorów.

W badaniach stwierdzono, że najwięcej padnięć indorów zaobserwowano w grupie otrzymującej dodatek kwasu mlekowego do wody pitnej, w której stwierdzono zwiększone ilości Ca, Cu, Zn i Fe. W mięśniu piersiowym indorów ze wszystkich grup zawartość Zn nie przekraczała 20 mg kg^{-1}, natomiast kumulacja miedzi we wszystkich grupach doświadczalnych, oprócz kontrolnej, przekraczała 10 mg kg^{-1}. Zwiększone dawki miedzi wpłynęły na podwyższenie koncentracji magnezu w mięśniu udowym, a suplementacja miedzi z kwasem mlekowym spowodowała wzrost kumulacji Cu w mięśniach piersiowym i udowym.

Słowa kluczowe: indyki, cynk, miedź, kwas mlekowy, mięsień piersiowy, mięsień udowy.

INTRODUCTION

Zinc is one of the trace elements that occur in organisms, mainly in the liver, kidneys and in the bones. The metabolism of zinc and of other elements is subject to hormonal regulation with participation of glycocorticoids, insulin and catecholamines. Also, zinc transformations are aided by interleukin-1, related with the immune system, inflammatory conditions and stress (MALINOWSKA 1993). Its most important function is participation maintaining the activity of numerous enzymes which determine the proper course of physiological processes. Zinc is the only element that is represented by at least one enzyme in all of the 6 main classes (KIDD et al. 1996, KORELEWSKI, ŚWIĄTKIEWICZ 2001). Absorption of zinc from feed varies between 10 and 40%, and takes place primarily in the proximal small intestine. When the zinc content in feed is low, its absorption in the intestines increases, while a high supply of zinc inhibits its absorption from the gastrointestinal system in poultry (NOY et al. 1994). Zinc absorbed in increased quantities accumulates in the bones, pancreas, liver, kidneys and intestines. When the tissue capacity is exhausted but the organism continues to receive zinc, its concentration in the plasma grows and first symptoms of zinc poisoning appear. As regards turkeys, these symptoms can appear at zinc levels of at least 2230 mg·kg^{-1} of feed (KIDD et al. 1996, NOY et al. 1994). The bio-availability of zinc depends on a number of factors, the presence of which may facilitate or inhibit its absorption. According to NOY et al. (1994) and WEDEKIND et al. (1992), the main inhibitors of zinc absorption include phytic acid, tannins, arginine, and such elements as Ca, Cd, Cu, Mg and P. The primary factor that determines the absorption of zinc is the water solubility of its compounds formed in the gastrointestinal tract. Ligands with smaller molecular weight, i.e. amino acids or organic acids, may promote the absorption of zinc.

Although turkeys do not need much copper (*ca* 2-4 mg·kg^{-1} of feed), this metal shapes the proper course of numerous processes and functions, i.e. the formation of cross-bonds in collagen, mineralisation of bones, biosynthesis of haem, oxidative phosphorylation, metabolism of glucose and cholesterol, immune functions, functioning of the central nervous system, and many others. Copper is frequently added in increased amounts to poultry feeds for prophylactic purposes, as a growth stimulator (PESTI, BAKALLI 1996). In practice, however, copper deficit in turkeys appears frequently due to the presence of phytins and phosphates in feed, and because of antagonisms between certain elements, e.g. Zn and Cu (PANG, APPLEGATE 2007). In respect of turkeys, copper deficit is especially dangerous, as it may be the cause of spontaneous rupture of arteries (MADEJ et al.1994). At present, with the currently binding prohibition on using antibiotics for prophylactic purposes in poultry, Cu appears to be a good substitute owing to its biological properties. However, copper should be applied at doses notably higher than the ones allowed by the current Polish poultry feeding standards (Standards of Poultry Feeding 2005).

Calcium appears in large amounts in birds because of its structural role in the bone structure. It also plays a highly important role in the regulation of numerous cellular functions. In an ionised form, it participates in numerous biochemical transformations in an organism that lead to a variety of effects manifested by contraction, secretion or proliferation. With a high supply of calcium in a diet and high calcium content in the organism, the absorption of this element is low and takes place mainly through passive transport from the intestinal lumen to the enterocyte space (ANDERSON 1991).

Apart from its structural function, magnesium in the organism constitutes the primary regulator of the cell growth cycle. The fundamental functions of magnesium include participation in the processes of synthesis and decomposition of high-energy compounds (ATP) and activation of most enzymes. The magnesium-adenosine-5'-triphosphate complex is a substrate for numerous enzymes participating in carbohydrate and fat transformations (AIKAWA 1981).

In animals, most iron occurs in metabolically active compounds, in a form bound with protein complexes. As a component of haemoglobin, myoglobin and many enzymes, it performs fundamental functions in oxygen transport and storage, de-saturation of fatty acids, tyrosine iodination, biosynthesis of prostaglandins. The amount of absorbed iron is variable and depends for example on the needs of an organism, i.e. it is more intensive in states of deficiency and decreases when the state of organism's saturation with iron is attained (NAGURNA-STASIAK, LECHOWSKI 1994).

In industrial aviculture of turkeys, it is a frequent practice to add small amounts of lactic acid (usually 0.2%-0.3%) on silica carrier to feed or directly to drinking water. Lactic acid is a strong prebiotic; by lowering pH to the level of 5.0-6.8 in particular sections of the gastrointestinal tract, it creates optimum conditions for colonisation by beneficial bacteria, and facilitates resorption of mineral components. In a study by BYRD et al. (2001) and HIGGINS at al. (2007), it was demonstrated that lactic acid given to birds in their drinking water notably reduced the multiplication of *Salmonella* s. Enteritidis and s. Typhimurium in their gastrointestinal tract, and lowered their content in the excrements. Lactic acid is classified as a strong acid and is capable of destroying metal objects (it even etches stainless and acid-resistant steel), which is why it is difficult to apply in aviculture.

The objective of the study was to determine the concentration of Ca, Mg, Zn, Cu and Fe in the breast and leg muscles of turkey cocks given increased doses of Cu, Zn, Fe and Ca in drinking water, in combination with lactic acid.

MATERIAL AND METHODS

The study was conducted on medium-weight turkey cocks type BUT-9 maintained under conditions that complied with those recommended for birds of this type. On the 2nd day of their life, the squealers were divided into 5 groups of ca 60 turkey chicks each that were fed typical full-dose mixes suitable for their age. An approximate content of basic mineral components in the full-dose feed mixes for turkeys, based on analyses performed by the manufacturer, is given in Table 1. From the 3rd week of the birds' life, application of copper compounds and lactic acid was begun. Group I was the control group, while drinking water for the other groups was enriched as follows: lactic acid in the form of 0.4% solution for group II, $CuSO_4$ in the amount of 30 mg $Cu \cdot dm^{-3}$ H_2O for group III, combined $CuSO_4$ (30 mg $Cu \cdot dm^{-3}$) and 0.4% solution of lactic acid for group IV and $CuSO_4$ at the level of 50 mg $Cu \cdot dm^{-3}$ for group V. The day after the first application of the experimental preparations, and on subsequent days, an increase was observed in the rate of mortality of turkeys in groups II and IV (Table 1). As

Table 1

Content of basic mineral components in full-dose feed mixes for turkeys (manufacturer's data)

Full-dose feed mixes	Ca	Mg	K	P	Zn	Fe	Cu	Mn
	$(g\ kg^{-1})$				$(mg\ kg^{-1})$			
I (weeks 0-3)	14.21	2.85	4.57	7.34	84.34	48.94	20.47	115.62
II (weeks 4-6)	15.37	3.21	3.94	8.06	87.42	52.14	24.82	119.26

a result, after 3 days the application of the preparations was discontinued and samples of drinking water were taken for chemical analyses. From each group, 10 birds were selected for slaughter, to take tissue samples (breast and leg muscles). Since then, From the turkeys were kept in their cages but they were no longer given the experimental preparations. In groups II and IV some young turkeys observed to have of poisoning (they had no appetite and tended to assume immobile positions with their wings lowered). These signs receded gradually, but were still observable in the 4th week of the birds' life. In the tissue samples taken from the breast and leg muscles of 3-week old turkeys, determinations were made of the content of Zn, Cu, Ca, Mg, Fe and K with the AAS method after prior wet mineralisation in a mixture of HNO_3 and $HClO_4$ (5:1) and dilution with distilled water. Also the samples of drinking water taken from the tanks of each experimental group were subjected to chemical analysis for the content of the same mineral components as those determined in the tissue samples, except mineralisation. The results were subjected to statistical analysis using Statistica version 5 software, with the ANOVA test of single-factor analysis of variance, adopting 0.01 and 0.05 levels of significance.

RESULTS AND DISCUSSION

Table 3 presents the content of mineral components in the drinking water given to the turkeys. The results indicate that in groups II and III the drinking water contained increased levels of Zn, Cu, Fe, Ca and Mg. The increased concentration of copper in the water was in agreement with the assumptions of the biological experiment and corresponded to the amount of copper added to the drinking water in the amounts of 30 mg in groups III and IV, and 50 mg in group V. In the water supplemented with lactic acid, increased concentrations of Zn, Fe, Ca and Mg were observed. Acidification of drinking water with strong lactic acid caused increased dissolution of zinc (group II – 550 $mg \cdot dm^{-3}$, and group IV – 682 $mg \cdot dm^{-3}$) and iron (group II – 24.73 $mg \cdot dm^{-3}$, and group IV – 20.09 $mg \cdot dm^{-3}$) from the water supply system which was made of zinc-coated iron alloy. Increase in the concentration of Cu, Zn and Fe in drinking water for turkeys, however, should not be life threatening, as these concentrations were still significantly below the levels assumed to be toxic, i.e. 500 mg kg^{-1} of feed for copper, 2230 mg kg^{-1} of feed for zinc, and 2000 mg kg^{-1} of feed for iron (Kidd et al. 1996). There was also a 4-fold increase in the level of Ca and 2-fold increase in that of Mg with relation to the mains drinking water. In the groups of turkeys that were given lactic acid, lack of appetite was observed immediately the day after the first application, which was followed by apathy and, finally, increased mortality (Table 2). After detailed examination of the dead birds, performed by a veterinarian, all causes of death other than poisoning were excluded. Zinc should be taken into consideration among factors that might have caused the poisonings, as its content in the drinking water was the closest to the toxic level.

Table 2

Deaths of turkeys during 5-week aviculture

Group	Number of deaths					
	week 1	week 2	week 3	week 4	week 5	total
I	5	2	1	0	0	8 (13.3%)
II	4	0	4	5	0	13 (27.7%)
III	18	2	0	0	0	20 (33.3%)
IV	12	1	23	5	0	41 (68.3%)
V	7	1	2	0	1	11 (18.3%)

Table 1 presents the mortality rate of turkeys during the 5-week aviculture. The highest incidence of bird deaths was recorded in the first week of their life. Immediately after transport the squealers were weighed, earmarked and assigned to the particular experimental groups. After three weeks, the application of the experimental preparations was begun, which resulted in an increase of mortality among turkeys in groups supplemented with lactic acid. The highest mortality rate was recorded in group IV (23 birds), where lactic acid was applied in combination with $CuSO_4$. However, the increased level of copper fed to the turkeys in that group probably had no influence on absorption of zinc in the form of lactate. Increased mortality was also observed in group II (8 birds), where 0.4% lactic acid was applied. The effects of poisoning were still observable in the 4th week of the birds' life, when 5 deaths each were recorded in groups II and IV.

Based on the mean daily feed consumption by turkey cocks, on the content of mineral components in full-dose feed mixes (Table 1) and on the concentration of these elements in drinking water (Table 3), it is possible to make a theoretical calculation of the daily intake of Zn and Cu by the turkeys. Thus, it was calculated that in the 3rd week of their life, the turkeys absorbed the following amounts: group I (control) 7.57 mg Zn and 1.91 mg Cu, group II: 91.59 mg Zn and 1.93 mg Cu, group III: 7.74 mg Zn and

Table 3

Content of mineral components in drinking water for turkeys

Sample	Mineral components ($mg \cdot dm^{-3}$)					
	Ca	Mg	K	Zn	Cu	Fe
Tap water	65.10	7.98	1.85	5.38	0.01	0.003
H_2O+lactic acid	269.10	12.30	1.22	550.00	0.15	24.73
$H_2O+CuSO_4$ (30 mg $Cu \cdot dm^{-3}$ H_2O)	57.30	6.54	1.63	6.72	32.25	0.005
$H_2O+CuSO_4$+lactic acid	274.80	11.80	4.20	682.00	34.50	2009
$H_2O+CuSO_4$ (50 mg $Cu \cdot dm^{-3}$ H_2O)	62.18	8.32	1.73	5.28	51.03	0.003

6.91 mg Cu, group IV: 111.9 mg Zn and 7.23 mg Cu, group V: 7.56 mg Zn and 9.78 mg Cu. On this basis, we can conclude that the dose of 111.9 mg Zn absorbed by group IV turkeys during one day was enough to be toxic as it caused increased rate of mortality (Table 2).

Table 4 presents the content of mineral components in the breast muscle of turkeys that received increased amounts of copper (30 and 50 mg $Cu \cdot dm^{-3}$ H_2O), zinc (550 and 682 mg $Zn \cdot dm^{-3}$ H_2O) as well as calcium and iron in their drinking water. The concentration of Ca in the breast muscle determined in all the experimental groups was similar except group V, where under the effect of $CuSO_4$ in the higher dose (50 mg $Cu \cdot dm^{-3}$ H_2O) there appeared a decrease in the concentration of this element, statistically significant versus the other groups of turkeys. The minerals (Zn, Cu, Ca and Fe) contained in drinking water had no significant effect on the content of Mg in the breast muscle. However, it should be noticed that the highest concentrations of Mg were observed in group III (30 mg $Cu \cdot dm^{-3}$ H_2O), and the lowest in the control group. The concentration of zinc, in turn, was similar in all the experimental groups, even though it was probably zinc that caused the poisoning of turkeys in groups II and V. Similar observations were made by Bou et al. (2005) in their study on chickens, which did not show any effect of increased doses of Zn in the diet on the content of this element in the muscles. However, as the Zn concentration in the diet increased significantly in the 3rd week of chickens' life, Sandoval et al. (1998) observed a linear relation between the concentration of Zn in muscles and its level in the feed. In none of the experimental groups, the mean zinc content in the breast muscle was over 20 mg Zn kg^{-1}.

In all the experimental groups, the concentration of copper was higher with relation to the control, and the differences obtained were confirmed statistically. It was observed that the level of copper accumulation in the breast muscle depended on its concentration in drinking water given to the turkeys (Table 3). The same dependence was observed by Makarski et al.

Table 4

Concentration of mineral components in breast muscle of turkeys

Group	Mineral components ($mg \cdot kg^{-1}$)				
	Ca	Mg	Zn	Cu	Fe
I	38.17^a	395.28	16.75	7.76^a	23.57
	±12.85	±42.16	±4.26	±1.22	±12.43
II	43.80^a	385.06	15.94	18.13^b	17.97
	±15.70	±99.48	±5.85	±7.80	±5.36
III	40.42^a	471.81	15.83	15.03^b	18.45
	±16.49	±109.83	±1.10	±7.80	±4.93
IV	36.69^a	439.56	15.45	18.55^b	27.53
	±13.23	±30.03	±2.44	±7.77	±17.66
V	24.08^b	460.55	14.22	27.84^b	17.48
	±0.40	±21.00	±0.58	±0.81	±2.20

a, b – differences significant at $p < 0.05$

2004, MAKARSKI, ZADURA 2006. Noteworthy is the result obtained for group II of turkeys receiving lactic acid, where increased accumulation of copper was observed in the breast muscle of the birds in spite of its absence in drinking water. In this group, the concentration of copper was 18.13 $mg \cdot kg^{-1}$ and was higher than in the control group (7.76 $mg \cdot kg^{-1}$), as well as in group III, where copper concentration in the breast muscle was 15.03 $mg \cdot kg^{-1}$. We can suppose that copper contained in feed, after its transformation into lactate, is more easily resorbed from the alimentary tract compared to other chemical forms of copper. In other studies (PANG, APPLEGATE 2007), antagonism between Cu and Zn was observed. In our study, it was demonstrated that the concentration of copper in the breast muscle of turkeys being administered additives of copper and lactic acid considerably exceeded the level of 10 mg Cu kg^{-1}. It was only in the control group that the concentration of copper remained at a low level.

The concentration of magnesium in the breast muscle of turkeys from all the experimental groups was higher than in the control group, but the differences were not statistically significant. In the case of iron concentrations, its highest value was observed in turkeys from group IV, and the lowest in birds from group V. The differences were not statistically significant.

Table 5 presents the content of mineral components in the leg muscle of turkeys. It was found that the highest concentration of Ca (134.48 $mg \cdot kg^{-1}$) in this muscle appeared in birds from group IV. In contrast, the maximum dose of copper applied in the experiment (50 mg $Cu \cdot dm^{-3}$ H_2O) caused

Table 5

Concentration of mineral components in leg muscle of turkeys

Group	Mineral components ($mg \cdot kg^{-1}$)				
	Ca	Mg	Zn	Cu	Fe
I	94.38	324.74^{b}	38.21^{a}	8.64^{a}	21.62
	±18.62	±17.26	±2.71	±1.53	±3.65
II	85.87	$351.27a^{b}$	22.70^{b}	11.14^{a}	24.80
	±12.77	±20.48	±6.17	±0.59	±6.83
III	93.02	371.97^{a}	37.54^{a}	10.92^{a}	23.74
	±44.30	±23.12	±4.00	±0.80	±0.85
IV	134.48	324.10^{b}	33.00^{a}	20.55^{b}	21.33
	±46.39	±87.48	±9.10	±16.12	±5.63
V	55.74	362.60^{a}	30.53^{a}	11.60^{a}	27.55
	±4.50	±17.46	±2.34	±0.87	±1.12

a, b – differences significant at $p < 0.05$

a decrease in the concentration of Ca from 94.38 $mg \cdot kg^{-1}$ (control) to the level of 55.74 $mg \cdot kg^{-1}$. The differences, however, were not confirmed statistically. Analogous variation in the concentration of Ca under the effect of the experimental factors was observed in the breast muscle of the turkeys (Table 4), where it was statistically confirmed. It should be noticed that the concentrations of Ca in the leg muscle were considerably higher than in the breast muscle. In the study by LEACH at al. (1990), a relationship was found between the intestinal absorption of copper and the content of calcium in the gastrointestinal tract, and it appears that the results obtained in our study can be attributed to such interaction.

Increased doses of Zn in groups II and IV had no effect on the concentration of Ca in the leg muscle. In the experiment, a significant increase in the concentration of Mg was observed in groups III and V (371.97 $mg \cdot kg^{-1}$ and 362.60 $mg \cdot kg^{-1}$, respectively) versus the control group (324.74 $mg \cdot kg^{-1}$). In these groups, the turkeys were administered a supplement of $CuSO_4$ in the amounts of 30 and 50 mg $Cu \cdot dm^{-3}$ H_2O. Under the effect of the experimental factors, a significant decrease in the concentration of zinc in the leg muscle appeared. These results are of particular significance to the consumers, as they indicate that increased doses of zinc and copper did not cause increased accumulation on Zn in the leg muscle of the turkeys. A decrease in the Zn concentration under the effect of increased doses of Cu in feed appears to support antagonism between these two elements observed in numerous studies (NOY et al. 1994, WEDEKIND et al. 1992, SANDOVAL et al. 1998, PANG, APPLEGATE 2007). Similarly to the effects observed on the breast muscle, all the experimental factors caused an increase in the concentration of copper in the leg muscle. A particularly high concentration of copper was observed in group IV under the effect of $CuSO_4$ and lactic acid, which would indicate that the lactic acid supplement enhanced Cu resorption from the gastrointestinal tract of turkeys. It also appears that the phenomenon of increased Cu resorption may be strongly affected by the content of Ca in drinking water, which was confirmed by LEACH at al. (1990). The concentration of Cu in the leg muscle in particular groups of turkeys, except the control, exceeded the level of 10 mg Cu kg^{-1}, which may constitute a hazard to consumers. With respect to Fe, the experimental factors did not cause any significant changes in the concentration of this element in the leg muscle of the turkeys.

CONCLUSIONS

1. The highest incidence of deaths of turkeys occurred in groups administered 0.4% lactic acid and when the administration of lactic acid was combined with copper supplementation.

2. The study showed that a daily intake of zinc in the amount of 111.9 mg was toxic to young turkeys.

3. The concentration of Zn in the breast and leg muscles of turkeys from all the experimental groups was at a constant level, while the concentration of Cu increased considerably in groups administered copper and lactic acid supplements.

4. Lactic acid added to drinking water for turkeys, due to its chemical properties, may constitute a hazard to birds' health, especially when water supply pipes are made of metal.

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INFLUENCE OF PARENTAL FORMS ON CHANGES IN THE CONTENT OF MINERAL ELEMENTS IN GRAIN OF NEW WINTER TRITICALE HYBRID STRAINS

Ewa Makarska[1], Anna Ciołek[1], Wanda Kociuba[2]

[1]Chair of Chemistry
[2]Plant Breeding and Biotechnology
University of Live Sciences in Lublin

Abstract

The paper presents a study on the content of Ca, Mg, K, Mn, Zn, Cu, and Fe in kernels (F5 generation) of winter triticale produced by crossbreeding three maternal with two paternal forms. As maternal forms, two strains were used, IGS 5101, FDT 975, as well as cultivar Alzo. The paternal components consisted of strains LAD 122 and F 8063. Morphologically established hybrid strains were selected at the Institute of Genetics, Plant Breeding and Biotechnology of the University of Life Sciences in Lublin. The component selection for crossbreeding was performed to achieve hybrids with great yield-forming potential and to improve the resistance to grain sprouting. Minerals were determined by the atomic absorption spectrometry (AAS) method. The results revealed differentiation in amounts of the analysed mineral components in hybrids depending on genotypes of the parental forms. Hybrid strains were usually characterized by a lower ash content in kernels than the parental forms. Among all the hybrids, strain IGS 5101 × F 8063 was distinguishable by its higher content of K, Ca, Mn, and Fe in grain versus both parental forms. It also excelled exceeded the other hybrids in respect of Ca, K and Mn. Tests on the mineral components in hybrid triticale strains indicate that it is possible to shape their levels through an appropriate selection of parental forms and selection during breeding work on X *Triticosecale* Wittmack genus to achieve cultivars of improved nutritional value.

Key words: triticale, hybrid strains, mineral elements.

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prof. dr hab. Ewa Makarska, Chair of Chemistry, University of Live Sciences, Akademicka 15, 20-950 Lublin, Poland, phone: 081 445 65 86, e-mail: ewa.makarska@wp.pl

WPŁYW FORM RODZICIELSKICH NA ZMIANY ZAWARTOŚCI SKŁADNIKÓW MINERALNYCH W ZIARNIE NOWYCH RODÓW MIESZAŃCOWYCH PSZENŻYTA OZIMEGO

Abstrakt

W pracy badano zawartość Ca, Mg, K, Mn, Zn, Cu, Fe w ziarnie (pokolenie F5) pszenżyta ozimego otrzymanego w wyniku krzyżowania trzech form matecznych z dwoma ojcowskimi. Jako formy mateczne użyto: 2 rody IGS 5101, FDT 975 i odmianę Alzo. Formami ojcowskimi były rody LAD 122 i F 8063. Rody mieszańcowe ustalone morfologicznie wyselekcjonowano w Instytucie Genetyki, Hodowli Roślin i Biotechnologii Uniwersytetu Przyrodniczego w Lublinie. Dobór komponentów do krzyżowania prowadzono w celu uzyskania mieszańców o dużym potencjale cech plonotwórczych oraz poprawienia odporności na porastanie ziarna. Zawartość składników mineralnych analizowano metodą absorpcyjnej spektrometrii atomowej (AAS). Wyniki badań wykazały zróżnicowanie poziomu badanych składników mineralnych w mieszańcach zależnie od genotypów form rodzicielskich. Rody mieszańcowe wykazywały zwykle niższą popiołowość ziarniaków w porównaniu z formami rodzicielskimi. Z badanych mieszańców wyróżniał się ród IGS 5101 × F 8063, w którego ziarnie stwierdzono wyższą zawartość K, Ca, Zn, Mn i Fe w odniesieniu do obu form rodzicielskich oraz Ca, K i Zn w porównaniu z pozostałymi rodami mieszańcowymi. Badania zawartości składników mineralnych w mieszańcowych rodach pszenżyta wskazują na możliwość kształtowania ich poziomu przez odpowiedni dobór komponentów rodzicielskich i selekcję w pracach hodowlanych w obrębie rodzaju X *Triticosecale* Wittmack dla uzyskania odmian o wyższej wartości żywieniowej.

Key words: pszenżyto, rody mieszańcowe, składniki mineralne.

INTRODUCTION

Triticale is a cereal plant that has modest requirements and is tolerant to low soil pH and water deficiency (Masłowski et al. 1994, Kaczmarczyk et al. 2000). It can therefore be grown on lighter soils, under soil and weather conditions typical of Poland. When evaluated for its nutritional value as fodder, triticale demonstrates many advantages, for instance high protein levels of higher biological value than proteins in wheat or rye, richer mineral composition, and smaller amounts of anti-nutritive substances than rye (Makarska, Gruszecka 1998, Kociuba, Wasak 1998). Breeders continue to be interested in r achieving stable triticale genotypes, characterized by high potential and good quality of kernels. Thus, new sources of genetic material for creative breeding are searched for in order to obtain genotypes of greater practical importance and potential, also as a baking grain, than the initial forms (Gustafson et al. 1989, Gruszecka et al. 2004, Makarska 2000, Sodkiewicz et al. 2002). Valuable forms can be obtained by introducing into crossbreeding components which supply genes that make plants resistant to diseases, tolerant to drought or high temperatures, or adaptable to be cultivated under different pH conditions (Masłowski et al. 1994).

There is growing interest in the breeding work in which where related wild forms (*Aegilops* sp. or *Agrotriticum*) are used for crossbreeding with triticale. Beside improving the resistance to environmental factors, the obtained material is expected to have wider genetic diversity in terms of chemical composition, including mineral make-up (MAKARSKA, GRUSZECKA 1996, 2000).

The study aimed at evaluating the content of nutrients in grains of hybrid triticale strains as compared to their parental forms.

MATERIAL AND METHODS

The material for study consisted of kernels of 5 hybrid winter triticale strains produced by crossbreeding strains and varieties originating from various collections as well as their parental forms (F5 generation) – Table 1. The hybrids were morphologically established and selected at the Institute of Genetics, Plant Breeding and Biotechnology of the University of Live Sciences in Lublin. The grain samples were taken from a field experiment set in 2 replications on 2 m^2 area plots on loess soil with brown subsoil. Common vetch was the forecrop. Mineral fertilization was applied at the following amounts: 60 kg N ha^{-1}, 80 kg ha^{-1} P_2O_5, 100 kg ha^{-1} K_2O.

Table 1

Material for study

Sample number	Forms	Strain / Cultivar
1	maternal form	IGS 5101
2		FDT 975
3		Alzo
4	paternal form	LAD 122
5		F 8063
6	hybrid strain	IGS 5101 × LAD 122
7		IGS 5101 × F 8063
8		FDT 975 × LAD 122
9		Alzo × LAD 122
10		Alzo × F 8063

The component selection for crossbreeding was performed to obtain hybrids of great yield-forming potential and to improve the resistance to grain sprouting. Since the qualitative traits of the hybrids had not been previously tested, such an analysis was performed. Qualitative determinations of early uniform populations of strains facilitate their proper selection for further breeding and may be a starting point for establishment of a new variety.

Minerals (Ca, Mg, K, Fe, Mn, Zn, Cu) were determined by the atomic absorption spectrometry (AAS) method using a UNICAM 939 apparatus after combustion in a muffle furnace and dissolution in hydrochloric acid (1:1). All the analysis were made in three replications. The results were statistically processed by calculating arithmetic means, while the verification of differences between mean values for particular hybrid strains and parental components was performed with variance analysis and Tukey's test at the level of significance p=0.05. The coefficient of variation was calculated according to the formula: (standard deviation/arithmetic mean) · 100%.

RESULTS AND DISCUSSION

The hybrids, as compared to the parental forms, were usually characterized by a lower ash content, except strain IGS 5101 × F 8063, which had the highest ash content in grain (1.98%) – Table 2. The analysis of the content of macronutrients and microelements in hybrid kernels and their parental forms showed variability of levels of these elements. The study focused on the influence of a genotype on the content of mineral nutrients in order to confirm the possibility of shaping their levels in hybrid strains through a proper selection of parental forms.

Beside the genotype effect, other authors (Feil, Fossati 1995) pointed to a significant effect of the field location and year on the uptake and accumulation of minerals in cereal grains. Such influence was determined for most of mineral elements, whose level was also affected by the interaction between location and weather conditions.

When evaluating the content of macronutrients in winter triticale, it was found that Ca concentration in kernels of the maternal form cv. Alzo was significantly higher than in the other maternal components. Among the analyzed paternal forms, kernels of LAD 122 strain were characterized by a higher Ca level, although the paternal components did not statistically differ. The level of Ca in hybrid strains – IGS 5101 x LAD 122 and FDT 975 × LAD 122 – was lower than in the parental kernels; they were a homogenous group with the maternal forms but had a significantly lower Ca content than the paternal components. For the strains Alzo x LAD 122 and Alzo x F 8063, the Ca content in grain was moderate; statistically it was a homogenous group with both parental forms. In the strain IGS 975 x F 8063, the Ca concentration in grain was considerably higher than in both parental forms (Table 2).

Table 2

Content of macroelements in kernels of winter triticale hybrids and their parental forms $(g \cdot kg^{-1})$

Strain/cultivar		Ash (%)	Ca	SD *CV* (%)	Mg	SD *CV* (%)	K	SD *CV* (%)
Maternal forms	IGS 5101	1.97	$0.149^{a,b,c}$	0.006 3.9	1.60^{b}	0.238 1.49	4.65^{d}	0.067 1.4
	FDT 975	1.86	$0.156^{b,c,d,e}$	0.003 2.1	$1.35^{a,b}$	0.078 5.7	$4.23^{b,c}$	0.200 4.7
	Alzo	1.85	0.181^{f}	0.017 9.6	$1.62^{a,b}$	0.282 17.4	$4.29^{b,c}$	0.075 1.7
Paternal forms	LAD 122	1.94	$0.168^{d,e,f}$	0.006 3.7	$1.65^{a,b}$	0.038 2.3	4.18^{b}	0.004 0.1
	F 8063	1.95	$0.153^{b,c,d}$	0.005 3.6	1.46^{b}	0.126 8.6	$4.46^{c,d}$	0.135 3.0
Hybrids	IGS 5101 × LAD 122	1.80	0.135^{a}	0.003 2.3	$1.49^{a,b}$	0.032 2.1	4.17^{b}	0.070 1.6
	IGS 5101 × F 8063	1.98	$0.171^{e,f}$	0.012 7.0	$1.49^{a,b}$	0.206 1.39	4.70^{d}	0.022 0.5
	FDT 975 × LAD 122	1.75	$0.140^{a,b}$	0.001 0.6	$1.43^{a,b}$	0.091 6.4	$4.05^{a,b}$	0.044 1.1
	Alzo × LAD 122	1.91	$0.169^{d,e,f}$	0.004 2.5	1.59^{b}	0.048 3.0	4.17^{b}	0.006 0.1
	Alzo × F 8063	1.84	$0.164^{c,d,e}$	0.004 2.4	1.21^{a}	0.004 0.37	3.86^{a}	0.266 6.9
$LSD_{0.05}$			0.017		0.33		0.27	

SD – standard deviation, *CV* – coefficient of variation, *a, b, c, d, e, f* – homogeneous groups at p=0.05

No significant differences in Mg were found between the parental forms. The content of Mg in kernels of three hybrids, i.e. IGS 5101 x LAD 122, Alzo x LAD 122 and Alzo x F 8063, was lower than in the parental components. The latter strain contained significantly less magnesium than the paternal form F 8063, whereas the other differences were not significant. For the hybrid strains IGS 5101 × F 8063 and FDT 975 x LAD 122, the Mg content in grain reached moderate values as compared to the parents (Table 2).

The maternal form IGS 5101 was distinguishable by its considerably higher potassium content than the other maternal components, in which it was similar to the paternal form F 8063. The hybrid strain IGS 5101 × F 8063 derived from these parental components was characterized by a significantly higher K concentration than the other hybrids. Furthermore, it was the only hybrid that contained more K than its both parents. The other hybrid strains, as compared to the corresponding parental forms, were characterized by lower potassium levels. In the case of Alzo x F 8063 strain, the difference was statistically significant versus both parental forms (Table 2).

The effect of different factors (e.g. fertilization) on content of macronutrients in triticale grain was analyzed by others authors. Higher nitrogen rates contributed to an increase in the potassium concentration (PILEJCZYK et al. 2004) and nitrogen magnesium fertilisation had a positive effect on the accumulation of potassium, magnesium and sodium in triticale grain (WYSZKOWSKI 2001).

Levels of microelements (Fe, Mn, Cu, Zn) in the analysed hybrid strains were diversified and depended on the parental forms (Table 3). The content of Fe and Mn in the hybrids was evidently affected by the strain LAD 122 (paternal form), which was distinguishable by the highest levels of these elements among grain samples. All the hybrids originating from this form (i.e. IGS 5101 x LAD 122, FDT 975 x LAD 122, and Alzo x LAD 122) contained significantly less manganese than the paternal strain and more than the maternal forms, although these differences were not significant. And similarly, a moderate content of Fe in the hybrid strains, relative to the parental forms, was not statistically significant at $p = 5\%$. The content of Fe and Mn was significantly lower in the hybrid strain Alzo x F 8063. Kernels of this strain contained less of these elements than the maternal and paternal forms (Table 3).

A considerably higher content of copper versus the other maternal forms was found in IGS 5101, in which it was similar to the paternal form LAD 122. The hybrid strain IGS 5101 × LAD 122 also contained a significantly higher Cu level as compared to the other hybrids. The content of Cu in kernels of four hybrid strains IGS 5101 x LAD 122, FDT 975 x LAD 122, Alzo x F 8063, and IGS 5101 x F 8063 was lower than in both parental forms. In the case of the hybrid strains IGS 5101 x LAD 122 and Alzo x F 8063, the difference was considerable as compared to the parental components;

Table 3

Content of microelements in kernels of winter triticale hybrids and their parental forms (mg·kg^{-1})

Strain/cultivar		Cu	SD *CV* (%)	Zn	SD *CV* (%)	Fe	SD *CV* (%)	Mn	SD *CV* (%)
Maternal forms	IGS 5101	5.06^{e}	0.069 1.3	$62.7^{a,b,c}$	0.21 0.3	36.6^{a}	5.98 16.3	$34.9^{b,c}$	1.60 4.6
	FDT 975	$4.07^{b.c}$	0.092 2.3	$53.5^{a,b}$	2.83 5.3	37.9^{a}	5.20 13.7	32.1^{b}	1.05 3.3
	Alzo	4.14^{c}	0.157 3.8	65.9^{b}	15.64 20.3	$50.1^{a,b}$	8.72 17.4	$35.2^{b,c,d}$	3.80 10.7
Paternal forms	LAD 122	5.20^{e}	0.185 3.5	72.3^{c}	0.20 0.2	55.4^{b}	9.72 17.5	45.7^{g}	0.34 0.7
	F 8063	4.29^{c}	0.076 1.8	$62.2^{a,b,c}$	0.27 0.4	39.1^{a}	6.89 17.6	$32.8^{b,c}$	1.86 5.7
Hybrids	IGS 5101 × LAD 122	4.70^{d}	0.051 1.1	$54.2^{a,b}$	3.52 6.4	$43.8^{a,b}$	5.69 13.0	$39.5^{e,f}$	1.47 3.7
	IGS 5101 × F 8063	$4.25^{b,c}$	0.105 2.5	$66.4^{b,c}$	9.89 14.8	39.8^{a}	4.35 10.9	$36.3^{c,d,e}$	1.19 3.3
	FDT 975 × LAD 122	3.82^{b}	0.049 1.3	$60.7^{a,b,c}$	9.71 16.0	$50.9^{a,b}$	2.16 4.2	40.9^{f}	0.31 0.7
	Alzo × LAD 122	4.23^{c}	0.040 0.9	$55.6^{a,b,c}$	10.73 19.3	$50.7^{a,b}$	8.91 17.5	$38.7^{d,e,f}$	0.33 0.9
	Alzo × F 8063	3.50^{a}	0.189 5.4	47.5^{a}	2.02 4.2	37.0^{a}	5.99 16.2	28.4^{a}	1.30 4.6
$LSD_{0.05}$		0.25		16.9		13.26		3.72	

SD – standard deviation, *CV* – coefficient of variation, *a, b, c, d, e, f* – homogeneous groups at p=0.05

the FDT 975 x LAD 122 hybrid contained significantly less Cu than its paternal form, and IGS 5101 x F 8063 – than its maternal form (Table 3). Our comparison of the hybrids IGS 5101 x LAD 122, FDT 975 x LAD 122 and Alzo x LAD 122 originating from the same paternal form showed an evident influence of the maternal component on the Cu level in kernels of the hybrid strains.

The level of Zn in the hybrids was the most advantageous in the strain IGS 5101 x F 8063, in which it significantly exceeded both parental forms (Table 3). Considerably less zinc was found in kernels of the hybrids IGS 5101 x LAD 122, Alzo x LAD 122 and Alzo x F 8063 when compared to parental components.

The content of the determined mineral components in triticale strains was compared to the levels found in triticale by SOUCI et al. (2000). The concentrations of Ca and Cu in the strains analyzed in our research were below the average values reported by the above authors. The zinc content was twice as high, while the iron level was similar to the values found by SOUCI et al. 2000.

When comparing the mean values of macronutrients and microelements in kernels of the analyzed strains with the results presented by VARUGHESE et al. (1996) for many triticale varieties, higher levels of Mg, Ca, Zn, and Mn but a similar content of Cu were observed.

When comparing the average content of minerals in grain of the analyzed strains with 14 promising strains cultivated in India, their differentiation depending on the genotype features can be seen (SEHGAL et al.1983). Our analysis of the mean levels of minerals for all the objects revealed that the analyzed strains were characterized by a higher content of Mn and Zn in grain, similar K level and lower concentrations of Ca, Cu, and Fe than the mentioned strains from India (SEHGAL et al. 1983). Similarly to other studies (SEHGAL et al. 1983), the grain of the strains we tested demonstrated positive correlations between the ash content and concentrations of K and Mn. Considering solely the genetic variability, FEIL and FOSSATI (1995) proved positive correlation between protein and mineral levels. When comparing the protein content in kernels of the analyzed strains (previously published data, MAKARSKA et al. 2008), positive correlation of this parameter with the content of minerals was determined in the hybrid strains. The hybrid strain Alzo x LAD 122 with the highest protein content was also characterized by the highest Zn, Fe, Mn, and Cu concentrations in grain.

CONCLUSIONS

1. Triticale hybrid strains, as compared to their parental forms, were usually characterized by a lower ash content in kernels, except the strain IGS 5101 x F 8063.

2. The IGS 5101 x F 8063 hybrid strain excelled both parental forms in terms of Ca, K, Zn, Fe, Mn levels; it was also distinguishable from the other hybrids by higher Ca, K and Mn levels.

3. The differentiation of mineral components in the hybrid strains depended on the selection of parental forms.

4. Tests on the mineral components in triticale strains suggest that it is possible to shape their levels through a proper selection of parental components and selection, which takes into account the positive features within X Triticosecale Wittmack genus.

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INVESTIGATION ON ADSORPTION OF FATTY AND BILE ACIDS IN THE PRESENCE OF DIETARY SUPPLEMENTS CONTAINING CHROMIUM

Jan Meler, Bożena Grimling, Janusz Pluta

Department and Division of Drug Form Technology
Wrocław Medical University

Abstract

Dietary supplements contain not only macro- and microelements, but also elements which affect human metabolism. Many products available on the market contain chromium compounds together with chitosan used as a dietary supplement enhancing the digestion of lipids. The studies involved natural chitosan from krill available on the market, with the deacetylation degree of 85 to 95%, and dietary supplements containing chitosan (Vitana®, Hitec Nutrition®) as well as a product containing ionic chromium with niacin and several aminoacids – Chromdiet®). The study has determined the capability of binding fatty and bile acids by dietary supplements containing chitosan and chromium. The process of lipids and bile acids adsorption was investigated by means of a dynamic method in a biopharmaceutical model imitating *in vitro* conditions. The findings prove that extracts of fatty acids and bile acids undergo adsorption by various kinds of adjuvant substances found in dietary supplements, which confirms a significant effect of these polymers on the bioavailability of fatty and bile acids in a human organism. The addition of chromium to a supplement does not effect the capability of chitosan to bind fatty and bile acids. Mean adsorption of bile acids by 1 g of the polymer (chitosan, inulin, fibre) ranges from 0.9 g to 1.79 g depending on the pH (which decreases the bioavailability of lipids by 15-30%).

Key words: chitosans, chromium, bioavability, adsorption, model in vitro.

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dr n. farm. Jan Meler, Division of Dispensing Pharmacy, Department of Applied Pharmacy, Szewska 38/39 Str., 50-139 Wrocław, Poland, email: meler@ktpl.am.wroc.pl

BADANIE SORPCJI KWASÓW TŁUSZCZOWYCH I CHOLOWYCH W OBECNOŚCI SUPLEMENTÓW DIETY ZAWIERAJĄCYCH CHROM

Abstrakt

Suplementy diety to nie tylko makro- i mikroelementy, ale też pierwiastki, które wykazują wpływ na przemiany metaboliczne organizmu. Obecnie na rynku jest sporo preparatów zawierających związki chromu wraz z chitozanem stosowanych jako dodatek żywieniowy wspomagający trawienie tłuszczowców. W pracy przebadano naturalny chitozan z kryla o stopniu deacetylacji od 85 do 95% występujący obecnie w sprzedaży rynkowej oraz preparaty zawierające chitozany, stosowane jako suplementy diety (Vitana®, Hitec Nutrition® oraz preparat zawierający chrom–Chromdiet®). Określono zdolności wiązania tłuszczowców i kwasów żółciowych przez suplementy diety zawierające chrom i chitozan. Zjawisko adsorpcji lipidów i kwasów żółciowych badano metodą dynamiczną w modelu biofarmaceutycznym imitującym warunki in vitro. Otrzymane wyniki dowodzą, że kwasy tłuszczowe i ekstrakty kwasów żółciowych ulegają adsorpcji przez różnego rodzaju substancje pomocnicze służące do wytworzenia suplementu diety, co potwierdza znaczący wpływ tych polimerów na biodostępność kwasów tłuszczowych i żółciowych w organizmie człowieka. Średnia wielkość adsorpcji kwasów żółciowych przez 1 g polimeru w zależności od pH środowiska mieściła się w granicach od 0,90 g do 1,79 g (daje to zmniejszenie biodostępności tłuszczowców o 15-30%).

Słowa kluczowe: chitozan, chrom, biodostępność, adsorpcja, model in vitro.

INTRODUCTION

In view of the rapid progress in pharmaceutical technology and a large variety of manufactured products, supplementation of certain microelements has gained an increasing significance. Dietary supplements not only include macro- and microelements, but also elements which affect human metabolism (MELER et al. 2002).

Many products available on the market contain chromium compounds used as a dietary supplement enhancing the digestion of lipids, and their use is based on studies indicating that chromium together with insulin increase the use of glucose (SHERMAN et al. 1968, UUSITUPA et al. 1992, AMATO et al. 2000, TROW et al. 2000, MIZERSKA et al. 2005). The process of digestion of lipids starts in the stomach under the effect of sublingual lipase and lipase secreted by the gastric mucosa. First of all, they decompose triacylglycerols with short fatty acids to monoacylglycerols. The main process in the breakdown of lipids takes place in the duodenum in the presence of pancreatic lipase, cholesterol esterase and phospholipase A_2. The digestion of lipids in the duodenum is facilitated by the products of lipid digestion in the stomach performed by phospholipids and bile acids, which emulsify triacylglycerols with long acid chains to form fine molecules, thus increasing the surface of the enzyme's contact with the substrate.

MATERIAL AND METHODS

The tests were performed with the use of natural chitosan from krill, available on the market, characterized by the deacetelylation degree of 85 to 95% and manufactured by Primex (85 and Chito-Clear-1015), France-Chitine (352 and 343) and Chitozan Huasu (Figure 1). Moreover, the study involved dietary supplements containing chitosans (Vitana®, Hitec Nutrition®) as well as a product containing ionic chromium with niacin and several aminoacids (Chromdiet®).

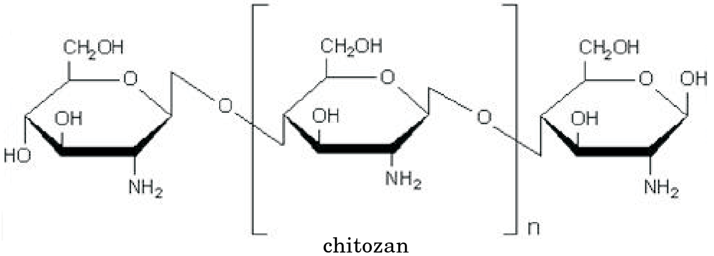

Fig. 1 Molecular structure of chitosan as a polymer

To calculate the amount of fatty and bile acids adsorbed by various chitosans, a biopharmaceutical model of the alimentary tract was used (Meler et al. 2003). Due to the scant amount of the product, the determination method was developed which used 30 milligrams (mg) of a sample mixed with the excess of oil mass. The measurements were based on six trials, in which mean results were determined, as well as on a thorough statistical evaluation.

The investigations were performed in water bath with a shaker, under the conditions imitating those in the human alimentary tract. The amplitude of vibrations (300 rpm) as well as the temperature of the process (37°C) were determined (Meler et al. 2003).

Next, 0.03 g of chitosan was added to 5 ml shaker vials, completed with 2 ml of 0.05 N HCl and shaken until dissolved. Afterwards, 0.05 N HCl was added in order to achieve pH 2, corresponding to the gastric pH when fasting. Next, the pH of the solution was raised to 6.4 by addition of 0.2 M Na_2CO_3. The mixture was shaken for 0.5 hours and completed with 100 mg of bile acids and 1 g of fatty acids in the form of 0.5 g of olive oil (Extra Vergine di Oliva Costa d'Oro, Spoleto, Italy) and 0.5 g of soya oil (Wielkopolskie Zakłady Przemysłu Tłuszczowego). The content of the vials was brought up to pH 7.0-7.6, which corresponds to the pH of intestinal juice in the small intestine and colon, and next incubated at 37°C, shaking (300 rpm) for

2.5 hours. The mixture was cooled to room temperature; the vials were weighed together with their content and centrifuged (2,100·g) for 20 minutes. Next, the mixture was left for 0.5 h to stabilize, the oil layer was collected and discarded and 1.5 ml of the mixture was collected from above the sediment, transferred to clean tubes and completed with 2 ml of 1 N NaOH. The absorbance was measured in a spectrophotometer and the amount of adsorbed bile acids was calculated. The remaining solution was brought to pH 3 with 0.05 N HCl, 2 ml of ethyl ester was added, shaken for 5 minutes and left until separation of the layers. The ether layer was collected with a syringe fitted with a needle to previously weighed weighing bottle. The extraction was repeated twice. Combined ether extracts were evaporated by heating to the temperature higher than the ether boiling point. After evaporation of ether, the bottle was weighed and the amount of fat bound by the chitosan sample was calculated from the difference in weight between the empty bottle and the bottle containing lipids. Accessory substances were prepared according to the standards of *Polish Pharmacopoeia VI (Farmakopea polska VI 2002).*

RESULTS AND DISCUSSION

Table 1 presents the results of binding oil mixture containing olive oil and soya oil available on the market by various kinds of chitosan.

Table 1

Viscosity and capability of binding fatty acids by chitosans and dietary supplements in a biopharmaceutical model of the alimentary tract

Specimen	Intrinsic viscosity $(\eta)/\mathrm{dm}^3\mathrm{g}^{-1}$	Average mass (g) of fatty acids bound by 1 g of chitosan	Standard deviation S (±g)	Relativity coefficient Rc (%)
Primex 85	0.2852	2.91	$4.889 \cdot 10^{-4}$	1.68
Chitosan type-343	0.6402	1.21	$1.416 \cdot 10^{-4}$	1.17
Chito-Clear TM fg 95 Batch TM 1015	0.2213	7.90	$8.434 \cdot 10^{-4}$	1.07
Chitosan type-352	0.2117	1.63	$2.156 \cdot 10^{-4}$	1.32
Chitosan HUASU	0.7437	2.50	$3.260 \cdot 10^{-4}$	1.30
Chitosan Chromdiet	0.1872	4.57	$5.567 \cdot 10^{-4}$	1.22
Chitosan Nutrisearch	0.1576	2.52	$4.678 \cdot 10^{-4}$	1.86
Chitosan Witana	0.1774	6.22	$9.134 \cdot 10^{-4}$	1.47

Statistical errors and relativity coefficients determining the repeatability of the findings were calculated for all investigated samples. As shown in Table 1, the standard deviation ranged from $1.416 \cdot 10^{-4}$ g to $9.134 \cdot 10^{-4}$ g, and the relativity coefficient ranged from 1.07% to 1.86%. The investigations have demonstrated that the initial chitosans are capable of binding from 1.21 g (Chitozan type-343) to 7.9 g (Chitosan Chito-Clear) of fat.

This is probably associated with the structure of chitosan as a polymer, which exerts electrostatic effect on lipid molecules and is capable of entrapping micelles containing bile acids and fatty acids. Partly protonated chitosan binds negatively charged acid molecules and forms ionic bonds between $-NH_3^+$ groups and $–COO^-$ groups of acids (PARRA-BARRAZA et al. 2005), which decreases the surface for subsequent enzymatic hydrolysis.

The applied method for binding lipids by chitosans in a biopharmaceutical model gives repeatable results, which was confirmed by the calculated relativity coefficients below 5% (probability higher than 95 %).

Table 2 presents the results of bile acids binding. The findings demonstrate that salts and extracts of bile acids are adsorbed by various accessory substances used to manufacture dietary supplements containing chitosan and chromium, which confirms a significant effect of polymers on the bioavailability of bile acids in a human organism. Mean bile acid adsorption by 1 g of the polymer ranged from 0.90 g to 1.79 g depending on pH (which reduces the bioavailability of lipids by about 15-30%)

The weight-reducing mechanism of the system can be explained by the effect of reduced amounts of bile acids, due to which dietary lipids undergo emulsification in the intestines (pH 7) rather than in the acid environment

Table 2

Viscosity and capability of binding bile acids by chitosans and dietary supplements in a biopharmaceutical model of the alimentary tract

Specimen	Intrinsic viscosity $(\eta)/dm^3g^{-1}$	Average mass (g) of fatty acids bound by 1 g of chitosan	Standard deviation S (±g)	Relativity coefficient Rc (%)
Primex 85	0.2852	1.30	0.0221	1.70
Chitosan type-343	0.6402	0.90	0.0046	0.51
Chito-Clear TM fg 95 Batch TM 1015	0.2213	1.79	0.0105	0.58
Chitosan type-352	0.2117	1.12	0.0136	1.21
Chitosan HUASU	0.7437	1.14	0.050	4.38
Chitosan Chromdiet	0.1872	1.31	0.0065	0.89
Chitosan Nutrisearch	0.1576	1.15	0.089	0.94
Chitosan Witana	0.1774	1.32	0.088	0.87

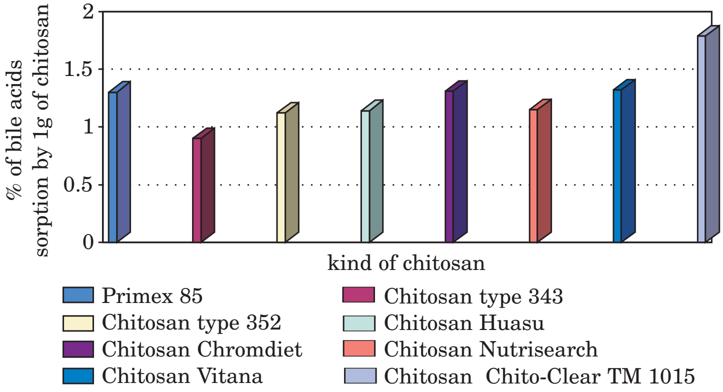

Fig. 2. Sorption of bile acids on dietary supplements including chitosans

of the stomach, but their surface is charged negatively as a consequence of dissociation of acids, which initiates electrostatic interactions between chitosan and droplets of lipids resulting in their adsorption and decreased amounts (Kanauchi et al. 1995, Paszko et al. 2000). Addition of chromium to a product containing chitosan, inulin and fibre (Chromdiet®) provides more effective support for weight reduction in comparison with monocomponent products. Chromium regulates the blood levels of glucose, lipids and proteins, but its effect on the binding of fatty acids and bile acids by adjuvant substances is very weak.

Summing up: Chromium present in a dietary supplement in the amount of 10 μg per dose has a minimal effect on sorption of lipids and bile acid compounds, with the highest adsorption of these substances occurring at pH above 7.

CONCLUSIONS

1. The developed method for investigations on interaction between small amounts of substances in the biopharmaceutical model of the alimentary tract enables evaluation of qualitative and quantitative changes occurring in the aqueous solution of chitosans and other accessory substances.

2. The investigation on absorption of fatty acids and bile acids confirms the previously hypothesized depressed amounts of these compounds in the intestinal passage (by 15-30% in the daily intake).

3. The binding of lipids and bile acids is accelerated at pH above 7.

4. Addition of 10 μg of chromium per dose has very little effect on the bioavailability of bile acids and lipids.

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CONTENT OF Ca, Mg, Na, K, P, Fe, Mn, Zn, Cu IN SOILS DEVELOPED FROM THE HOLOCENE DEPOSITS IN NORTH-EASTERN POLAND*

Mirosław Orzechowski, Sławomir Smólczyński

Chair of Soil Science and Soil Protection
University of Warmia and Mazury in Olsztyn

Abstract

Mineral alluvial and deluvial soils, mineral-organic mucky soils and organic peat-muck soils were developed from the Holocene deposits in northern Poland. The total content of Ca, Mg, K, P, Na, Fe, Mn, Zn and Cu was analysed in the following soils: alluvial soils which were formed from riverine deposits in delta and riverine landscapes, deluvial soils which were developed from slope deposits in a landscape of moraine hills and plains and in a riverine landscape as well as in mucky soils and peat-muck soils located in a landscape of moraine hills and plains and delta landscape. The aim of the paper was to determine total amount of the elements in a soil profile and catena sequence in the mentioned landscapes.

Total content of analysed elements in the soils developed from the Holocene deposits varied considerably among the soil types, in a soil profile, among catena sequences and landscapes. The highest content of Fe, Mn, Mg, K, Zn and Cu was reported in alluvial soils whereas Ca reached the highest level in peat-muck soils. In the riverine landscape, the highest accumulation of total Ca, Mg, K, P, Na, Fe, Mn, Zn and Cu was stated for alluvial soils, having the lowest location in the catena. In the landscape of moraine hills and plains, total content of K, Zn and Cu was the highest in deluvial soils and mucky soils located in the lower part of the slope. The total content of Ca and P was the highest in peat-muck soils having the lowest location in the catena. Statistically significant differences in the content of Ca, Mg, K, and Cu were stated between alluvial soils in the riverine and delta landscapes. The differences in the content of analysed elements, excluding Cu, were also statistically significant between alluvial and deluvial soils.

Key words: macro- and microelements, alluvial soils, deluvial soils, mucky soils, peat-muck soils.

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dr inż. Mirosław Orzechowski, Chair of Soil Science and Soil Protection, University of Warmia and Mazury in Olsztyn, pl. Łódzki 3, 10-727 Olsztyn, Poland, e-mail: miroslaw.orzechowski@uwm.edu.pl

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ZAWARTOŚĆ Ca, Mg, Na, K, P, Fe, Mn, Zn, Cu W GLEBACH WYTWORZONYCH Z OSADÓW HOLOCEŃSKICH W POLSCE PÓŁNOCNO-WSCHODNIEJ

Abstrakt

Z holoceńskich osadów na terenie północnej Polski wykształciły się mineralne gleby aluwialne i deluwialne, mineralno-organiczne gleby namurszowe oraz organiczne gleby torfowo-murszowe. Badania całkowitej zawartości Ca, Mg, K, P, Na, Fe, Mn, Zn, Cu prowadzono w glebach aluwialnych wytworzonych z osadów rzecznych w krajobrazie deltowym i dolin rzecznych. Gleby deluwialne powstały z osadów stokowych w krajobrazie równin i wzniesień morenowych oraz krajobrazie dolin rzecznych. Analizowane gleby namurszowe i torfowo-murszowe były położone w krajobrazie równin i wzniesień morenowych oraz deltowym. Celem podjętych badań było określenie całkowitej zawartości pierwiastków – w ujęciu profilowym i katenalnym – w wymienionych krajobrazach.

W glebach wytworzonych z osadów holoceńskich stwierdzono duże zróżnicowanie całkowitej zawartości badanych pierwiastków między wydzielonymi typami gleb, w układzie profilowym oraz w obrębie wydzielonych katen i krajobrazów. Największą całkowitą zawartość Fe, Mn, Mg, K, Zn i Cu stwierdzono w glebach aluwialnych, natomiast Ca – w glebach torfowo-murszowych. W krajobrazie dolin rzecznych największą kumulację całkowitego Ca, Mg, K, P, Na, Fe, Mn, Zn i Cu stwierdzono w glebach aluwialnych położonych najniżej w katenie. W krajobrazie równin i wzniesień morenowych całkowita zawartość K, Zn i Cu była największa w glebach deluwialnych i namurszowych położonych w dolnej części stoku, natomiast Ca i P – w glebach torfowo-murszowych usytuowanych najniżej w katenie. Stwierdzono statystycznie istotne różnice w zawartości całkowitego Ca, Mg, K i Cu między glebami aluwialnymi w krajobrazie deltowym i dolin rzecznych oraz w zawartości wszystkich badanych pierwiastków, oprócz Cu, między glebami aluwialnymi i deluwialnymi.

Słowa kluczowe: makro- i mikroelementy, gleby aluwialne, gleby deluwialne, gleby namurszowe, gleby torfowo-murszowe.

INTRODUCTION

The landscape of north-eastern Poland was shaped during the Vistula glaciation and is distinguished from other landscapes by the morphogenesis, lithogenesis and soil cover (KONDRACKI 1988). The land relief, various texture of soil parent materials as well as geological processes and soil-forming processes contributed to spatial diversity of the soil cover. In young glacial landscape, soil properties, soil chemical composition and soil cover are modified by natural and anthropogenic denudation processes (WOCŁAWEK 1973, BIENIEK 1997, ORZECHOWSKI 1999, ORZECHOWSKI et al. 2004b). In the soils which are not changed by human activities, natural content of elements depends mainly on parent material, weathering and soil-forming processes (KABATA-PENDIAS 1981, CZARNOWSKA 1996, ZGŁOBICKI 2008). Changes in the environment induced by diverse human activity increased the amounts of macro- and microelements not only in soil surface layers but also in the slope and riverine deposits (CZARNOWSKA 1996, ZGŁOBICKI 2008). The soils which were formed from the Holocene alluvial and deluvial deposits as well as from organic formations play an important role in nutrient cycles. Moreover, changes in the total content of elements are imprinted in these deposits.

The aim of the paper was to determine the total content of some elements in the soils which were formed from the Holocene deposits. The study was carried out in various landscapes of north-eastern Poland, which differed in distribution of the elements in a soil profile and in a catena sequence.

MATERIALS AND METHODS

Total content of Ca, Mg, K, P Na, Fe, Mn, Zn and Cu was analysed in alluvial soils, deluvial soils, mucky soils and peat-muck soils. The chemical elements were analysed in genetic horizons of 26 soil profiles. Alluvial soils and mucky soils in a delta landscape were located in Nowa Kościelnica and Jegłownik in the region of Żuławy Wiślane and in Baranowo in Staropruska Lowland (KONDRACKI 1988). The soils were formed from fine alluvial delta deposits in the Vistula and Pasłęka River basins. The soils in the delta landscape were situated on a plain, at the sea level. These soils had high moisture content and were drained with open ditches. Peat-muck soils in Jegłownik were developed from drained, silted alder and reed peats.

In the riverine landscape, the following sites were chosen: Smolajny on the Łyna River, Równina Górna on the Guber River and Krzeczewo on the Liwna River. The river valleys were surrounded by the following soil types: arenosols, lessive soils and black earths. On the slopes of the valley, proper and humic deluvial soils occur. In land depressions, alluvial and organic soils were situated. In the mentioned landscape, the soils were formed as a result of slope and alluvial processes under the impact of groundwater as well as of the water flowing down and across.

In the landscape of moraine hills and plains, Reszel in Sepopol Plain was chosen. In this area, the risk of erosion is low, the slope gradient is up to 3°, and slopes are gentle. The study comprised black earths developed from loam, located in the upper parts of slopes, humic deluvial soils situated in the middle of and in the lower part of slopes as well as mucky soils and peat-muck soils situated at the bottom of slopes and in a depression.

The research was carried using soil catena method, with which it is possible to determine the toposequence and spatial diversity of soils.

Total content of Ca, Mg, K, P, Na, Fe, Mn, Zn and Cu was measured in 96 soil samples after digestion in a mixture of $HClO_4$ and HNO_3. Calcium, potassium and sodium were measured photometrically using a Jenway flame photometer; phosphorous was analysed colorimetrically using a Specol EK 1 spectrocolorimeter and magnesium was measured using an AAS 1 Zeiss Jena analyzer. The total content of Fe, Mn, Zn and Cu was estimated applying the AAS techniques on a 30 Zeiss Jena analyser.

Statistical analyses were conducted using Statistica 8.0. The significance tests and correlation analyses were applied (Statistica pl for Windows 1997).

RESULTS AND DISCUSSION

Mineral alluvial and deluvial soils, mineral-organic mucky soils and organic peat-muck soils were developed from the Holocene deposits in northern Poland. Alluvial soils in the delta and riverine landscapes had similar amounts of clay (Ø <0.002 mm) and silt (Ø 0.05-0.002 mm). The content of these mineral fractions was higher than in deluvial soils. The differences in the content of clay and silt were statistically significant between alluvial and deluvial soils (Table 1).

The highest amounts of Mn and Fe were recorded in alluvial soils developed from silt, loam and clay formations in the delta and riverine landscapes (Table 1). The amounts of the mentioned elements were 1.5-2-fold higher than in deluvial soils and organic soils. Considerable concentrations of iron (35.1 $g \cdot kg^{-1}$) and manganese (681.3 $mg \cdot kg^{-1}$) in alluvial soils were accompanied by a high groundwater level and changeable redox processes (ORZECHOWSKI et al. 2004a). Iron and manganese undergo processes of oxidation and reduction, which affect their solubility and mobility (GLIŃSKI, STĘPNIEWSKI 1984). The highest amounts of Fe and Mn were reported in surface horizons in the aerobic zone. The content of iron in alluvial soils was significantly positively correlated with the clay content (r = 0.485*). For silt content, however, the correlation was negative (r = –0.360*) – Table 2. High soil moisture may increase solubility and simultaneously activate the mobility of some trace elements, for instance manganese (DIATTA 2008).

Among the analysed soils, alluvial soils in the riverine landscape had the highest total content of Mg, K, Zn and Cu. An average total content of magnesium and potassium in these soils was 8.9 $g \cdot kg^{-1}$, being significantly positively correlated with the clay content (Table 2). Peat-muck soils contained on average three-fold less magnesium (2.9 $g \cdot kg^{-1}$) and four-fold less potassium (2.1 $g \cdot kg^{-1}$).

The deposits which were accumulated at the bottom of river valleys reveal the changes in the catchment area. Some trace elements which were accumulated in alluvial deposits reflect the influence of climate, human settlement as well as agricultural and industrial human activity (CZARNOWSKA 1996, ZGŁOBICKI 2008). The highest average content of Zn (124.8 $mg \cdot kg^{-1}$) and Cu (25.9 $mg \cdot kg^{-1}$) was reported in alluvial soils in the riverine landscape (Table 1). In the profiles of alluvial soils, the highest concentration of these elements was recorded in surface horizons which were developed from the late Holocene deposits. Such high concentrations might have been induced by anthropogenic transformation of the environment (ZGŁOBICKI 2008).

Table 1

Content of Ca, Mg, Na, K, P, Fe, Mn, Zn, Cu in alluvial soils, deluvial soils and peat-muck soils in different landscapes of north-eastern Poland

Specification	Value	Alluvial soils[•]			Deluvial solis	Peat-muck soils
		delta landscape and riverine landscape $n = 39$	delta landscape $n = 16$	riverine landscape $n = 23$	riverine landscape, landscape of moraine hills and plains $n = 29$	delta landscape, landscape of moraine hills and plains $n = 28$
<0.002 (mm) Percentage of fraction in diameter	x	25.7^{+}	24.8	26.4	19.0^{+}	–
	S	14.61	14.51	14.97	10.21	
	CV (%)	56.8	58.5	56.7	53.7	
0.05-0.002	x	53.7^{++}	53.3	54.1	35.1^{++}	–
	S	10.54	9.14	11.60	12.93	
	CV (%)	19.6	17.1	21.4	36.8	
Organic matter (g·kg^{-1})	x	42.0	35.3	46.6	50.2	637.5
	S	24.70	16.30	28.60	42.3	212.60
	CV (%)	58.8	46.1	61.3	84.2	33.3
Ca	x	13.9^{++}	9.5^{**}	17.0^{**}	8.4^{++}	34.3
	S	5.19	3.20	3.92	4.96	20.90
	CV (%)	37.3	33.6	23.0	59.0	60.9
Mg	x	7.4^{++}	5.2^{**}	8.9^{**}	4.7^{++}	2.9
	S	3.65	1.30	4.01	1.97	1.60
	CV (%)	49.3	25.0	45.0	41.9	55.1
K (g·kg^{-1})	x	8.0^{+}	6.8^{*}	8.9^{*}	6.2^{+}	2.1
	S	3.06	1.30	3.62	2.75	1.40
	CV (%)	38.2	19.1	40.6	44.3	66.7
P	x	1.3^{+}	1.0	1.5	0.9^{+}	1.4
	S	0.79	0.20	0.97	0.61	1.00
	CV (%)	60.7	20.0	64.6	67.7	71.4
Na	x	0.4^{++}	0.4	0.5	0.2^{++}	0.3
	S	0.12	0.10	0.14	0.04	0.10
	CV (%)	30.0	25.0	28.0	20.0	33.3
Fe	x	35.1^{++}	35.5	34.8	20.1^{++}	20.8
	S	9.56	11.00	8.67	8.38	10.92
	CV (%)	27.2	30.9	24.9	41.6	52.5
Mn	x	681.3^{++}	732.7	645.5	368.4^{++}	411.9
	S	259.27	206.22	289.57	137.92	238.61
	CV (%)	38.0	28.1	44.8	37.4	57.9
Zn (mg·kg^{-1})	x	107.6^{++}	82.9	124.8	50.9^{++}	28.2
	S	69.38	25.50	83.97	18.33	11.63
	CV (%)	64.4	30.7	67.2	36.0	41.2
Cu	x	20.0	11.6^{**}	25.9^{**}	18.3	17.8
	S	16.72	8.96	18.42	6.53	9.04
	CV (%)	83.6	77.2	71.1	35.6	50.8

[•] Taxonomy of Polish soils (1989), x – mean, S – standard deviation, CV – coefficient of variation
* $\alpha = 0.05$; ** $\alpha = 0.01$ significant between alluvial soils in delta and riverine landscape;
$^{+}$ $\alpha = 0.05$; $^{++}$ $\alpha = 0.01$ significant between alluvial soils and deluvial solis; n – number of soil samples

The total content of zinc and copper in alluvial soils was positively correlated with the content of < 0.002 mm fraction, although this relationship was statistically insignificant (Table 2).

In the alluvial soils, the total content of K and Mn was similar to that in alluvial soils of Poland investigated by ANDRUSZCZAK and CZUBA (1984), and the content of Mg, Zn, Cu was slightly higher than the amounts reported by those authors. The content of Fe, Mn, Zn and Cu was considerably higher than in alluvial soils or in parent materials of Polish soils, which are considered as geochemical background levels (CZARNOWSKA 1996).

Peat-muck soils, which have the lowest location in the catena in the landscape of moraine hills and plains and in the delta landscape, contained the highest amounts of calcium (on average 34.3 $g \cdot kg^{-1}$). Calcium compounds which are dissolved during erosion are translocated with river waters as well as with waters flowing down to peat-muck soils situated lower in the catena. With regard to a high cation exchange capacity, these soils accumulate calcium in the ionic form Ca^{2+} (ORZECHOWSKI et al. 2004a). When compared to the surrounding alluvial and deluvial soils, peat-muck soils contained 2-4-fold more calcium (Table 1) and considerably less total Mg, K, Zn. The content of Mg, K, Fe, Zn and Mn in peat-muck soils was significantly negatively correlated with organic matter content (Table 2). In these soils, total content of Ca and Na was higher than the amounts in organic soils in the delta of the Vistula River reported by PIAŚCIK et al. (2001). However the amounts of Mg, K, P, Fe, Cu were similar and Zn, Mn lower than those reported by PIAŚCIK et al. (1998, 2001).

Alluvial soils and peat-muck soils contained similar average amounts of phosphorus and sodium whereas deluvial soils contained less P and Na (Table 1). In the analysed catena sequences, these elements were accumulated in the lowest land relief parts, which were occupied by peat-muck soils, mucky soils and alluvial soils (Table 3).

The analysed soils, which were formed from the Holocene deposits, contained more Ca, Mg, P and alluvial soils additionally had more Fe, Zn and Cu as compared to an average content of these elements in surface layers of Polish soils studied by DUDKA (1992).

In the riverine landscape, humus horizons of alluvial soils, located in the lowest parts of catena, contained three-fold more calcium, magnesium and potassium and 1.5-2-fold more iron, manganese, zinc and copper than deluvial soils and the soils surrounding the valleys (Table 3). However, in Reszel, in the catena of the landscape of moraine hills and plains, the highest concentration of K, Zn and Cu was reported in slope deposits which formed deluvial soils and mucky soils. Permanent and repeating losses of microelements from eroded soils in Masurian Lakeland were noticed by WOCŁAWEK (1973). The author qualified Cu, Mn and Zn as elements sensitive to transformation and translocation with flowing water. In peat-muck soils, calcium and phosphorus were predominant. SOWIŃSKI et al. (2004) and SMÓLCZYŃSKI

Table 2

Pearson's correlation coefficients between silt, clay, organic matter and total content of analysed elements

Properties	Ca	Mg	K	P	Na	Fe	Mn	Zn	Cu
	$g \cdot kg^{-1}$						$mg \cdot kg^{-1}$		
Alluvial soils, $n = 39$									
Clay (fraction Ø< 0.002 mm)	0.184	0.402*	0.342*	-0.166	0.290	0.485*	-0.070	0.137	0.197
Silt (0.05-0.002 mm)	-0.161	-0.546*	-0.326*	-0.299	-0.399*	-0.360*	0.077	-0.520*	-0.538*
Organic matter ($g \cdot kg^{-1}$)	0.329*	0.454*	-0.147	0.626*	0.408*	0.023	-0.305	0.616*	0.582*
Deluvial soils, $n = 29$									
Clay (fraction Ø< 0.002 mm)	0.567*	0.895*	0.800*	0.206	0.295	0.780*	0.613*	0.235	0.230
Silt (0.05-0.002 mm)	0.469*	0.872*	0.666*	0.248	0.338	0.632*	0.839*	0.108	0.031
Organic matter ($g \cdot kg^{-1}$)	0.669*	0.260	0.230	0.588*	-0.206	0.286	-0.185	0.109	0.659*
Peat-muck soils, $n = 28$									
Organic matter ($g \cdot kg^{-1}$)	0.065	-0.448*	-0.494*	-0.209	-0.232	-0.450*	-0.432*	-0.594*	-0.348

*correlations significant at $p \le 0.05$

Table 3

Average amounts of elements in surface horizons (A, AO, Mt) of soils in catena sequences in the landscape of moraine hills and plains and in the riverine landscape

Horizon and soils	Ca	Mg	K	P	Na	Fe	Mn	Zn	Cu
	$g \cdot kg^{-1}$						$mg \cdot kg^{-1}$		
Landscape of moraine hills and plains – catena Troksy									
Ap (black earths)	10.4	6.2	1.0	0.6	0.2	26.8	506.9	46.4	15.6
A (deluvial soils)	11.0	5.9	8.1	1.1	0.2	27.3	417.9	56.4	22.1
AO (mucky soils)	13.8	6.6	6.0	1.6	0.4	26.8	578.8	68.7	26.0
Mt (peat-muck soils)	69.7	4.5	2.8	2.5	0.4	13.7	542.8	33.2	17.4
Riverine landscape – catenae Smolajny, Krzeczewo and Równina Górna									
Ap (arensols, black earths, lessives soils)	3.9	3.5	3.0	1.2	0.2	14.0	387.0	72.4	18.3
A (deluvial soils)	6.3	3.7	4.7	0.7	0.2	14.2	328.2	46.5	15.2
A (alluvial soils)	16.9	9.2	9.1	1.6	0.5	35.5	628.3	128.3	26.8

Table 4

Correlation coefficients between the content of macro- and microelements in the mineral Holocene deposits (1) and organic Holocene deposits (2)

Elements	Mg	K	P	Na	Fe	Mn	Zn	Cu
Ca (1)	0.627*	0.249*	0.338*	0.501*	0.514*	0.311*	0.426*	0.407*
(2)	0.026	-0.266	0.286	0.088	-0.443*	0.081	-0.166	-0.111
Mg (1)		0.427*	0.419*	0.537*	0.627*	0.185	0.654*	0.576*
(2)		0.765*	0.660*	-0.235	-0.116	0.076	0.450*	0.087
K (1)			0.080	0.191	0.327*	0.250*	0.213	0.155
(2)			0.297	0.001	0.114	0.206	0.476*	0.214
P (1)				0.466*	0.268*	-0.101	0.747*	0.732*
(2)				-0.494	-0.299	0.351	0.112	0.448*
Na (1)					0.606*	0.320*	0.738*	0.449*
(2)					0.309	0.351	0.359	0.449*
Fe (1)						0.530*	0.431*	0.246*
(2)						0.527*	0.545*	0.693*
Mn (1)							0.037	-0.186
(2)							0.598*	0.591*
Zn (1)								0.806*
(2)								0.703*

*correlations significant at $p \le 0.05$

et al. (2004) stated that in moraine landscapes, in a catena sequence, the highest content of macroelements and microelements was found in the soils of midmoraine depressions (mucky soils and peat-muck soils).

The examined soils were not contaminated with zinc and copper. It was only in the Livna riverine landscape, in the surface horizon (Aa) of alluvial soils, that increased levels of Zn (71.1 $mg \cdot kg^{-1}$) and Cu (303.0 $mg \cdot kg^{-1}$) were recorded – I° of contamination (KABATA-PENDIAS et al. 1993). High concentration of Zn and Cu appeared to be related to the humus content (98.1 $g \cdot kg^{-1}$). The content of these chemical elements in alluvial soils was significantly correlated with organic matter content (Table 2). ORZECHOWSKI (1999) reported that in the delta landscape of Żuławy Wiślane, there are small areas with humic alluvial soils developed from clay containing increased amounts of Zn, Cu and Ni.

Differences in the total content of Ca, Mg and Cu between alluvial soils in delta and riverine landscapes proved to be statistically significant (at significance level α=0.01). The differences for potassium were statistically significant at significance level α=0.05. Also the differences in the content of analysed elements, excluding Cu, were statistically significant between alluvial and deluvial soils (Table 1).

The correlation analyses proved that in both alluvial soil and deluvial soil, total content of Mg, K, and Fe was significantly positively correlated with the content of clay and the amounts of Ca, P and Cu were significantly positively correlated with organic matter content (Table 2). Total content of iron and calcium in the mineral Holocene deposits was significantly positively correlated with other elements. However, in organic deposits the content of iron was significantly positively correlated with manganese, zinc and copper. The content of calcium was not correlated with the other analysed elements (Table 4).

CONCLUSIONS

1. Alluvial soils, deluvial soils, mucky soils and organic soils were formed from the Holocene deposits in northern Poland. Alluvial soils which were developed from riverine deposits contained more silt and clay than deluvial soils which were formed from slope deposits.

2. In the soils which were developed from the Holocene deposits, considerable differences in total amounts of Ca, Mg, K, P, Na, Fe, Mn, Zn and Cu were recorded. Qualitative differences in the content of the analysed elements were also reported for the distribution in the soil profile and among catenae and landscapes.

3. The highest amounts of Fe, Mn, Mg, K, Zn and Cu were noted in alluvial soils whereas Ca was the most abundant in peat-muck soils.

4. In the riverine landscape, the highest content of Ca, Mg, K, P, Na, Fe, Mn, Zn and Cu was reported in alluvial soils, which are situated in the lowest part of the catena. In the landscape of moraine hills and plains, the highest level of K, Zn and Cu was reported in deluvial soils and mucky soils, which are found in lower parts of slopes and at the bottom of the slope. Calcium and phosphorus were largely accumulated in peat-muck soils located in the lowest part of catena.

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SPECIATION ANALYSIS OF PHOSPHORUS IN BOTTOM SEDIMENTS – COMPARISON OF TWO METHODS

Hanna Siwek

Department of General and Ecological Chemistry
West Pomeranian University of Technology, Szczecin

Abstract

The purpose of this paper was to compare two methods of fractionation of reactive phosphorus forms (RP) in bottom sediments of polymictic water reservoirs located in rural areas. As a criterion for estimation, quantities RP extracted in analyzed fractions versus the general content of Al, Fe and Ca were used. The sediments were collected in springtime, from rural areas with different land use in the drainage basin (arable fields, uncultivated land, farmyards). The sediments were collected with a KC-Denmark core sampler for bottom sediments, which makes it possible to collect samples leaving the upper structure of 6 cm in thickness intact. After mineralization, each sample was analyzed for the general phosphorus content and a speciation analysis was performed by two methods. With the first method (M1), the RP bonded to Al (Al_RP), Fe (Fe_RP) and Ca (Ca_RP) was determined. The second method (M2) was applied to determine fractions of reactive phosphorus released from the sediments under reduced conditions, mainly from bonds with iron and manganese (BD_RP), adsorbed on aluminium oxides mainly Al_2O_3 and other surfaces (NaOH_RP) and apatite phosphorus and bonds with carbonates.

In almost all the sediments, most RP was determined in the phosphorus fractions with Ca extracted with acid solution. For the fractions obtained via the M1 method, more of the general RP, as well as phosphorus bonded to calcium and iron was found than for fractions obtained with the M2 method, and the differences might be attributed to translocation of phosphorus from organic to inorganic compounds during fractioning. The fraction of reactive phosphorus determined with the M1 method is correlated more strongly with the general content of the metal in the sediments than reactive phosphorus fractions determined with the M2 method. The strong correlation between the content of iron and the fraction of BD_RP in mineral sediments shows that it can be an indicator of the sensitivity of sediment to changes in the redox potential.

Key words: bottom sediments, kettle holes, phosphorus, fractionation, multivalent metals.

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dr inż. Hanna Siwek, Department of General and Ecological Chemistry, West Pomeranian University of Technology, Szczecin, J. Słowackiego 17, 71-434 Szczecin, Poland, e-mail: hanna.siwek@zut.edu.pl

ANALIZA SPECJACYJNA FOSFORU W OSADACH DENNYCH – PORÓWNANIE DWÓCH METOD

Abstrakt

Celem pracy było porównanie dwóch metod frakcjonowania reaktywnych form fosforu (RP) w osadach dennych polimiktycznych zbiorników wodnych zlokalizowanych na terenach wiejskich. Jako kryterium oceny przyjęto ilość RP wyekstrahowanego w badanych frakcjach w odniesieniu do zawartości ogólnej Al, Fe i Ca. Osady do badań pobrano w sezonie wiosennym, z sześciu oczek wodnych zlokalizowanych na terenach wiejskich o różnym sposobie zagospodarowania zlewni (pola uprawne, odłóg, gospodarstwa rolne). Osady pobierano próbnikiem rdzeniowym osadu dennego KC-Denmark, który umożliwia pobór prób z nienaruszoną strukturą wierzchnią warstwy osadów o grubości 6 cm. W każdym osadzie oznaczono, po mineralizacji, ogólną zawartość fosforu (TP), Fe, Ca, Al oraz przeprowadzono analizę specjacyjną fosforu dwoma metodami. Metodą 1 (M1) oznaczono reaktywny fosfor (RP) związany z: Al (Al_RP), Fe (Fe_RP) i Ca (Ca_RP). Metodą 2 (M2) oznaczono frakcje reaktywnego fosforu uwalnianego z osadów w warunkach zredukowanych głównie z połączeń z tlenkami żelaza i manganu (BD_RP), zaadsorbowanego na tlenkach metali głównie na Al_2O_3 i innych powierzchniach (NaOH_RP), fosfor apatytowy i związany z węglanami (HCl_RP). Prawie we wszystkich osadach najwięcej RP oznaczono we frakcjach fosforu związanego z Ca ekstrahowanego roztworem kwasu. We frakcjach otrzymanych metodą M1 oznaczono większe ilości ogólnego RP oraz fosforu związanego z wapniem i z żelazem niż w metodzie M2, a powstałe różnice mogą wynikać z przemieszczania się fosforu ze związków organicznych do nieorganicznych w trakcie frakcjonowania. Frakcje fosforu reaktywnego oznaczonego metodą M1 są silniej skorelowane z ogólną zawartością tych metali w osadzie niż frakcje fosforu reaktywnego oznaczonego metodą M2. Silna korelacja między zawartością żelaza i frakcji BD_RP w osadach mineralnych wskazuje, że może ona być wskaźnikiem wrażliwości osadu na zmianę potencjału redoks.

Słowa kluczowe: osady denne, oczka wodne, fosfor, frakcjonowanie, metale wielowartościowe.

INTRODUCTION

The influence of phosphorus on the trophic state of water depends not only on its concentration but also on the cycling of phosphorus compounds in an entire aquatic ecosystem. In the processes of transformation and accumulation of phosphorus in aquatic environments, bottom sediments play the main and unquestionable role, and depending on the physical and chemical conditions in a reservoir, they may either be a trap for phosphorus or a source of autochthonous phosphorus (PSENNER et al. 1988, KENTZER 2001). As a result of sedimentation of organisms, absorption of insoluble phosphates on silty materials and precipitation of insoluble complexes, phosphorus translocates from oxygenated surface waters to bottom sediments. In the topmost bottom sediments, phosphorus occurs in its organic form and is chemically bonded to multivalent ions of metals, including Al, Fe and Ca, together forming hardly soluble salts and complex compounds. The basic processes which control translocation of phosphorus between water and sediments include sorption, desorption, dissolution and precipitation, which highly depend on physical and chemical qualities of the bottom water layer and sediments, e.g. oxygen conditions, redox potential, forms of phosphorus in both phases and the structure and hydration of sediments.

The quantitative and qualitative description of how phosphorus is incorporated in the bottom sediments is an important indicator which helps to predict and limit eutrophication. Therefore, many methods have been developed for speciation analysis of this element. Generally, all analyses are modifications of the methods used for fractioning and determination of phosphorus in soil and in biologic materials. The methods help to determine the total content of mineral and organic phosphorus (GOŁACHOWSKA 1977), organic compounds of phosphorus (SHAPIRO et al. 1971, GOŁACHOWSKA 1978, GOLTERMAN et al. 1998) and its mineral forms (GOŁACHOWSKA 1978, HIETLTJES, LIJKLEMA 1980, PSSENER et al. 1985). The latest methods for speciation analysis of phosphorus involve specific chelating agents – NTA lub EDTA (GOLTERMAN, BOOMAN 1988, RUTTENBERG 1992, GOLTERMAN 1996). The basic objective of speciation analysis of phosphorus in sediments is to characterize the sediment as a potential internal source of this element in an aquatic ecosystem. Therefore, the methods for fractioning of inorganic compounds of phosphorus, which are bio-assimilable and dissolve in water better than its organic compounds, play a more important role. This conclusion finds support in limnology, where inorganic phosphorus is defined as reactive and organic phosphorus as non-reactive (PSENNER1988). Because the specificity of applied extractants, compared to the defined connections of phosphorus, depends on the type of sediments (e.g. content of organic matter and limestone), selection of an appropriate method requires their earlier comparison (PSENNER1988). The purpose of this paper was to compare two methods for fractionation of reactive phosphorus forms (RP) in bottom sediments of polymictic water reservoirs located in rural areas. As a criterion for estimation, quantities of RP extracted in analyzed fractions versus the total content of chosen metals were used.

MATERIAL AND METHODS

Sediments for the research were sampled in springtime from water ponds near the golf course in Binowo (O1, O2), from kettle holes in Kołowo (O3 – located near some farms and O4 – in the middle of a field), from Lake Rusałka, a barrier lake in Kasprowicza Park in Szczecin (O5), and from a field kettle hole near Stobno (O6). The samples were collected from bottom sediments with a KC-Denmark core sampler, which enables collection of samples from the topmost layer. Thus, the upper layer of sediments, 6 cm thick, was examined. Each sample underwent speciation analysis of phosphorus with two methods: one suggested by Gołachowska (M1) (1977) and the other one elaborated by Psenner et al. (M2) (1984). With the first method, the following fractions of reactive phosphorus were determined: loosely bonded or absorbed in sediments – extracted with a 1 M solution of NH_4Cl; bonded to aluminum (Al_RP) – extracted with a 0.5 M solution of NH_4F; bonded to iron (Fe_RP) – extracted with a 0.1 M solution of NaOH and bonded to calcium (Ca_RP) – extracted with a 1 M solution of H_2SO_4. With the other method (M2), the following fractions of reactive phosphorus were determined: loosely bonded or adsorbed in the sediment – extracted with a 1 M solution of NH_4Cl; released from sediments under reduced conditions mainly from bonds with iron and manganese oxides – extracted with a mixture $NaHCO_3$ and $Na_2S_2O_4$ (RP_BD); adsorbed on metal oxides, mainly on Al_2O_3 and other surfaces, interchangeable with OH^- – extracted with a solution of NaOH (NaOH_RP); apatite and bonded to carbonates – extracted with a solution of HCl (HCl_RP). Having completed speciation analysis, in each of the sediment samples, hardly releasable phosphorus (RES_P) was determined so as to account for fractions of phosphorus which are practically insoluble, containing mainly fluorapatite and some forms of organic matter. Such fractions of phosphorus were determined after wet mineralization in a mixture of acids H_2SO_4 i $HClO_4$ (Gołachowska 1977). Likewise, the total content of phosphorus in sediments before extraction was determined (TP). The content of phosphorus dissolved in individual extraction solutions was determined by spectrophotometry on a spectrophotometer UV/VIS 8500 (Techcomp). Based on the results, the total content of extracted reactive and non-reactive phosphorus was calculated for each of the methods. For each sediment sample, after mineralization with concentrated nitric acid (V) in a microwave oven, the total concentrations of Fe, Ca and Al were determined using the atomic absorption method (AAS) with a flame spectrophotometer Solaar S (ThermoElemental).

RESULTS AND DISCUSSION

The results of assays of the content of inorganic phosphorus fractions in sediment samples are shown in Table 1. With both the methods, the first fraction of phosphorus, loosely bonded with the sediment, was below the minimum determination limit and thus it was neglected. In each of the fractions tested, most reactive phosphorus (RP) was found in sample O5 with method M1 and in sample O3 with method M2. For method M1, fraction Fe_RP was an exception in that that a higher content of RP was determined in sample O1 than O5 (22.64 and 12.03% RP in the total TP phosphorus content, respectively). For method M2, fraction HCl_RP an exception was, as higher content of RP was found in sediments O2 and O5 than in sediment O3 (18.07, 15.91 and 13.56% RP in TP, respectively). In the ana-

Table 1

The content of phosphorus in tested sediments

Sediment	TP (mg P·g^{-1} d.m.)	Part of RP fraction in the total content of phosphorus (%)					
		method M1			method M2		
		Al_RP	Fe_RP	Ca_RP	BD_RP	NaOH_RP	HCl_RP
O1	1.11	2.52	22.64	9.44	0.91	4.02	3.04
O2	0.62	2.45	11.86	34.51	14.27	7.78	18.07
O3	1.47	3.00	3.73	13.04	23.18	12.48	13.56
O4	0.93	2.27	9.59	18.70	7.77	6.05	9.40
O5	0.95	9.64	12.03	33.65	5.19	10.06	15.91
O6	0.69	5.38	10.58	15.14	7.50	9.76	3.64
Average	0.963	4.21	11.74	20.75	9.80	8.36	10.60

lyzed sediments, most RP was determined with method M1 in a fraction extracted with a solution of acid, which is usually the largest fraction extracted with all methods for speciation analysis of reactive phosphorus (GOŁACHOWSKA 1977, HIELTJAS, LIJKLEMA 1980, GOLTERMAN, BOOMAN 1988). An exception was sediment O1, where most RP was determined with method M1 in fraction Fe_RP (22.64%), and sediment O3, where most RP was determined with method M2 in a fraction of phosphorus released from sediments under reduced conditions BD_RP (23.18%). The differences may have resulted from different content of organic matter in the examined sediments, related to Ca, as most of the tested samples were mineral ones (containing 2-3% of organic matter), expect mineral-humic sediment O3 (containing 3.7% of organic matter) and organic O1 (containing 30.31% of organic matter). The results confirm that the specificity of applied extractants towards the determined bonds of phosphorus depend on the type of sediment (PSENNER et al. 1988).

Figure 1 shows the percentage share of RP, non-reactive (NRP) and general phosphorus in all fractions (FTP), in the total content of phosphorus in the sediment (TP). The percentage of RP fractions determined with method M1 varied within the range of 19.4 to 54.27% and in almost all the cases was higher than determined with method M2, where it fluctuated between 8.0 and 51%. An exception was sediment O3, where this percentage was 19.9% as determined with method M1, and 51% – with method M2. This difference was due to a big quantity of RP extracted under reduced conditions, making up for 23% (Table 1). This sediment was the only one which was sampled from a pond surrounded by farms, which are the main source of pollution. With the two methods being compared, different amounts of general RP and NRP were extracted from individual samples, whereas the amounts of extracted TP were similar (Figure 1). The percentage of NRP

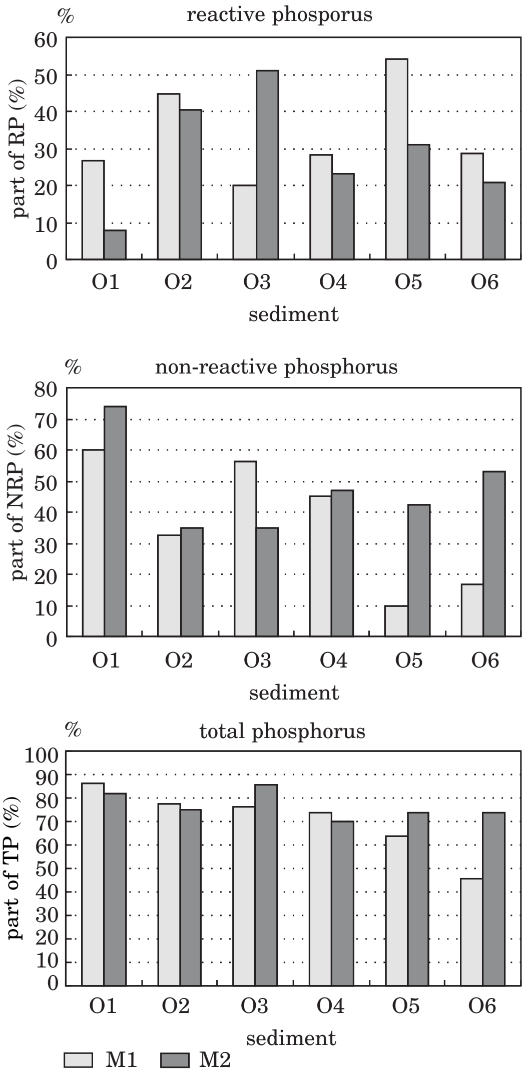

Fig. 1. Total sum of phosphorus in the fractions extracted by methods M1 and M2

fractions marked with method M1 ranged from 9.7 to 70.9%, while determined with method M2 – from 34.8 to 74.0%. According to M1 and M2 methods, the difference between the total extracted FTP varied from 1.0 to 10.0%, reaching as much as 28.0% only for sediment O6. This indicates the changes which may take place during fractionation, which are accompanied by migration of phosphorus between the organic and inorganic fraction of sediment. Because for the fractions obtained with method M1, higher amounts of the general RP and phosphorus bonded to Ca and Fe were determined, the differences might have resulted from translocation of phosphorus from organic to inorganic compounds. An exception was sample O3, where the amount of extracted phosphorus under reduced conditions and with application of NaOH was several-fold higher than in the Fe_RP fraction obtained with method M1. In the same sediment, in fractions extracted with acids, comparable amounts of RP bonded to Ca were determined, reaching 0.192 mg $P \cdot g^{-1}$ d.m. (13.04%) with method M1 and 0.200 mg $P \cdot g^{-1}$ d.m (13.56%) with method M2 Table 1). The process of extraction may cause dramatic changes in the sediment structure, e.g. NaOH may dissolve organic compounds of phosphorus, which cover the sediment particles and form a barrier to the release of inorganic fractions of phosphorus (GUNATILAKA et al. 1988). Extraction with a solution of NH_4F and NaOH may lead to the formation of fluorapatites or hydroxiapatites (GOLTERMAN, BOOMAN 1988), which are extracted in the last fraction. Excessively prolonged extraction in NaOH may increase the amount of phosphorus in fractions of reactive phosphorus by hydrolysis of organic compounds of phosphorus in the sediment, or may reduce the amount of extracted phosphorus by its adsorption to $CaCO_3$ (ROMERO-GONZALEZ et al. 2001).

In order to evaluate the speciation methods used, significance of the correlations between the content of RP determined in fractions and the content of metals in the sediments was examined. Significant correlations between Fe and RP content determined with method M1 in Fe_RP fraction (excluding the fraction extracted from sediment O2) and with method M2 in BD_RP fraction (excluding the fraction from sediment O3) were calculated; the determination coefficient was 0.996 and 0.823, respectively. Linear regression functions which illustrate these relationships are shown in Figure 2. The strong correlation between Fe content and BD_RP fraction in mineral sediments suggests that it can be an indicator of the sediment sensitivity to changes in the redox potential. The content of compounds of phosphorus with iron is regulated mainly by the oxidative-reductive potential. Under oxidized conditions, they tend to form hardly dissolvable but easily sedimenting compounds, and when the redox potential reaches 200-300 mV, they are mostly dissolved and phosphorus may migrate to the solution. Significant positive correlations between the content of Ca in sediments and the content of RP as determined with method M1 in fraction Ca_RP and with method M2 in fraction HCl_RP were calculated. Here, the determination coefficient was 0.865 and 0.464, respectively. Linear regression functions which illustrate these relationships are shown in Figure 2. The strong correlation between the content of Al and the content of RP was calculated for the Fe_RP fraction only, and an exception was sediment O3, where the lowest level of Al was determined. The lack of significant correlations between the content of Al and the Al_RP fraction might be related to the low percentage of this fraction in the general content of TP, which on average was 4.2% (Table 1), and that confirms that, unlike in soils, in bottom sedi-

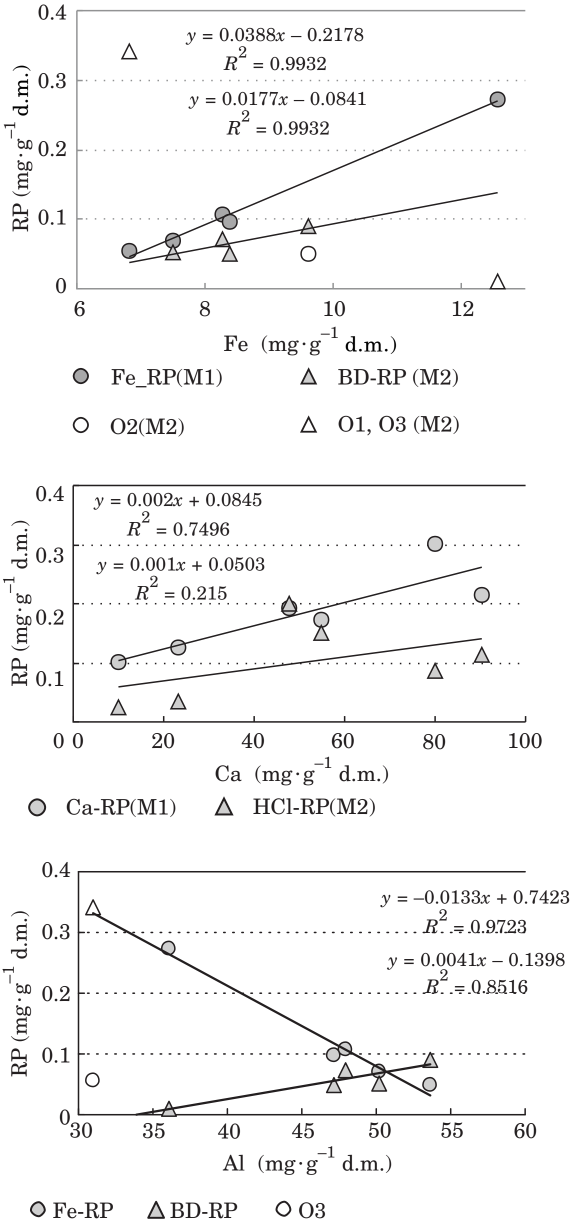

Fig. 2. Relationships between content of the metals and reactive phosphorus in selected fractions

ments aluminum does not play an important role in processes of binding RP and may be important only in extremely acidic waters (ULRICH 1997).

CONCLUSIONS

1. In sediment samples, most reactive phosphorus (RP) was determined in the phosphorus fractions with Ca extracted with acid solution. For the fractions obtained with the M1 method, more of general RP as well as phosphorus bonded to calcium and iron was found than with the M2 method, and the differences might be attributed to translocation of phosphorus from organic to inorganic compounds during fractionation.

2. The fraction of reactive phosphorus determined with the M1 method is correlated with the general content of the metal in the sediments more strongly than the reactive phosphorus fractions determined with the M2 method. The strong correlation between the content of iron and the fraction of BD_RP in mineral sediments shows that it can be an indicator of the sensitivity of sediments to changes in the redox potential

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CHANGES IN THE CONTENT OF SULPHATE SULPHUR AND ARYLSULPHATASE ACTIVITY IN SOIL UNDER POTATO CAUSED BY FERTILIZATION

Anetta Siwik-Ziomek, Jan Koper

Chair of Biochemistry
University of Technology and Life Sciences in Bydgoszcz

Abstract

Biological processes which shape soil fertility are affected by microorganisms and enzymes they produce as well as the rate of biogeochemical transformations in the cycling of elements. One of the enzymes is arylsulphatase (EC 3.1.6.1.), which hydrolyses sulphate esters with aromatic radical, releasing sulphate ions according to the equation: $R\text{-}C\text{-}O\text{-}SO_3^- + H_2O \rightarrow R\text{-}C\text{-}OH + SO_4^{2-} + H^+$. The enzyme plays an essential role in the sulphur cycle in soil and it can be an indicator of sulphur mineralization in soil. For his study, soil was sampled from a field under potato fertilized with different doses of farmyard manure (0, 20, 40, 60 and 80 $t \cdot ha^{-1}$) and mineral nitrogen (0, 45, 90, 135 kg $N \cdot ha^{-1}$). The activity of arylsulphatase was assayed according to Tabatabai and Bremner, while sulphate (VI) sulphur was determined as described by Bardsley and Lancaster. The content of organic carbon in the soil ranged from 8.168 to 10.96 $g \cdot kg^{-1}$ and depended on FYM fertilization, while the content of total nitrogen ranged from 0.889 to 1.012 $g \cdot kg^{-1}$ with an average of 0.960 $g \cdot kg^{-1}$ for FYM and mineral nitrogen doses. The effect of fertilisation on changes in the amount of sulphate sulphur and the activity of arylsulphatase in the soil was noted. The content of sulphate sulphur throughout the research ranged from 21.49 to 24.83 $g \cdot kg^{-1}$. The higher the FYM doses, the higher the content of the fraction of sulphur available to plants. The soil provided a good supply of sulphur to plants. The activity of the enzyme ranged from 0.010 to 0.024 μM $pNP \cdot g^{-1} \cdot h^{-1}$. Its highest activity (an average 0.018 μM pNP $g^{-1} \cdot h^{-1}$) was recorded in the samples fertilised with nitrogen at the amount of 45 $kg \cdot ha^{-1}$. Both parameters changed during the potato vegetation period.

Key words: arylsulphatase, fertilization, soil, sulphate sulphur (VI).

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dr inż. Anetta Siwik-Ziomek, Chair of Biochemistry, University of Technology and Life Sciences, Bernardyńska 6/8 St., 85 029 Bydgoszcz, Poland, e-mail: ziomek@utp.edu.pl

OKREŚLENIE ZMIAN ZAWARTOŚCI SIARKI SIARCZANOWEJ I AKTYWNOŚCI ARYLOSULFATAZY W GLEBIE SPOD UPRAWY ZIEMNIAKA W ZALEŻNOŚCI OD NAWOŻENIA

Abstrakt

Procesy biologiczne kształtujące żyzność gleby są związane m.in. z drobnoustrojami i wydzielanymi przez nie enzymami oraz tempem przemian biogeochemicznych w krążeniu pierwiastków. Arylosulfataza (EC 3.1.6.1.) jest enzymem, który hydrolizuje estry siarczanowe z rodnikiem aromatycznym, uwalniając jony siarczanowe, zgodnie z reakcją: $R\text{-}C\text{-}O\text{-}SO_3^-$ + H_2O → $R\text{-}C\text{-}OH$ + SO_4^{2-} + H^+. Odgrywa on istotną rolę w obiegu siarki i może być wskaźnikiem mineralizacji jej związków w glebie. Celem pracy było zbadanie wpływu zróżnicowanych dawek obornika (0, 20, 40, 60 i 80 $t \cdot ha^{-1}$) i azotu mineralnego (0, 45, 90, 135 kg $N \cdot ha^{-1}$) na aktywność arylosulfatazy i zawartość siarki siarczanowej (VI) w glebie podczas uprawy ziemniaka. Aktywność arylosulfatazy oznaczono wg metody Tabatabai i Bremnera, a zawartości siarki siarczanowej (VI) wg metody Bardsleya-Lancastera. Zawartość węgla organicznego w badanej glebie mieściła się w zakresie 8,168-10,96 $g \cdot kg^{-1}$ i zależała od nawożenia obornikiem. Zawartość azotu ogółem wynosiła 0,889-1,012 $g \cdot kg^{-1}$, średnio 0,960 $g \cdot kg^{-1}$, dla dawek obornika i azotu mineralnego. Stwierdzono wpływ nawożenia na zmiany ilości siarki siarczanowej oraz aktywność arylosulfatazy w badanej glebie. Aktywność badanego enzymu wynosiła 0,010-0,024 μM pNP $g^{-1} \cdot h^{-1}$. Najwyższą aktywność arylosulfatazy (średnio 0,018 μM pNP $g^{-1} \cdot h^{-1}$) stwierdzono w próbkach nawożonych azotem w ilości 45 $kg \cdot ha^{-1}$. Zawartość siarki siarczanowej w glebie w okresie wegetacyjnym ziemniaka wynosiła 21,49-24,83 $g \cdot kg^{-1}$. Zwiększające się dawki obornika powodowały wzrost koncentracji frakcji siarki przyswajalnej dla roślin. Badaną glebę cechuje dobre zaopatrzenie roślin w siarkę. Aktywność arylosulfatazy i zawartość siarki siarczanowej (VI) w glebie zmieniały się w sezonie wegetacyjnym ziemniaka.

Słowa kluczowe: arylosulfataza, nawożenie, gleba, siarka siarczanowa (VI).

INTRODUCTION

Biological processes which shape soil fertility are mainly associated with microorganisms and enzymes they excrete as well as the ratio of biogeochemical processes in elemental cycles. The level of soil enzymatic activity has been considered a sensitive index of its fertility and cultivation potential (Myśków et al.1996). Organic and mineral fertilization introduces nutrients into soil and thus determines the development and activity of soil microorganisms. Soil sulphatases are mainly synthetized by bacteria and fungi. An example of a soil sulphatase is arylsulphatase (EC 3.1.6.1.), which hydrolyses sulphate esters with aromatic ring, releasing sulphate ions according to the equation $R\text{-}C\text{-}O\text{-}SO_3^-$ + H_2O → $R\text{-}C\text{-}OH$ + SO_4^{2-} + H^+. This enzyme plays an important role in the cycling of sulphur and can be an indicator of the mineralization of sulphur compounds in soil (Germida et al. 1992).

The objective of the study was to assess effects of fertilization with farmyard manure and mineral nitrogen applied in various doses on the content of sulphate sulphur (VI) and arylsulphatase activity in soil under potato.

MATERIALS AND METHODS

Soil samples were collected from a long-term experiment carried out at the Grabowo Agricultural Experimental Station by the Department of Plant Nutrition and Fertilization of the Institute of Tillage, Fertilization and Soil Science in Puławy. Considering its fractional composition, the soil was classified as light clay (brown podzolic soil). The crop rotation was: potato, winter wheat, spring barley and clover. The sampling was done four times (10 April, 04 June, 20 July, 12 September 2004) during the vegetation period of potato (medium-early cv. Wiking). The fertilization consisted of farmyard manure (FYM) in the doses: 0, 20, 40, 60 and 80 $t \cdot ha^{-1}$, or mineral nitrogen as ammonium nitrate in the doses: N_0– 0, N_1– 45, N_2– 90, and N_3– 135 kg $N \cdot ha^{-1}$. Arylsulphatase activity was assayed according to Tabatabai and Bremner (1970), while sulphate sulphur was measured as described by Bardsley–Lancaster and modified by COMN-IUNG (1960). Concentrations of the other nutrients were determined by the commonly used methods (Lityński et al. 1976). The significance of the content of sulphate sulphur(VI) and activity of arylsulphatase was evaluated with the use of Tukey's half-zones of confidence (p=0.05). Calculations were done using the FR-ANALWAR (Microsoft Excel) software.

RESULTS AND DISCUSSION

The content of organic carbon in the soil ranged within 8.168-10.96 $g \cdot kg^{-1}$ and depended on fertilization with FYM The lowest concentration of this bioelement was found in soil samples collected from the control objects. As the rates of FYM rose, increasing accumulation of organic carbon in the investigated soil was noticed (Table 1). A similar effect of FYM on the content of organic carbon was observed earlier in many experiments by other authors, e.g. Mercik et al. (1995). Concentration of total nitrogen was less differentiated and fluctuated within the range 0.889-1.012 $g \cdot kg^{-1}$ (mean for FYM and mineral nitrogen doses – 0.960 $g \cdot kg^{-1}$) – Table 1. Small differences were found in the C:N ratio calculated for the samples under study. The highest value of this ratio (12) was observed for samples taken from plots fertilized with manure at the dose 60 $t \cdot ha^{-1}$ and nitrogen at the dose N_0 (Table 1). These values indicated that the process of mineralization was stronger than nitrogen immobilization in the soil.

The values of pH measured in 1 $mol \cdot dm^{-3}$ KCl ranged within 5.3-5.8 (Table 1), which meant that the soil was acidic or slightly acidic. The lowest pH was recorded for the soil fertilized with the highest dose of nitrogen (Table 1). It has been reported earlier that the soil reaction between 5.5 and 6.2 is best for arylsulphatase activity (Tabatabai, Bremner 1970).

Table 1

The content of total organic carbon, total nitrogen, the C:N ratio and sulphate sulphur (VI) as well as activity of arylsulphatase in soil and soil pH

FYM (I factor) ($t \cdot ha^{-1}$)	Nitrogen fertilization II factor ($kg\ N \cdot kg^{-1}$)													
	N_0	N_1	N_2	N_3	$\overline{x}$	N_0	N_1	N_2	N_3	$\overline{x}$	N_0	N_1	N_2	N_3
	C ($g \cdot kg^{-1}$)					N ($g \cdot kg^{-1}$)					C:N			
0	8.168	8.494	8.602	8.233	8.374	0.889	0.966	0.924	0.966	0.936	9	9	9	9
20	9.057	8.732	8.862	9.490	9.035	0.980	0.956	0.956	0.956	0.962	9	9	9	10
40	9.837	9.512	10.66	10.64	10.16	0.991	1.012	0.942	0.987	0.983	10	9	11	11
60	10.77	10.36	10.23	9.945	10.32	0.931	0.970	0.956	0.921	0.944	12	11	11	11
80	10.70	10.57	10.96	10.29	10.61	0.959	0.966	0.980	1.005	0.977	11	11	11	10
$\overline{x}$	9.705	9.533	9.863	9.720	9.705	0.950	0.974	0.951	0.967	0.960	-	-	-	-
$LSD_{0.05}$	I – 0.435			II – n.s		I – n.s			II – n.s					
	SO_4^{2-} ($mg \cdot kg^{-1}$)					arylsulphatase activity (µM pNP $g^{-1} \cdot h^{-1}$)					pH $mol \cdot dm^{-3}$ KCl			
0	22.00	22.33	22.04	21.49	21.96	0.022	0.010	0.012	0.010	0.013	5.8	5.7	5.5	5.3
20	22.08	22.62	21.79	22.11	22.19	0.012	0.013	0.016	0.011	0.013	5.3	5.4	5.4	5.3
40	22.55	22.34	21.79	22.87	22.39	0.024	0.020	0.019	0.011	0.018	5.4	5.4	5.4	5.2
60	23.94	24.83	22.02	22.77	22.39	0.011	0.027	0.014	0.015	0.017	5.4	5.5	5.5	5.3
80	23.87	21.85	23.14	22.95	22.95	0.018	0.021	0.019	0.010	0.017	5.4	5.5	5.5	5.4
$\overline{x}$	22.89	22.79	22.20	22.44	22.58	0.017	0.018	0.016	0.011	0.016	-	-	-	-
$LSD_{0.05}$	I – 0.571 I/II – 1.082			II – 0.476 II/I – 1.065		I – n.s I/II – 0.011			II – 0.050 II/I – 0.011					

n.s. – non-significant

Concentrations of sulphate sulphur (VI) ranged within 21.49-24.3 mg·kg^{-1} and were significantly affected by fertilization, both with FYM and mineral nitrogen (Table 1). Most of Polish arable soils contain sulphur concentrations below 25 mg·kg^{-1} soil. About 70% of Polish arable land contain from 5 up to 20 mg·kg^{-1} of this fraction of sulphur (LIPIŃSKI et al. 2003). In our samples, we found on average 22.58 mg sulphate sulphur (VI) per kg soil, which, in terms of soil abundance in sulphur, allows us to classify it as of a very high sulphur content. Therefore, a good supply of this element (S-SO_4^{2-}) to plants seems ensured (LIPIŃSKI et al. 2003). An increase in the sulphate sulphur (VI) concentrations in brown podzolic soil was observed along with the increasing doses of farmyard manure (Table 1). However, fertilization with ammonium sulphate did not give such an unambiguous effect on this sulphur fraction. The highest concentration of SO_4^{2-} (22.89 mg·kg^{-1}) was found in soil samples collected from plots without nitrogen fertilization, while the lowest amount (22.20 mg·kg^{-1}) was recorded for the dose 95 kg N·ha^{-1} (Table 1).

The resources of soil sulphate sulphur (VI) fluctuated during the vegetation period of potato. The highest concentration of this fraction was noticed in soil samples collected on the third sampling date (25.14 mg·kg^{-1}), while the corresponding amounts recorded on the first sampling date were lower by 22 % than these found in the samples taken in July (Figure 1).

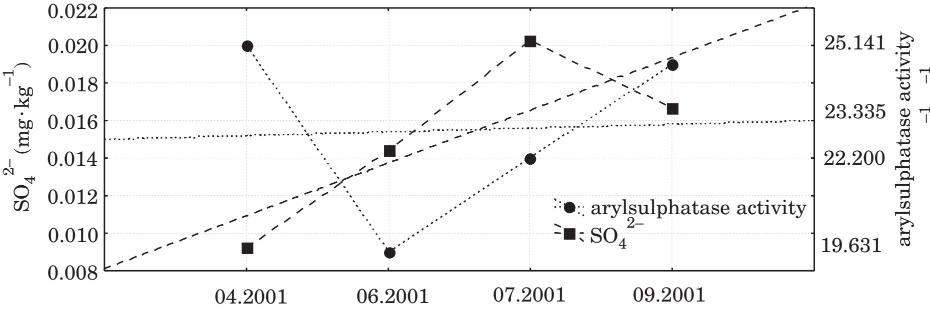

Fig. 1. Sulphate sulphur (VI) content and arylsulphatase activity in potato soil

The activity of arylsulphatase ranged from 0.010 to 0.024 µM pNP g^{-1}·h^{-1}. Fertilization with ammonium nitrate affected this parameter. The use of nitrogen fertilizer in the dose of 135 kg·ha^{-1} caused a decrease in arylsulphatase activity in the investigated soil. Its highest activity (mean 0.018 µM pNP g^{-1}·h^{-1}) was observed in samples fertilized with nitrogen in the dose 45 kg·ha^{-1} (Table 1). Such results indicated an inhibitory effect of ammonium nitrate on the enzyme activity. A negative effect of some ions (NO_3^-, NO_2^-, PO_4^{3-}, SO_4^{2-}, Cl^-) on soil enzymatic activity was reported by many other authors (DICK et al.1988, GANESHAMURTHY, NIELSEN 1990, GERMIDA et al. 1992). Significantly dynamic changes in the activity of this enzyme was observed in our study during the potato vegetation period. The highest activity of the enzyme was found at the beginning of this period, followed by a 22% decline in July and a gradual increase until the end of the vegetation of potato (Figure 1).

CONCLUSIONS

1. The analyzed brown podzolic soil revealed a very high content of sulphur, which should ensue a very good supply of this bioelement to the plants included in the rotation of crops.

2. The highest activity of arylsulphatase and concentration of sulphate sulphur (VI) was found after fertilization with ammonium nitrate at the dose of 45 $kg \cdot ha^{-1}$.

3. A decrease in the enzyme activity accompanied by a high content of the sulphur fraction available to plants and increasing doses of ammonium nitrate suggested an inhibitory effect of sulphate (VI) and nitrate (V) ions on arylsulphatase activity in the investigated soil.

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DISTRIBUTION OF ELEMENTS IN SOILS OF MORAINE LANDSCAPE IN MASURIAN LAKELAND*

Sławomir Smólczyński, Mirosław Orzechowski

Chair of Soil Science and Soil Protection
University of Warmia and Mazury in Olsztyn

Abstract

The research was carried out in three catenas, in which the soils made a typical toposequence of the moraine landscape in Masurian Lakeland, i.e. eroded soils, deluvial soils, mucky soils as well as slightly and strongly silted peat-muck soils. The total content of Ca, Mg, K, P, Na, Fe, Mn, Zn and Cu was analyzed in these soils. The amounts of soil organic matter and measured elements showed catenal variation. Mucky soils had the highest content of Mg, K, Fe and Na whereas strongly and slightly silted peat-muck soils contained the highest amounts of P, Mn, Ca and Cu. The content of Ca was positively correlated with organic matter content. The amount of Mg, K, Cu and Mn was positively correlated with the amount of clay fraction. As the studied soils are located at the bottom of a slope, downwards water flow, where mineral compounds are accumulated in deposits or dissolved in water, mucky soils and silted peat-muck soils contain high amounts of the analyzed elements. Mucky soils and silted peat-muck soils play a role of biogeochemical barriers protecting wetlands against nutrients from surrounding moraine uplands.

Surface horizons of deluvial soils were depleted of the analyzed elements (excluding P) whereas AO horizons of mucky soils and Mt horizons of peat-muck soils were abundant in the measured elements (excluding Ca). Various amounts of the analyzed elements in the pedons of the catena sequences should be taken into consideration when planning land use in the moraine landscape, which has unique natural values.

Key words: macro- and microelements, moraine landscape, toposequence, eroded soils, deluvial soils, mucky soils, strongly and slightly silted peat-muck soils.

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dr inż. Sławomir Smólczyński, Chair of Soil Science and Soil Protection, University of Warmia and Mazury, 10-719 Olsztyn, Poland, phone: 089 5234846, e-mail: slawomir.smolczynski@uwm.edu.pl

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ROZMIESZCZENIE PIERWIASTKÓW W GLEBACH KRAJOBRAZU MORENOWEGO POJEZIERZA MAZURSKIEGO

Abstrakt

Badania przeprowadzono w 3 katenach, w których gleby tworzyły typową dla krajobrazu morenowego Pojezierza Mazurskiego toposekwencję: gleb erodowanych, deluwialnych, namurszowych oraz gleb torfowo-murszowych silnie i słabo zamulonych. W glebach oznaczono całkowitą zawartość Ca, Mg, K, P, Na, Fe, Mn, Zn, Cu. Badane gleby wykazywały katenalną zmienność zawartości materii organicznej i oznaczonych pierwiastków. Gleby namurszowe wyróżniały się najwyższą zawartością Mg, K, Fe, Na, a silnie i słabo zamulone gleby torfowo-murszowe – P, Mn, Ca i Cu. Zawartość wapnia była dodatnio skorelowana z ilością materii organicznej, natomiast zawartość Mg, K, Cu, Mn z ilością frakcji ilastej. Stwierdzona największa zawartość większości oznaczonych pierwiastków w glebach namurszowych i zamulonych glebach torfowo-murszowych jest uwarunkowana ich usytuowaniem u podnóża stoków, na drodze spływów erozyjnych, gdzie następuje akumulacja składników mineralnych przemieszczanych wraz z masą glebową lub rozpuszczonych w wodzie. Gleby namurszowe i zamulone gleby torfowo-murszowe w krajobrazie morenowym pełnią funkcję barier biogeochemicznych chroniących siedliska mokradłowe przed przenikaniem pierwiastków biogennych z otaczających wysoczyzn.

Poziomy powierzchniowe gleb deluwialnych wykazywały zubożenie w oznaczone pierwiastki (z wyjątkiem P), natomiast poziomy AO gleb namurszowych i Mt torfowo-murszowych wzbogacenie (z wyjątkiem Ca). Zróżnicowanie zawartości pierwiastków w pedonach badanych katen powinno być uwzględniane podczas użytkowania gleb w krajobrazie morenowym, który ma unikatowe walory przyrodnicze.

Słowa kluczowe: makro- i mikroelementy, krajobraz morenowy, toposekwencja, gleby erodowane, gleby deluwialne, gleby namurszowe, silnie i słabo zamulone gleby torfowo-murszowe.

INTRODUCTION

The moraine landscape, shaped during the Vistula glaciation, is located in the middle of Masurian Lakeland and occupies the largest area (Piaścik 1996a). In this landscape, the relief, soil cover and soil formations are diversified. The zone of moraine uplands is represented by hills and rolling ground moraines with numerous depressions filled with wetlands, which have unique natural values (Piaścik, Gotkiewicz 2001). In the moraine landscape, which has diversified land relief, processes of translocation of soil formations from slopes and their accumulation at the foot of slopes, on the surface of organic formations filling depressions, are common. In consequence, certain toposequences of soils are found, e.g. eroded soils, deluvial soils along sloped and mucky soils, silted peat-muck soils in depressions (Piaścik et al. 2001b, Piaścik, Sowiński 2002).

In the moraine landscape, slope-related processes have great influence on the carbon cycle and content of mineral compounds (Wocławek 1973, Smolska et al. 1995, Stepa 1996, Kruk 2000, Sowiński at al. 2004), which locally, in young glacial areas, in soils of mid-moraine depressions, may reach dangerous levels (Bieniek 1997, Smólczyński et al. 2004). Deluvial and upper silted organic soils (mucky soils as well as slightly and strongly silted peat-muck soils) play an important role in the nutrient cycle (Gotkiewicz et al. 1990, Sowiński et al. 2004a,b). These soils were formed in the ecotone zones between wetlands and mineral soils of the surrounding slopes. Hillbricht-Ilkowska (2005) stressed that ecotone zones are a very typical element of the landscape of lakeland hills of north-eastern Poland and are particularly important for sustaining its stability and function.

The aim of this paper was to determine the total amount of elements in the examined soils and depict the distribution of the elements in soil profiles as well as in typical toposeqences of eroded soils, deluvial soils, mucky soils and slightly and strongly silted peat-muck soils of the moraine landscape of north-eastern Poland.

MATERIAL AND METHODS

The research was carried out in the zone of the moraine landscape shaped during Poznań phase (Prusinowo site in Mrągowo Lakeland) and Pomeranian phase (Lutry I and Lutry II sites in Olsztyn Lakeland) of the Vistula glaciation. At the studied sites, along the transects, from the slope to the depression, soil profiles were made (in Prusinowo and Lutry II catenas – 7 soil profiles each, in Lutry I catena – 8 soil profiles). The following alternation of soils was found: eroded soils in the upper part of the slope, deluvial soil in the lower part, mucky soils at the bottom of the slope, slightly and strongly silted peat-muck soils in a depression. Eroded soils were developed from sandy loam and their parent material had loam texture. Deposits of deluvial soils had thickness of 40-110 cm and silty sandy loam or silty loam texture. In mucky soils of the studied sites, the surface layer of thickness of 30 cm was composed of mineral-organic deposit, which contained 10-20% of organic matter. In peat-muck soils, the thickness of muck horizons was 25-33 cm and the muck-forming process was medium advanced.

As the textures of eroded soils and deluvial deposits were similar at the studied sites, an average content of the elements in the studied pedons at three catena sequences is shown in Tables 1 and 4. As bulk density of mineral and organic formation is different, the amount of the analyzed elements was expressed per dm^3 (calculated on the basis of bulk density) and is presented in Table 4.

The total content of Ca, Mg, K, P, Na, Fe, Mn, Zn and Cu was measured after digestion in a mixture of $HClO_4$ and HNO_3. Calcium, potassium and sodium was measured photometerically using a Jenway flame photometer, while phosphorous was measured colorimetrically using a Specol EK 1

Table 1

Mean content of elements in surface horizons of studied soils

Soil horizon	Value	Percentage of mineral fraction (diameter in mm)			Organic matter	Ca	Mg	K	Na	P	Fe	Cu	Zn	Mn
		<0.02	0.02-0.002	<0.002	$g \cdot kg^{-1}$							$mg \cdot kg^{-1}$		
1 A	*X*	25.0	17.0	8.0	22.7	3.1	2.7	3.7	0.2	0.5	23.1	7.30	59.87	290.37
	S	7.5	4.6	3.5	8.4	1.9	1.2	1.4	0.1	0.1	18.8	4.04	40.01	71.05
	CV	30.0	27.1	43.8	37.0	61.3	44.4	37.8	50.0	20.0	81.4	55.3	66.8	24.5
2 Ad	*X*	29.7	20.5	9.2	28.9	5.1	3.0	3.0	0.3	0.5	15.0	8.15	36.97	298.78
	S	11.0	6.2	2.7	17.0	3.4	0.6	0.7	0.06	0.2	3.5	3.01	9.41	157.92
	CV	36.9	29.9	29.3	58.8	66.8	20.0	23.3	20.0	40.0	23.3	36.9	25.5	52.8
3 AO	*X*	37.1	30.0	7.1	185.2	12.2	4.7	3.7	0.4	0.8	24.0	12.06	52.36	456.46
	S	6.4	10.7	1.0	56.5	3.0	0.9	0.6	0.09	0.2	7.7	2.61	8.04	159.06
	CV	17.3	35.7	13.9	30.5	24.6	19.2	16.2	22.5	25.0	32.1	21.6	15.4	34.9
4 Mtsz	*X*				357.9	15.6	3.5	3.3	0.3	1.0	20.7	9.79	55.51	665.63
	S				93.5	0.9	0.4	0.6	0.05	0.2	2.9	3.29	23.13	301.14
	CV				26.1	5.8	11.4	18.2	16.7	20.0	14.0	33.6	41.7	45.2
5 Mtz	*X*				639.2	39.7	2.2	1.3	0.4	0.8	20.5	12.75	34.20	385.27
	S				76.3	16.5	0.6	0.9	0.01	0.5	12.4	2.87	16.10	176.40
	CV				11.9	41.6	27.3	69.2	2.5	62.5	60.5	22.5	47.1	45.8
Statistically significant differences $\alpha = 0.05$						2<3<4<5	2<3 3>4>5	2<3 4>5	1<2	2<3	2<3	2<3	1>2 2<3	2<3

Explanations: A – humus horizon of eroded soils; Ad – humus horizon of deluvial soils; AO – humus horizon of mucky soils, Mtsz – strongly silted muck, Mtz – slightly silted muck, *X* – mean, *S* – standard deviation, *CV* – coefficient of variation

< – statistically significant differences between studied soil horizons

spectrocolorimeter and magnesium was measured using an AAS 1 Zeiss Jena analyzer. The total content of Fe, Mn, Zn and Cu was measured applying the AAS techniques on a 30 Zeiss Jena analyzer.

Statistical calculations (mean, correlation coefficients, standard deviation) were conducted using Statistica 8.0.

RESULTS AND DISCUSSION

In the soils of the analyzed catena sequences, the content of organic matter, which is a criterion for classification of hydrogenic soil formations, increased towards the depression. Based on the criteria suggested by OKRUSZKO (1974), the surface formations of mucky soils containing on average 185.2 $g \cdot kg^{-1}$ of organic matter were classified as mineral-organic and muck formations as strongly silted mucks (357.9 $g \cdot kg^{-1}$ of organic matter) or slightly silted mucks (639.2 $g \cdot kg^{-1}$ of organic matter) – Table 1. Deluvial deposits were richer in clay fraction (<0.02 mm) and colloidal clay (<0.002 mm) than eroded soils (Table 1).

Among all the analyzed elements, calcium and iron were predominant in the soils. In the surface horizons, the average content of calcium increased towards the depression and when the amount of organic matter increased from 3.1 $g \cdot kg^{-1}$ in the humus horizons of eroded soils to 39.7 $g \cdot kg^{-1}$ in slightly silted mucks (Table 1). The differences in the content of these elements proved to be statistically significant between deluvial deposits, surface horizons of mucky soils and slightly and strongly silted mucks. The positive relationship between the amounts of calcium and organic matter is proved by high values of correlation coefficients (Table 2) as well as the results of studies on soils in other landscapes (PIAŚCIK et al. 2001a). According to BIENIEK (1997), compounds of calcium are dissolved in water and translocated during erosion. In hydrogenic soils, calcium occurs mainly in an exchangeable form and its accumulation in peat-muck soils is connected with high cation exchange capacity (PIAŚCIK 1977). The calculated coefficients, shown in Table 3, indicate that the content of calcium in surface horizons of the examined soils was lower than in deeper layers of the soil profile. Considerabledepletion of Ca was reported for mucky and peat-muck soils. It suggests that the process of decalcitation, reported in the literature, takes place (PIAŚCIK 1977, 1996b, PIAŚCIK et al. 1998, 2001a).

The smallest content of iron was recorded in deluvial soils (on average 15.0 $g \cdot kg^{-1}$) – Table 1. In mineral-organic formations of mucky soils, the average content of this element was statistically significantly higher (24.0 $g \cdot kg^{-1}$). Similar amounts were reported in humus horizons of eroded soils (on average 23.1 $g \cdot kg^{-1}$). In muck horizons, the average content of iron was not related to the degree of silting (Table 1) and reached

Table 2

Correlation coefficients for analysed elements in the surface layers

Specification	Ca	Mg	K	Na	P	Fe	Cu	Zn	Mn
Organic matter	0.863*	-0.030	-0.428*	0.443*	0.558*	0.308*	0.498*	0.089	0.408*
<0.02	0.464*	0.606*	0.622*	0.129	0.375*	0.304	0.615*	-0.022	0.802*
0.02-0.002	0.514*	0.488*	0.407*	0.092	0.585*	0.175	0.276	0.120	0.496*
<0.002	-0.272	0.294	0.295	-0.041	0.420*	-0.063	0.001	-0.261	0.070
Ca		-0.089	-0.363*	0.385*	0.518*	0.326*	0.408*	0.042	0.190
Mg			0.474*	0.411*	0.214	0.395*	0.527*	0.275	0.364
K				0.003	0.160	0.083	0.029	0.226	0.201
Na					0.382	0.310	0.532*	0.165	0.176
P						0.360	0.396*	0.209	0.400*
Fe							0.488*	0.721*	0.391
Cu								0.122	0.526*
Zn									0.148

*significance level at α=0.05

20.5-20.7 $g \cdot kg^{-1}$. Iron was accumulated in surface layers of all the soils except deluvial ones (Tab. 3). As compared to calcium, distribution of iron in the soil profile was different. Solubility and mobility of this element depended on the oxidation and reduction processes and Fe was accumulated in aerobic zone of the soil profile. In muck horizons, iron is released during mineralisation as well as is precipitated from groundwater in the form of hydroxides (Piaścik, Bieniek 2001).

The content of magnesium was the highest in mineral-organic mucky soils (AO), reaching 4.7 $g \cdot kg^{-1}$ (Table 1). In deluvial deposits as well as in slightly and strongly silted mucks, the amount of this element was significantly lower.

The highest average content of potassium was reported in humus horizons of eroded and mucky soils (Table 1). In muck formations, the content of potassium decreased together with the degree of silting. Distribution of magnesium and potassium in the studied soil profiles was similar (Table 3). The humus horizons of eroded and deluvial soils was poor in Mg and K, which were largely accumulated in surface horizons of mucky soils and peat-muck soils (Table 3). The content of magnesium and potassium was positively correlated with the <0.02 mm and 0.02-0.002 mm fractions (Table 2). According to Chodak et al. (2005), magnesium is subjected to translocation with the soil solid phase and potassium partly with the soil solution.

The mean content of sodium oscillated between 0.2 $g \cdot kg^{-1}$ in the humus horizons of deluvial soils and 0.4 $g \cdot kg^{-1}$ in the mineral-organic forma-

Table 3

Coefficients enrichment or impoverishment in elements for the surface layers (5-10 cm) in relation to deeper horizons 30-40 cm (I) and 50-60 cm (II)

Soil unit		Ca	Mg	K	Na	P	Fe	Cu	Zn	Mn
Eroded soils	I	0.87 0.62-1.06	0.74^{-} 0.48-0.91	0.93 0.55-1.30	0.96 0.83-1.04	1.50^{+} 1.00-2.00	1.45^{+} 0.87-2.27	1.00 0.53-1.54	1.53^{+} 1.03-2.29	1.26^{+} 0.90-1.46
	II	0.33^{---} 0.20-0.58	0.49^{---} 0.32-0.61	0.77^{+} 0.57-1.15	0.67^{--} 0.43-0.87	1.42^{+} 0.75-2.00	1.25^{+} 0.36-2.37	0.75^{-} 0.47-1.23	1.75^{+} 0.82-2.74	1.03 0.98-1.11
Deluvial soils*	I	0.93 0.71-1.11	0.82^{-} 0.66-1.00	0.93 0.72-1.14	0.86 0.67-1.00	1.28^{+} 0.67-2.00	0.83^{-} 0.59-1.02	0.80^{-} 0.52-1.26	0.82^{-} 0.64-1.07	0.88 0.54-1.14
	II	0.81^{-} 0.30-1.13	0.93 0.71-1.11	1.21^{+} 0.83-1.65	0.91 0.77-1.20	1.60^{+} 1.00-2.00	0.86 0.66-1.09	1.01 0.56-1.51	0.92 0.54-1.09	1.29^{+} 0.65-2.70
Mucky soil with mineral-organic layer in the top horizon*	I	0.59^{--} 0.28-1.20	1.31^{+} 0.67-2.62	1.41^{+} 0.84-2.06	0.94 0.69-1.14	1.11 0.80-1.25	1.39^{+} 0.94-2.54	1.04 0.57-1.79	2.19^{++} 1.26-4.17	2.27^{++} 1.08-3.42
	II	0.38^{---} 0.14-0.64	3.91^{++} 1.21-10.83	3.32^{++} 1.10-5.00	0.63^{--} 0.11-1.45	2.32^{++} 0.83-4.00	10.38^{+++} 1.16-21.40	1.92^{+} 1.02-2.97	6.92^{+++} 1.34-11.52	11.51^{+++} 1.08-33.14
Peat – muck soils* strongly silted	I	0.70^{--} 0.36-0.90	1.41^{+} 0.79-3.08	2.75^{++} 0.77-3.30	1.51^{+} 0.58-2.70	1.17 0.71-2.20	3.22^{++} 0.36-6.54	1.30^{+} 0.66-1.82	3.25^{++} 1.12-7.98	1.77^{+} 1.08-3.07
	II	0.24^{---} 0.22-0.29	3.96^{++} 2.62-5.29	3.87^{++} 3.30-4.13	0.89 0.77-1.00	3.25^{++} 1.50-5.00	2.58^{++} 1.20-3.39	0.91 0.37-2.28	8.89^{+++} 5.98-9.22	7.62^{+++} 0.82-14.41
Peat – muck soils*, slightly silted	I	0.74^{-} 0.70-0.77	3.00^{++} 1.67-4.33	2.32^{++} 2.30-2.33	0.67^{--} 0.58-0.75	1.15 1.00-1.30	4.88^{++} 2.25-7.51	2.21^{++} 0.92-3.49	5.44^{+++} 2.88-8.00	4.94^{++} 4.20-5.67
	II	0.73^{-} 0.57-0.90	2.40^{++} 1.54-3.25	2.31^{++} 2.30-2.33	1.20^{+} 0.88-1.52	0.69^{--} 0.50-0.88	2.51^{++} 1.62-3.40	2.05^{++} 1.43-2.66	3.61 2.77-4.45	4.95^{++} 3.36-6.53

Explanations: 1.20-2.00 – evident enrichment (+); 2.01-5.00 – strong enrichment (++); > 5.00 very strong enrichment (+++); 0.85-0.71 – evident impoverishment (–); 0.70-0.51 – strong impoverishment (– –); <0.50 very strong impoverishment – – –).

*according to Polish Soils Classification System (1989) tions of mucky soils and slightly silted mucks (Table 1). The distribution of sodium in the soil profile was variable, which has been manifested by the varied values of coefficients presented in Table 3.

The smallest content of phosphorous was reported in eroded soils and deluvial soils (0.5 $g \cdot kg^{-1}$) – Table 1. In AO horizons of mucky soils and in muck formations, the amount of this element was significantly higher and oscillated between 0.8 $g \cdot kg^{-1}$ and 1 $g \cdot kg^{-1}$. In the analyzed soils, phosphorous was accumulated in surface layers (5-10 cm) of the soil profile (Table 3), which was also noted by SAPEK et al. (1991) and PIAŚCIK et al. (2001a).

Copper was largely accumulated in surface layers of peat-muck soils and mucky soils (Table 3), and its content (9.8-12.8 $g \cdot kg^{-1}$) – Table 1 was higher than in eroded soils and deluvial soils (7.3-8.2 $g \cdot kg^{-1}$). The content of this element was positively correlated with the content of organic matter and <0.02 mm fraction (Table 2).

The average content of zinc was the highest in A horizons of eroded soils (59.9 $g \cdot kg^{-1}$) – Table 1. In deluvial soils, its content was significantly lower (37.0 $g \cdot kg^{-1}$) and increased in AO horizons of mucky soils (54 $g \cdot kg^{-1}$) as well as in strongly silted mucks (55.5 $g \cdot kg^{-1}$) – Table 1.

The content of manganese increased from 290.4 $g \cdot kg^{-1}$ in A horizons of eroded soils to 665.6 $g \cdot kg^{-1}$ in strongly silted mucks (Table 1). The amount of this element was positively correlated with the amount of organic matter and clay fraction (<0.02 mm) – Table 2. In the soil profile, manganese is mobile and precipitated at the point of changes in the redox potential (SAPEK et al. 1991). The distribution of Mn in the soil profile was similar to the zinc distribution. Peat-muck soils and mucky soils showed considerable enrichment of surface layers whereas eroded soils demonstrated impoverishment in the analyzed elements (Table 3).

According to the data in Table 4, the content of the measured elements, except calcium, was higher in deluvial soils and mucky soils than in peat-muck soils. This can be explained by a 2-4-fold higher degree of silting of mineral and mineral-organic formations in comparison to mucks. However, SAPEK et al. (1991) stressed that due to high soil exchange capacity and low density, peat soils may be contaminated on the surface with heavy metals. A statistically significant decrease in the analyzed elements in muck formations as compared to humus horizons (AO) of mucky soils (Table 3) is typical. It distinguished mucky soils and is an argument in favour of classifying them as a separate soil unit in the soil classification system. In the moraine landscape, mucky soils are a pedo-ecotone between and deluvial soils and hydrogenic soils.

In the examined catena sequences, the content of the analyzed elements in eroded soils and deluvial soils is parallel to the amounts in similar soils of Masurian Lakeland examined by BIENIEK (1997). However the muck formations contained more magnesium and potassium than unsilted peat-muck soils of Masurian Lakeland (PIAŚCIK 1977). The content of zinc and copper

Table 4

Mean content of elements in surface horizons of studied soils (expressed per dm^3 of dry soil)

Soil horizon	Value	Ca	Mg	K	Na	P	Fe	Cu	Zn	Mn
		g·dm^{-3}							mg·dm^{-3}	
1 A	*X*	4.8	4.1	5.7	0.3	0.8	35.4	11.20	91.43	440.71
	S	3.0	1.9	2.4	0.1	0.2	29.2	6.57	62.49	114.57
	CV	62.5	39.6	42.1	33.3	25.0	82.5	58.7	68.4	26.0
2 Ad	*X*	7.1	4.2	4.2	0.4	0.7	20.9	11.27	52.05	415.65
	S	4.65	0.90	0.89	0.08	0.28	4.38	3.42	8.96	213.90
	CV	65.5	21.4	21.2	22.0	40.0	20.9	30.4	17.2	52.7
3 AO	*X*	10.2	4.0	3.3	0.3	0.7	19.7	10.09	42.55	405.84
	S	2.37	0.68	1.31	0.08	0.19	3.45	1.82	13.17	201.89
	CV	23.2	17.0	39.7	26.7	27.2	17.6	18.1	31.0	49.8
4 Mtsz	*X*	7.8	1.7	1.7	0.16	0.5	10.9	4.61	27.34	308.9
	S	1.88	0.51	0.57	0.06	0.17	1.81	1.01	132.12	98.31
	CV	24.1	29.7	34.6	37.5	32.7	16.6	22.0	48.3	31.2
5 Mt	*X*	14.6	0.8	0.5	0.13	0.3	6.9	4.59	11.81	135.12
	S	6.06	0.24	0.43	0.01	0.23	3.54	0.79	4.29	53.30
	CV	41.7	29.6	86.0	7.7	74.2	5.1	17.3	36.4	39.5
Statistically significant differences $\alpha = 0.05$		3>4>5*	3>4>5	1>2>3>4>5	2>3>4	3>4	1>2 3>4	3>4	1>2>3>4>5	4>5

Explanations as in Table 1

was at a natural level (Kabata-Pendias et al. 1993), although locally, in mid-moraine depressions of Masurian Lakeland, the soils contained increased levels of some heavy metals (Smólczyński et al. 2004). As the studied soils are located at the bottom of a slope, downwards water flow, where mineral compounds are accumulated in deposits or are dissolved in water, mucky soils and silted peat-muck soils contain the highest amounts of the analyzed elements (expressed as percentages). This verifies the role of these soils as geochemical barriers for nutrients in moraine landscapes. Similar relationships were reported by Okruszko and Chruska (1998) in grassland soils. Among all the soil formations these authors studied, the most abundant in macro- and microelements were silted organic and mineral-organic formations, especially at sites enriched in mineral compounds with surface flow from surrounding fields. Another study, carried out in an agricultural catchment area of young glacial landscape, proved that land relief was the main determinant of nutrient concentration (Ca, Mg, Na) in both groundwater (Szymczyk, Cymes 2005) and mid-field water bodies (Cymes, Szymczyk 2005).

CONCLUSIONS

1. The content of elements in the studied toposequences, typical for the moraine landscape of Masurian Lakeland, of eroded soils, deluvial soils, mucky soils as well as slightly and strongly silted peat-muck soils varied in a catena sequence.

2. The highest amounts of Mg, K, Fe, Na were reported in mucky soils, P and Mn in strongly silted peat-muck soils, Ca and Cu in slightly silted peat-muck soils.

3. Surface horizons of deluvial soils were poor in the analyzed elements (excluding P). The measured elements (excluding Ca) were largely accumulated in surface horizons of mucky soils and peat-muck soils.

4. In the moraine landscape, mucky soils and silted peat-muck soils play a role as geochemical barriers protecting wetlands against nutrients from surrounding uplands.

5. Various content of elements in the pedons of the examined toposequences should be taken into consideration when planning land use in the moraine landscape, which has unique natural values.

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INFLUENCE OF THE TYPE OF SOIL DEWATERING AND LAND USE ON THE DYNAMICS OF CONCENTRATIONS AND VOLUME OF NITROGEN DISCHARGED FROM AGRICUTLURAL AREAS

Sławomir Szymczyk

Chair of Land Reclamation and Environment Management
University of Warmia and Mazury in Olsztyn

Abstract

Studies on the dynamics of concentrations and discharge of nitrogen load through draining systems were carried out in Olsztyn Lake District (Pojezierze Olsztyńskie) from 1994 to 2007. For the tests, three basins were selected: an agricultural basin drained with a drainage system, an agricultural and forested basin drained with a network of ditches and drains, and an agriculrual basin with a large forest cover (ca 30%), drained with ditches. The discharge of water through the draining facilities was measured every two weeks, and once a month, $N\text{-}NO_3$, $N\text{-}NO_2$, $N\text{-}NH_4$ as well as Kjeldahl nitrogen were determined. It has been demonstrated that the volume of discharged water carried away through draining systems depends primarily on the amount and distribution of atmospheric precipitation, epsecially during the winter half-year. The dynamics of concentrations and loads of nitrogen in water discharged via draining systems varied with time and depended not only on the amount and distribution of precipitation over a year and in the multi-year period, but also on the type of land use in a given basin and a draining system. It was also found out that intensive rainfall in summer only slightly increased the discharge of water from the basinm drained with the drainage system. The load of total nitrogen flowing away through the draining systems from agricultural basins was closely connected with the amount of discharged water and water levels of mineral nitrogen compounds, especially $N\text{-}NO_3$. The highest nitrogen loss from drained areas appeared in spring, which was associated with the seasonal character of water outflow, culmunating in March-April. The highest concentration of total nitrogen (16.69 mg dm^{-3}) was determined in water discharged through the network of ditches and drains, but due to a more intensive dewatering of so-

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dr inż. Sławomir Szymczyk, Chair of Land Reclamation and Environment Management, University of Warmia and Mazury, Olsztyn, pl. Łódzki 2, 10-718 Olsztyn, Poland, e-mail: szymek@uwm.edu.pl

ils, a higher total nitrogen load (on average 33% more) was discharged through drains. Per 1 ha of an agricultural basin, the annual outflow of total nitrogen was up to 13.13 kg, including 12.04 kg of mineral nitrogen.

Key words: nitrogen, drains, ditches, agricultural basin, draining systems.

WPŁYW SPOSOBU ODWODNIENIA I UŻYTKOWANIA GLEB NA DYNAMIKĘ STĘŻENIA I WIELKOŚĆ ODPŁYWU AZOTU Z OBSZARÓW ROLNICZYCH

Abstrakt

Badania nad dynamiką stężenia i odpływu ładunku azotu systemami melioracyjnymi prowadzono na Pojezierzu Olsztyńskim w latach 1994-2007. Do badań wytypowano zlewnie: rolniczą odwadnianą siecią drenarską, rolniczo-leśną odwadnianą siecią rowów i drenów oraz rolniczą o dużym udziale obszarów leśnych (ok. 30%), odwadnianą rowami melioracyjnymi. Odpływ wód z urządzeń melioracyjnych mierzono co dwa tygodnie, a raz na miesiąc w wodzie oznaczano N-NO_3, N-NO_2, N-NH_4 oraz azot metodą Kjeldahla. Wykazano, że o wielkości odpływu wody systemami melioracyjnymi decydują głównie ilość i rozkład opadów atmosferycznych, a szczególnie ich ilość w półroczu zimowym. Dynamika stężeń i ładunków azotu w wodach odpływających systemami odwadniającymi była zmienna w czasie i zależała nie tylko od ilości i rozkładu opadów w roku i wieloleciu, ale również od sposobu zagospodarowania zlewni i systemu odprowadzającego wodę. Stwierdzono, że intensywne opady w sezonie letnim zwiększają odpływ wody w niewielkim stopniu, i tylko w przypadku sieci drenarskiej. Ładunek azotu ogólnego odpływającego systemami melioracyjnymi ze zlewni rolniczych jest ściśle uzależniony od ilości odpływającej wody oraz od stężenia w niej mineralnych związków azotu, szczególnie N-NO_3. Największe straty azotu z terenów zmeliorowanych występowały w okresie wiosennym, co wiąże się z sezonowym odpływem wód i jego kulminacją w miesiącach marzec-kwiecień. Największe stężenie azotu ogólnego (16,69 $mg \cdot dm^{-3}$) występowało w wodach odpływających siecią rowów i drenów, ale ze względu na intensywniejsze odwodnienie gleb większy jego ładunek, średnio o 33%, odpływał drenami. Z 1 ha zlewni rolniczej drenami odpływało do 13,13 kg azotu ogólnego rocznie, w tym do 12,04 kg azotu mineralnego.

Słowa kluczowe: azot, dreny, rowy, zlewnia rolnicza, systemy odwadniające.

INTRODUCTION

Threats posed by man's activity to the environmental quality of rural areas are a consequence of a combination of many natural and anthropogenic factors. Among the major man-made stress factors, which can be controlled and modified, are the type of land use in a catchment basin, including the share and location of arable lands, permanent grassland, forests, peatbogs and wetlands, water pools, tree assemblages and thickets, as well as the intensity of agricultural practice, including the amounts and forms of fertilizers (ALLAN, CHAPMAN 2001, HERZOG et al. 2008, GRABIŃSKA et al. 2005, HEATHWAITE et al. 1998, OENEMA et al. 2005, SPRUILL 2004). More intensive farming, often resulting in turning more grounds into arable land, has long been inseparebly connected with the development of draining systems, the aim of which in Poland is mainly to accelerate water outflow (LIPIŃSKI 2002). Thus, draining does not usually affect much groundwater, but just carries away excess of precipitation (LIPIŃSKI 2003). However, the fact that water shortages, which may appear following a prolonged after-drought period, cannot be replenished raises the risk of inferior crop yields and initiates many soil processes, especially mineralization of organic matter. Excessive quantities of released nutrients, due to the limited access to water, do not undergo biosorption, which means they are more likely to be leached by rainfall into deeper layers of the soil profile. They enter groundwater, and through drainage systems, quickly travel to surface waters, where they can cause eutrophication and pollution (KOC et al. 2007, SZYMCZYK, SZYPEREK 2005, SZYMCZYK et al. 2005, VAGSTAD et al. 2000). The dependence of the outflow of water through drainage ditches on atmospheric precipitation is very complex, being shaped by such elements as the amount and time distribution of precipitation, structure and permeability of a soil profile, level of groundwater, influx water supply, temperature and species of crops (LIPIŃSKI 2002, SZYMCZYK, SZYPEREK 2005). Precipitation water contains elements which are already dissolved, and this facilitates their migration within the environment and participation in physicochemical processes of ion exchange in soil. During intensive rainfall, the nitrogen contained in rainfall water quickly migrates to groundwater, and with the surface and subsurface flow, enters surface water (LIPIŃSKI 2002). Large changeability in the concentration of nitrogen in water and the outflow of nitrogen load through draining systems is connected with both the type of a draining system and the intensity of farming practice in a given agricultural catchment basin (KOC et al. 2007, KOPACZ et al. 2007, PULIKOWSKI et al. 2008, SZYMCZYK, SZYPEREK 2005). The outflow of nitrogen load from agricultural basins can be drastically limited by such structural, spatial and economic transformations that lead to changes in land use. As a result of such modifications, the area of farmed land decreases (more idle and fallow land) or some farmland is transformed so as to sustain low-cost, organic farming (KOPACZ et al. 2007).

The objective of this study has been to determine the dynamics of concentrations and loads of nitrogen carried away from basins which differed in the type of land use, soil compactness and draining system. The analysis was performed on the backdrop of changing meteorological conditions in the multi-year period from 1994 to 2007.

MATERIAL AND METHODS

In 1994-2007, a study was carried out in Olsztyn Lake District (Pojezierze Olsztyńskie) on the dynamics of nitrogen outflow from agricultural areas, with water drained by different types of draining systems. Three basins, which differed in the land use, soil compactness and draining systems, were selected for the study. The first basin was an agricultural one; it covered 34 ha (arable lands – 98%) and was drained with a drainage system. The prevailing soils were medium-compact and light ones, developed from sandy, loamy and silty formations. The second basin was agricultural and forested in character. It covered about 250 ha and was drained with an irregular network of drains and open ditches. The predominant form of land use (over 60%) was arable land, while about 34% of the total area was covered by forests and field tree assemblages. The basin comprised light and very light soils developed from sands, with some loams and silty, sandy or loamy formations. The third basin, which covered over 280 ha, was drained with an irregular network of drainage ditches. About 50% of the basin's area was covered by arable land, 15% belonged to grasslands (since 1993 some set aside) and about 30% was overgrown with forests and trees. The soils in this basin were mainly medium-compact and light ones, developed from medium loams and loamy sands. A small area of the basin lies on silty soils and peat. Later in this paper, the three basins are characterised according to the type of a draining system.

The flow of water in draining facilities was measured at two-week intervals, and once a month water samples were taken for the following determinations: nitrate nitrogen (V) – $N\text{-}NO_3$, by colorimetry with disulfofenolic acid; nitrate nitrogen (III), by colorimetry with sulfanilic acid; ammonia nitrogen – $N\text{-}NH_4$, by colorimetry with Nessler's reagent and Kjldahl nitrogen – N_{Kj} (ammonia nitrogen + organic nitrogen) by distillation after mineralization in sulfuric acid. The following concentrations were calculated for the analyzed water samples: mineral nitrogen (N_{min}) from the formula [$N_{min} = N\text{-}NH_4 + N\text{-}NO_3 + N\text{-}NO_2$] and total nitrogen ($N_{og}$) – [$N_{og} = N\text{-}NH_4 + N\text{-}NO_3 + N\text{-}NO_2 + N_{org}$}. Based on the measured flows, first a monthly outflow of water was calculated, and then loads of the determined forms of nitrogen. The results were processed statistically using *Statistica 8PL* software, generating the following values: linear (Pearson's) correlation coefficients (*r* Pearson's), normal distribution of data using Shapiro-Wilk test, a $p \leq 0.05$ and statistically homogenous groups using Kruskal-Wallis test.

RESULTS AND DISCUSSION

In the hydrological years 1994-2007, the total annual precipitation in the environs of Olsztyn ranged from 408 mm (1996) to 827 mm (2007) – Figure 1. Compared to the multi-year period 1951-2000 (average 616 mm), half of the analyzed 14-year period (7 years) had moderate/normal (±10%) rainfall; the other years could be classified as: one very dry year (1996), 3 dry years (1998, 2003 and 2005) and 3 humid years (1995, 2004 and 2007).

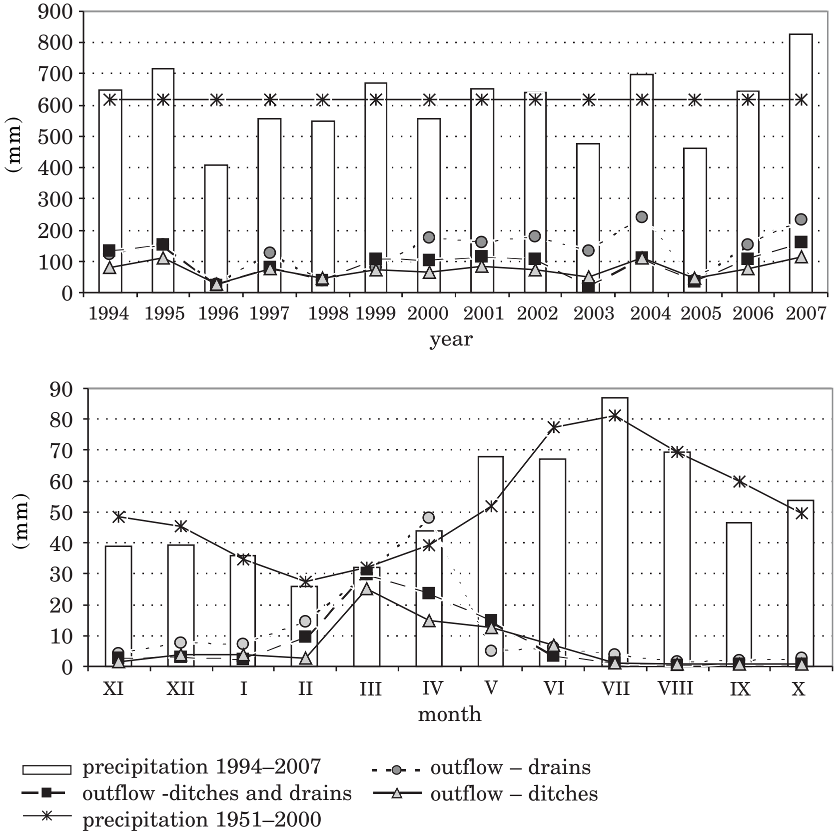

Fig. 1. Dynamics of the annual and monthly water discharge through draining systems from agricultural areas against the background of atmospheric precipitation in 1994-2007 (mm)

The monthly total rainfall was characterized by large variability over time (Figure 1). On average, during the 14-year period, the driest month was February (average precipitation 26 mm) and the wettest was July (86 mm). With respect to the multi-year period covered by this study, demonstrably less rainfall occurred in November (on average, by 9 mm less), December (by 6 mm), June (by 10 mm) and September (by 13 mm), and more rainfall appeared in April (on average by 5 mm more), May (by 16 mm), July (by 6 mm) and October (by 4 mm). In respect of the total precipitation, the analyzed period was dominated by normal (40%) and dry months (18%). Compared to an analogous multi-year period (1951-2000), in the winter half-years of the analyzed period the precipitation was lower by 11 mm (Table 1). The lowest precipitation in this half-year occurred in 1996 (on average, 104 mm) and the highest was recorded in 1994 (on average 335 mm). In the summer half-year, the atmospheric precipitation was on average 3 mm

Table 1

Discharge of water through the analysed draining systems in the winter and summer hydrological half-years versus the meteorological conditions

Year	Average temperature (°C)			Precipitation (mm)		Outflow of water (mm)					
						drains		drains and ditches		ditches	
	XI-X	XI-IV	V-X	XI-IV	V-X	XI-IV	V-X	XI-IV	V-X	XI-IV	V-X
1994	8.6	2.1	15.1	335	314	113	10	126	8	53	28
1995	8.2	0.9	15.5	274	443	115	38	137	15	77	35
1996	5.8	-1.9	13.4	104	305	21	6	10	14	12	16
1997	7.3	0.6	13.9	136	420	102	22	69	12	43	35
1998	7.3	1.0	13.5	197	353	31	9	30	9	22	25
1999	8.2	1.8	14.5	269	403	45	35	89	18	49	24
2000	8.6	3.2	14.0	208	350	144	32	85	17	59	6
2001	7.4	0.5	14.4	202	451	128	32	93	20	69	15
2002	8.4	1.6	15.1	224	417	158	23	80	26	56	18
2003	7.5	0.9	14.1	119	359	127	5	13	7	27	23
2004	7.3	1.1	13.6	255	441	213	26	59	52	38	72
2005	8.3	1.5	15.2	196	264	26	11	25	10	40	5
2006	7.9	0.4	15.4	193	452	124	29	43	63	68	9
2007	8.5	2.5	14.6	316	511	215	18	131	29	110	6
Average	7.8	1.2	14.4	216	392	112	21	71	21	51	23
1951-2000	7.1	2.1	12.1	227	389						

higher than in a comparable half-year from the 1951-2000 multi-year period. The rainfall was characterized by a very large amplitude, from 264 mm in 2005 to 511 mm in 2007.

In general, the examined multi-year period was warmer (by 0.7°C) than the 1051-2000 period, although it had colder winter half-years (on average by 0.9°C), but warmer (on average by 2.3°C) summer half-years (Table 1).

The amount and distribution of atmospheric precipitation in the particular years and time periods analyzed had a considerable influence on the volume of water carried away through the draining systems, although the effect was found to be significant only for the winter half-year (Table 1, Figure 2). In the 14-year period (1994-2007), the annual water outflow ranged from 20 mm (mixed system, 2003, dry year) to 239 mm (drains, 2004, wet year). Regarding the volume of water carried away from the basin, the drainage systems should be ordered as follows: drains (on average 133 mm) > ditches and drains (on average 92 mm) > ditches (on average 74 mm).

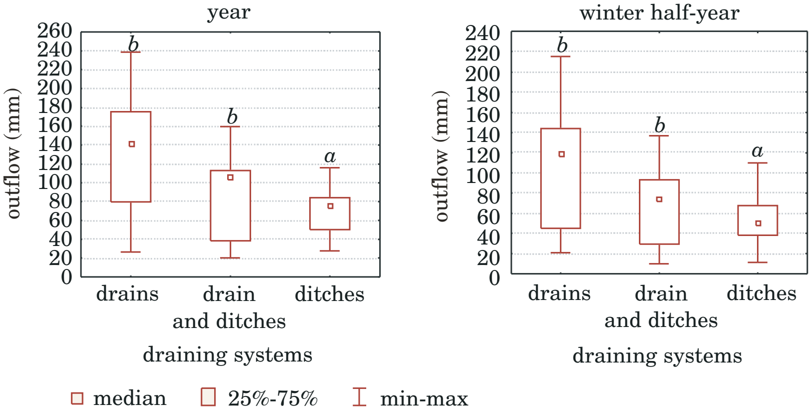

Fig. 2. The influence of the draining systems on the volume of discharged water

The smallest outflow of water, less than 50 mm, appeared during dry years, when a very small portion of the rainfall (4-10%) was carried away with the draining systems. The outflow of water was particularly strongly reduced in the summer half-year despite relatively high precipitation occurring at that time. This suggests that most of the rainfall underwent evapotranspiration. Most of the precipitation (up to 34%), in turn, was carried away from the basins during the wet years. A clear tendency appeared for a larger outflow in the basins drained with the drainage system (21-34%0 than with the mixed system (16-21%) or with ditches alone (14-16%).

Our study has demonstrated that the water outflow through draining systems grows considerably in winter, reaching the peak value in spring (Figure 3).

It has also been found that more intensive water draining through the drainage system compared to the ditches or the mixed system can result in a rapid increase in the water outflow, so that the amount of water discharged can exceed the current rainfall.

The discharge of water through the drainage system was higher than the one recorded for the mixed network or through the ditches. However, the statistical analysis proved that it was significantly higher only in summer versus the system of drains and ditches and in autumn and winter compared to the system of ditches (Figure 4).

In the analyzed multi-year period, this tendency featured particularly strongly in the spring in 2003, 2004 and 2007, when the amount of water discharged through the drainage system was higher than the current rainfall. This was caused by a larger – compared to the basins drained with the mixed system or with the ditches – share of more compact soils, which were almost totally (98%) used as arable lands. With the higher retention of these soils, more water was accumulated in the soil profile during autumn and winter and more was discharged in spring. This tendency con-

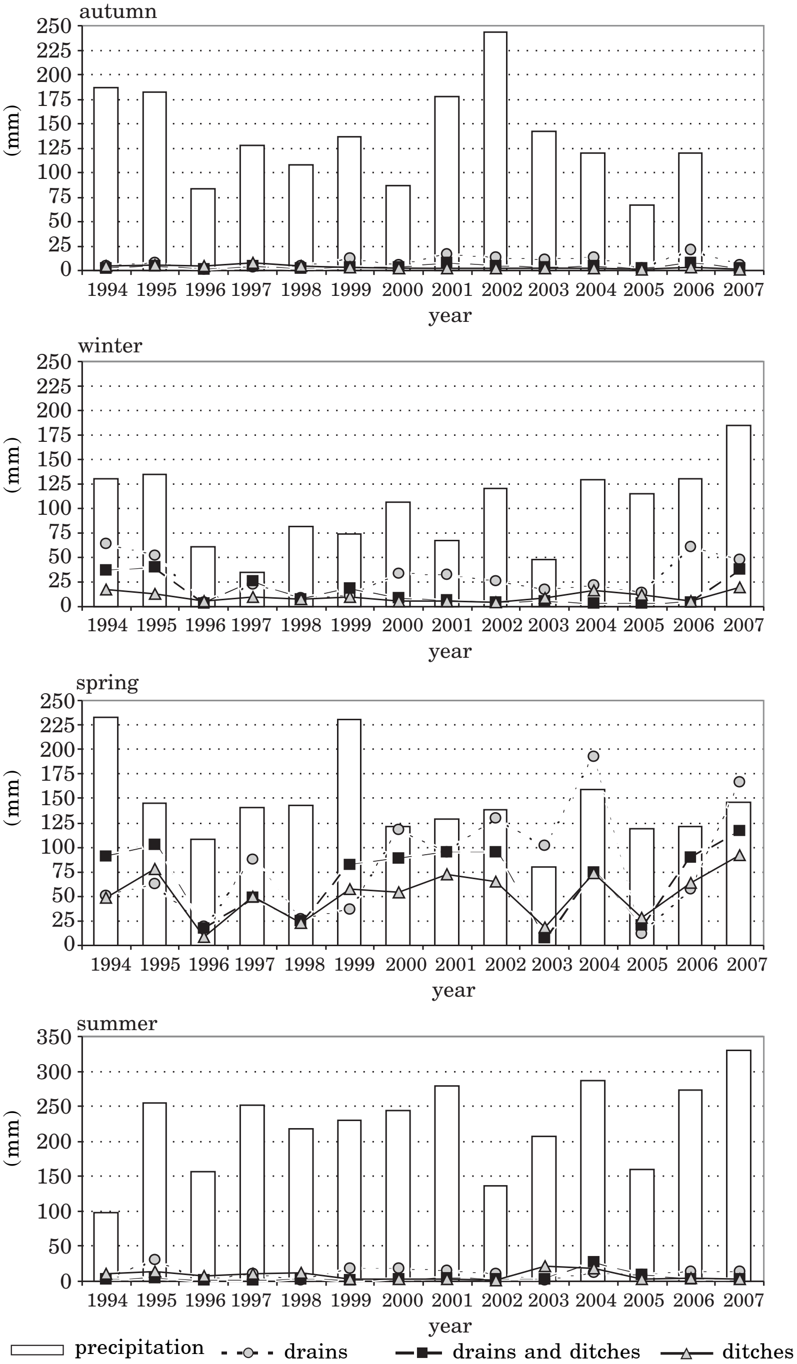

Fig. 3. Dynamics of the seasonal water discharge from agricultural areas though draining systems versus against the background of atmospheric precipitation in 1994-2007 (mm)

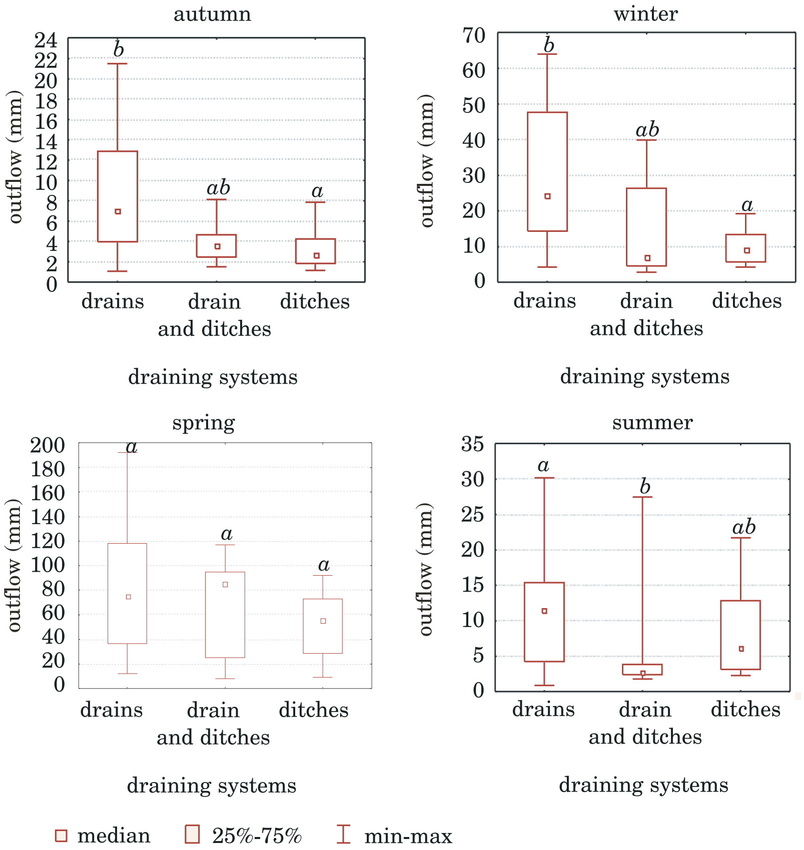

Fig. 4. The influence of a draining system on the seasonal dynamics of water discharge from an agricultural basin

firms how important is the role of forests and thickets present in the other two basins (34 and 30%) in regulating and maintaining an equilibrium of the annual water outflow. It was only in the autumn and summer that the examined draining systems discharged similar amounts of water and demonstrated a similar response to the volume and distribution of atmospheric precipitation. This finding supports the claim that the relationship between water discharge and rainfall is a very complicated one and depends on a number of factors, which condition the intensity of water migration in the environment (LIPIŃSKI 2002).

The content and outflow of nitrogen with the systems which drained the agricultural basins varied over a wide range and depended on both the type and intensity of land use, type of a draining system and meteorological conditions, which influenced the amount and intensity of translocation of water, and that also meant translocation of water-dissolved elements within the enviornment. Depending on such factors present in the three agriculturally used catchment basins, which differed in the extent of man-made stress as well as the type of a water draining system, the amount of total nitrogen varied from 0.16 to 16.69 mg dm^{-3}, and its annual outflow was 0.36 to 13.13 kg ha^{-1} (Table 2).

Most of the nitrogen in water was mineral nitrogen (up to 97%), of which the dominant form was N-NO$_3$. This confirms the assumption that one of the major factors affecting the intentsity of eutrophication and quality of water is the leaching of N-NO$_3$ from soils (Koc et al. 2009).

The highest variations in the concentration of nitrogen (0.43-16.69 mg dm^{-3}) as well as higher levels of nitrogen (on average 4.68 mg dm^{-3}) were found in water drained through a draining system which consisted of drains and ditches. This was associated with the fact that this catchment basin

Table 2

Extreme values and mean concentrations and loads of nitrogen in water discharged from agricultural basins

Draining systems	Form of nitrogen	Min.	Max.	Average	Median	V (%)
Concentration (mg·dm^{-3})						
Drains	N-NO$_3$	0.08	9.67	2.72	2.67	41
	mineral N	0.14	10.22	3.21	2.95	34
	total N	0.16	10.53	3.56	3.24	33
Drains and ditches	N-NO$_3$	0.16	11.97	3.46	3.57	23
	mineral N	0.38	15.03	4.11	3.99	26
	total N	0.43	16.69	4.68	4.60	25
Ditches	N-NO$_3$	0.02	0.85	0.53	0.46	38
	mineral N	0.60	1.60	0.93	0.91	29
	total N	0.71	1.90	1.09	1.08	30
Load (kg·ha^{-1})						
Drains	N-NO$_3$	1.13	11.66	5.02	4.28	60
	mineral N	1.44	12.04	5.45	4.81	59
	total N	1.60	13.13	5.98	5.28	58
Drains and ditches	N-NO$_3$	0.78	7.35	3.27	2.70	62
	mineral N	0.83	8.89	3.70	3.12	61
	total N	0.94	9.81	3.99	3.55	60
Ditches	N-NO$_3$	0.08	1.51	0.51	0.31	87
	mineral N	0.31	1.78	0.75	0.60	60
	total N	0.36	2.08	0.87	0.70	60

V – coefficient of variation

comprised more light soils compared to the area drained only with drains. However, a more intensive water dewatering of drained soils, irrespective of a lower nitrogen concentration (on average 3.56 mg dm^{-3}), resulted in a larger (by 50%) load of discharged total nitrogen. Lower concentrations and the smallest outflow of total nitrogen as well as its main fractions (especially N-NO$_3$) occurred in water carried away through ditches. This was connected with the less intensive use of the basin and a relatively small, versus the drains, outflow of water.

The dynamics of the concentrations of mineral nitrogen compounds in water carried away by the analyzed systems varied with time and depended not only on the amount and distribution of rainfall during a year and in

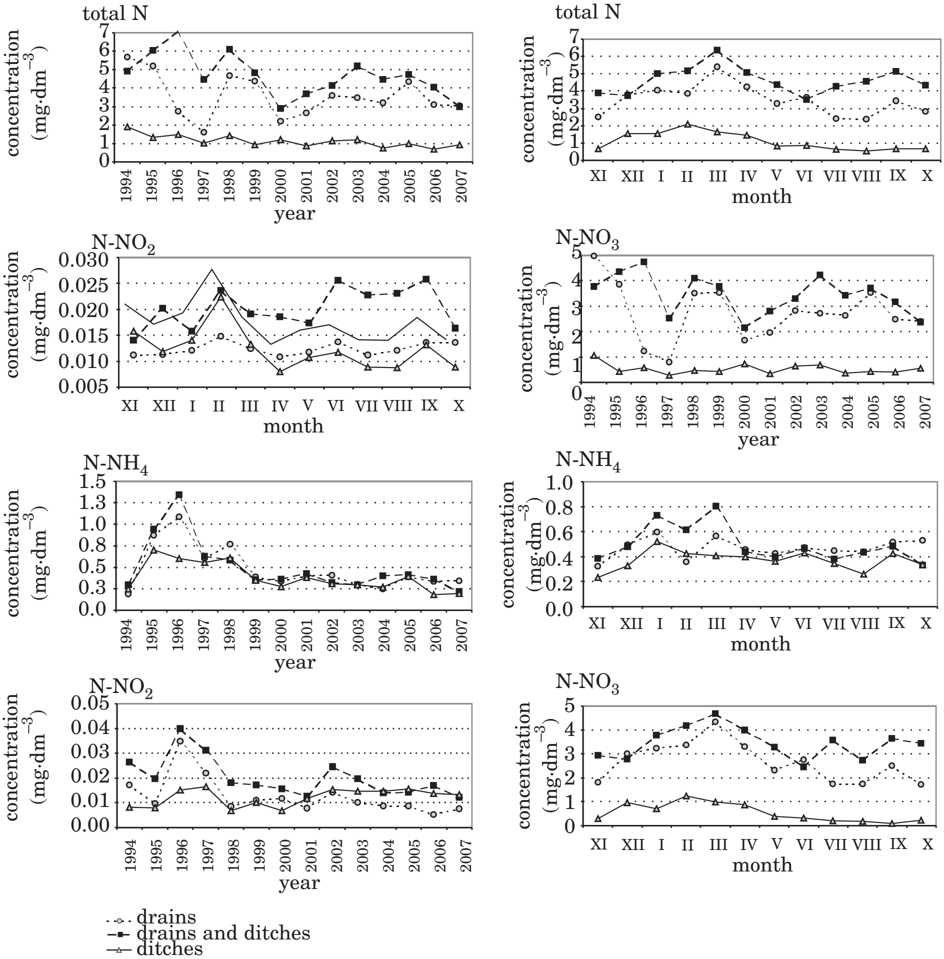

Fig. 5. Dynamics of the annual and monthly nitrogen concentrations in water discharged from agricultural areas in 1994-2007 (mg dm^{-3})

the multi-year period, but also on the land use in a given catchment basin and its draining system (Figure 5).

The smallest variability in the concentration, especially for N-NO_3, which made up the dominant part of mineral nitrogen, appeared in water drained through ditches with a supplementary network of drains. In the water discharged through ditches and drains, N-NO_3 made up 88% of mineral nitrogen and 81% of total nitrogen, and in the water discharged through drains, it constituted 68% of mineral N and 59% of total N. This finding suggests that the leaching of nitrogen from soil takes place after mineralization of organic nitrogen and oxidation to N-NO_3. This is a consequence of the fact that draining facilitates oxygen supply to a soil profile, which induces a series of soil processes, for example it raises the intentity of mineralization of organic substances (LIPIŃSKI 2002). Thus, improved oxigenation of drained soils under agricultural use may be a cause of an increased threat of water becoming polluted with nitrogen compounds, especially during a more intensive water draining of soils than recorded in draining systems comprising or consisting of cditches. Therefore, for the sake of environment conservation, the existing or planned drainig systems should be equipped with facilities which will regulate water discharge (LIPIŃSKI 2002).

The present study has not demonstrated any significant influence of the type of a draining system on the concentration of N-NH_4 in the analyzed water samples. In the water discharged through drains, compared to the samples taken from ditches, an elevated concentration of N-NO_3 appeared alongside levels of mineral N and total N (associated with this parameter) as well as N-NO_2, compared with the mixed system (drains and ditches – Figure 6).

A relatively high concentration of N-NH_4 was found in 1996, which was the driest year during the 14-year period. The highest levels of this nitrogen form, unseen in the other years, were determined then in water discharged through the drainage system (on average 1.09 mg dm^{-3}) and from the mixed system (on average 1.34 mg dm^{-3}). Over 20% higher concentration of N-NH^4 in water discharged through the mixed system may have been caused by a more intensive decomposition of organic matter at the bottom of ditches, where periodically oxygen deficiency can occur and the decomposition of organic substance slows down and is limited to ammonification. Similar relationships have been observed for N-NO_2. Small concentrations of N-NO_2, relative to N-NO_3, which occurred especially in water carried away through the drainage system, confirm good oxygenation of the soils drained with this system. The highest N-NH_4 level in the water drained through ditches (on average 0.70 mg dm^{-3}) was found in 1995, a wet year, characterized by a relatively large discharge of water, including nutrients, during the early vegetative growth period.

The dominant share of N-NO_3 in total nitrogen in water discharged through draining systems confirms that its variability runs a similar course to the variability of total nitrogen in all seasons of the year (Figure 7). The

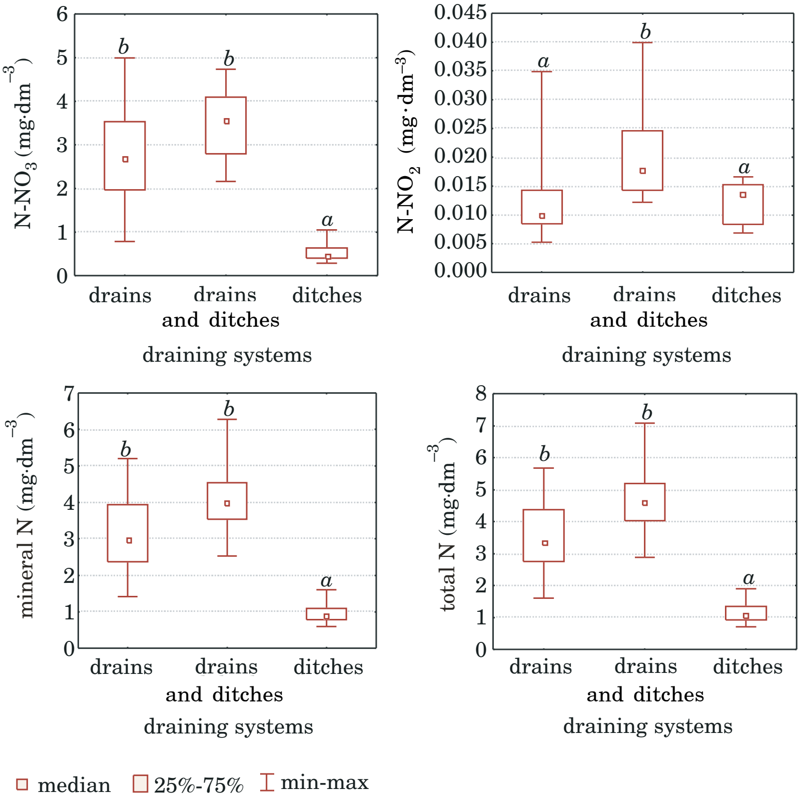

Fig. 6. Dynamics of concentrations of nitrogen compounds in water discharged from agricultural areas

highest concentrations of total nitrogen and N-NO_3 in water discharged through the drainage system or ditches with drains (the mixed system) appeared in spring, while in the ditches alone, the highest levels of these nitrogen forms were recorded in winter.

With respect of ammonia and nitrate (III) nitrogen, variations in the levels of these nitrogen forms followed a slightly different course. Nonetheless, because of their small concentrations, as compared to the levels of N-NO_3, they did not produce any significant effect on the variation of total nitrogen concentrations in water.

The correlation dependences between meteorological conditions (temperature and precipitation) and volume of discharged water demonstrated that the effect they produced on the concentration and load of discharged nitrogen depended on the type of a draining system (Table 3). Negative correlations between temperature and the concentration and load of nitrogen suggest that during the plant growing season (rising air temperature) the amount of nitrogen discharged from drained agricultural basins decreases,

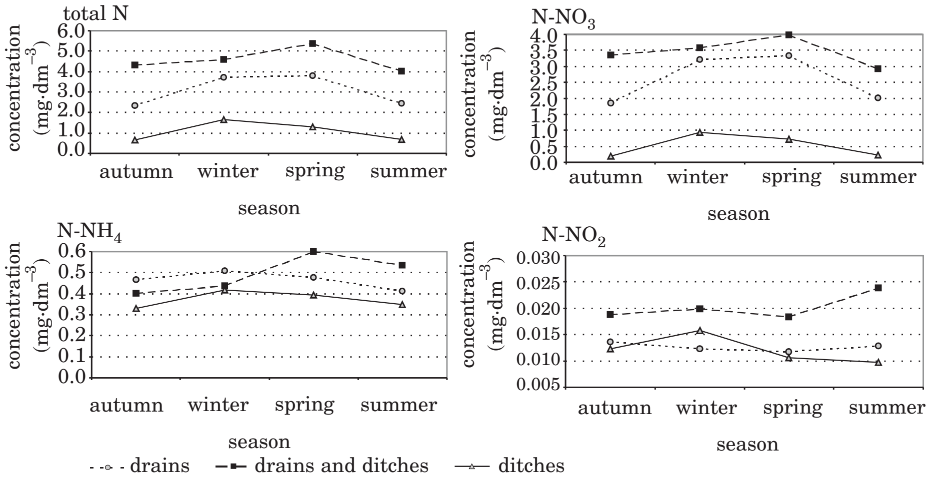

Fig. 7. Seasonal dynamics of nitrogen concentrations in water discharged from agricultural areas in 1994-2007 (mg dm^{-3})

which is a result of nitrogen bioaccumulation. This dependence became particularly evident when soils were drained with the drainage system or with the ditches.

Similar tendecies were also determined for atmospheric precipitation, most of which fell during summer (Table 3). Also, a tendency was demonstrated for the lowering of N-NH$_4$ and N-NO$_3$ in water and increased discharge of these forms of nitrogen through the analyzed draining systems.

A positive correlation has been demonstrated between the concentration of N-NO$_3$, which represented the major portion of nitrogen contained in water, and the volume of discharged water. This led to a significant increase in the load of N-NO$_3$ and, consequently, mineral and total nitrogen. Such findings are confirmed by the results reported by Pulikowski et al. (2008), who demonstrated that a larger load of nitrogen is discharged from drained basins mostly under agricultural land use, which was mainly attributed to a high concentration of nitrates in the drainage effluent.

The volume of nitrogen load discharged with the draining systems from the agricultural basins during the whole analyzed period as well as in individual years was mainly conditioned by the amount of discharged water, its flow rate and concentration of N-NO$_3$ (Figure 8). The most important was the water discharge in winter half-year (from October to April, Figure 2), i.e. during the time when water supplies in a basin are replenished (from November to February), as implied by a relatively small water outflow versus precipitation. The largest water outflow occurred during its annual culmination, mainly in March and April (Figure 1).

Thus, particularly large loads of N-NO$_3$, and consequently total nitrogen, appear in March and April in the basin drained with the drainage sys-

Table 3

Correlations between temperature. precipitation and water outflow versus concentration and load of nitrogen in water from draining systems (at $p < 0.05$; $N = 150$)

Variable	Temperature			Precipitation			Load		
	drains	drains and ditches	ditches	drains	drains and ditches	ditches	drains	drains and ditches	ditches
Concentration									
$N\text{-}NO_3$	-0.18*	–	-0.40*	–	-0.18*	-0.22*	–	–	0.17*
$N\text{-}NH_4$	–	-0.26*	–	–	-0.16*	–	–	–	–
$N\text{-}NO_2$	–	–	-0.20*	–	–	–	–	–	–
Load									
$N\text{-}NO_3$	-0.21*	–	–	–	–	–	0.70*	0.77*	0.57*
$N\text{-}NH_4$	-0.18*	–	–	–	–	–	0.59*	0.67*	0.74*
$N\text{-}NO_2$	-0.18*	–	–	–	–	–	0.74*	0.76*	0.30*
Mineral N	-0.22*	–	-0.17*	–	–	–	0.72*	0.81*	0.66*
Total N	–	–	-0.18*	–	–	–	0.72*	0.82*	0.66*

*essential correlation; (–) lack of essential dependences

tem, and in March in the basins drained with the mixed system or with the ditches (Figure 8).

Much larger water discharge and water levels of nitrogen compounds meant that a significantly larger nitrogen load (except $N\text{-}NO_2$) was discharged by the drainage and mixed systems than through the ditches (Figure 9).

The present study has also demonstrated that much more $N\text{-}NH_4$ is discharged through draining ditches in years characterized by a more even annual water discharge. Possible sources of $N\text{-}NH_4$ include atmospheric precipitation polluted with ammonia (LIPIŃSKI 2002), which directly feed surface waters, or its supply from surface effluents, especially in spring. In the summer season, in turn, much $N\text{-}NH_4$ reaches a basin after intensive rains. An additional source of $N\text{-}NH_4$ in water drained through ditches could be the products of decomposition of organic matter derived from dying plants and bottom sediments. This can be associated with the release of this form of nitrogen from plants and bottom sediments.

The load of nitrogen discharged through the draining systems (Figure 8) was closely connected with the seasonality of water flow (Figure 1, Table 1). Consequently, the highest loads of total and of the determined mineral nitrogen forms were the highest in the spring and the lowest in the autumn and winter. In respect of total nitrogen in spring, the largest loads were dsicharged through drains (4.0 kg ha^{-1}), followed by the network of ditches

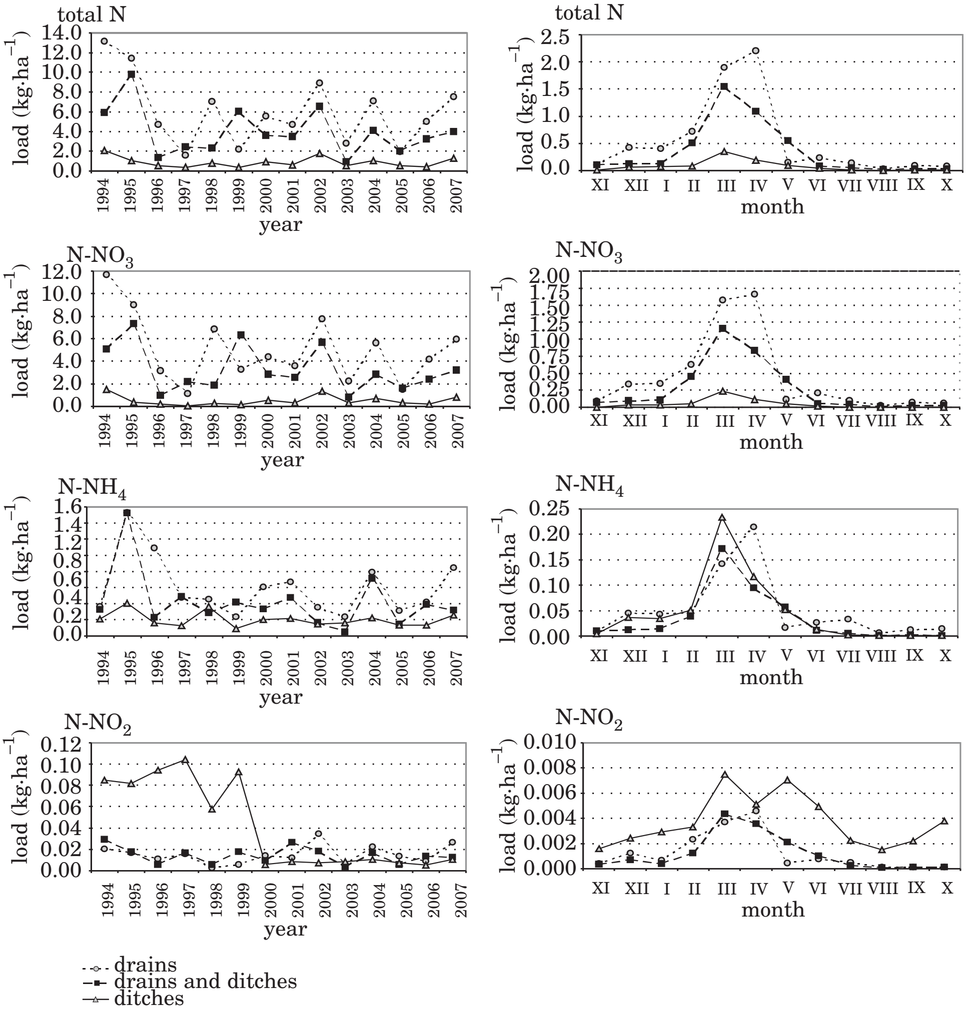

Fig. 8. Dynamics of annual and monthly outflow of nitrogen through draining systems in agricultural areas in 1994-2007

and drains (3.0 kg ha^{-1}) and the smallest ones – through ditches (0.6 kg ha^{-1}) – Figure 10.

Similar tendencies were observed for the outflow of loads of N-NO_3 (3.23 kg ha^{-1} through drains > 2.40 kg ha^{-1} through ditches and drains > 0.38 kg ha^{-1} through ditches) and N-NH_4 (0.37 kg ha^{-1} through drains > 0.32 kg ha^{-1} ditches and drains > 0.12 kg ha^{-1} through ditches). As for N-NO_2, an over two-fold larger load of this form of nitrogen was discharged through ditches than through drains or ditches and drains. The smallest discharge of N-NO_2 occurring in the spring in the basin drained with the

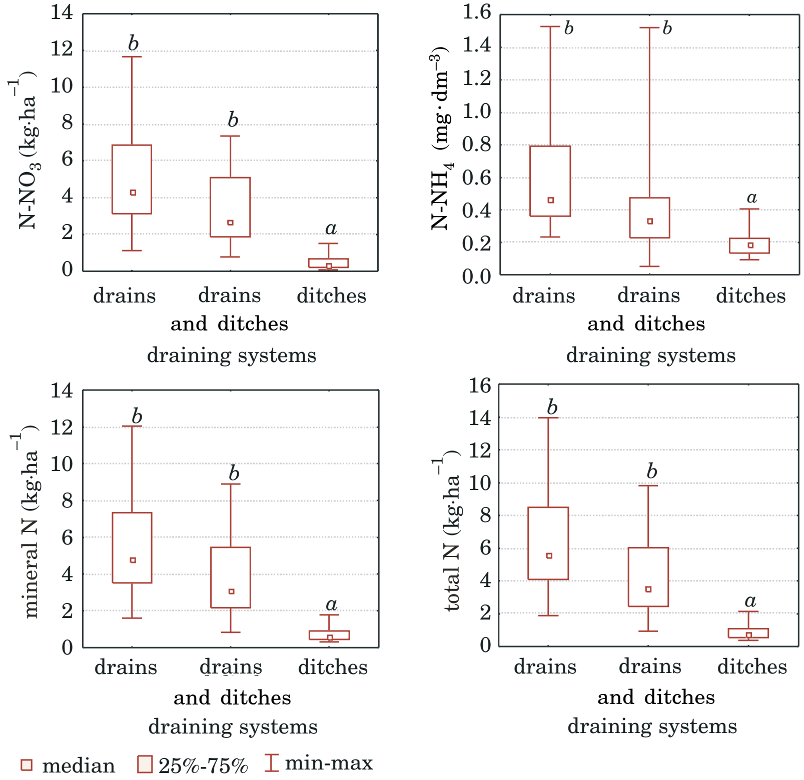

Fig. 9. Dynamics of loads of nitrogen compounds in water discharged from agricultural areas

drainage system should be associated with a much more rapid water outflow and better oxygenation of drained soils as compared to soils dewatered with ditches (Koc et al. 2009). This was also confirmed by the seasonal dynamics of the load of N-NO_3.

The statistical analysis verified the predominant influence of meteorological conditions, mainly atmospheric precipitation and air temperature (significant seasonal variability) on the volume of the outflow of forms of nitrogen through the draining systems dewatering agricultural basins (Figures 11-13). Significantly higher nitrogen loads, than in autumn, were discharged in spring. However, the seasonal dynamics of the outflow of particular forms of nitrogen was found to differ slightly between the three tested draining systems. In the case of the drainage systemn and the network of ditches, significantly larger loads of nitrogen compounds were discharged in spring.

The results of our tests have demonstrated that the discharge of nitrogen compounds from agricultural areas is largely dependent on the meteor-

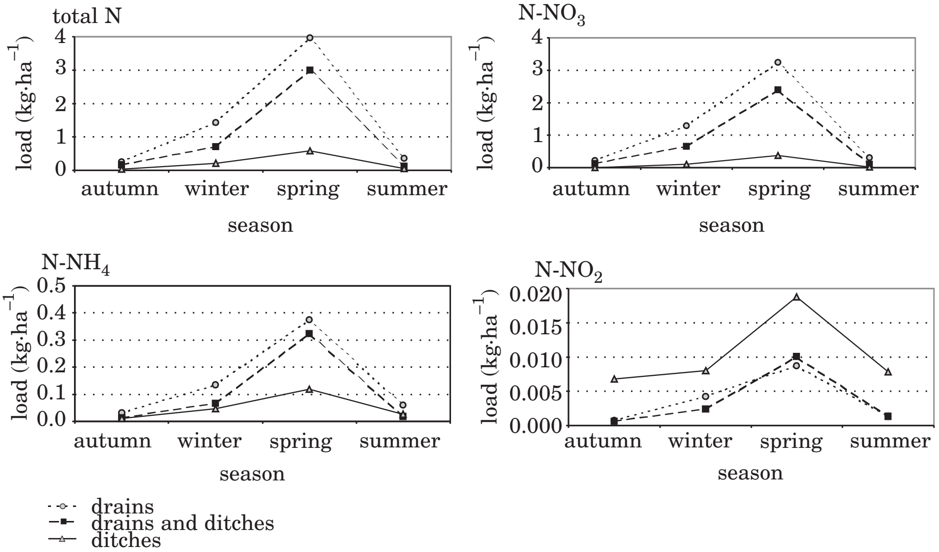

Fig. 10. Seasonal dynamics of nitrogen outflow through draining systems in 1994-2007 (kg ha^{-1})

ological conditions, draining system ,type of soils and type of lanbd use. At the same time, they seem to confirm the claim that the influence of agriculture on chemism of groundwater and surface water can be both positive and negative, and the amount of pollutants originating from area sources can be considerably reduced by proper water management (Heathwaite et al. 1998, Herzog et al. 2008, Oenema 2005). This can be achieved by reducing the intensity of fertilization and limiting surface area of cropped land (Kopacz et al. 2007) because, particularly in spring, during the intensive growth and development of plants, bioaccumulation of nitrates exceeds the sum of nitrates reaching a bsinc from the atmosphere and originating from mineralization and nitrofication of organic compounds of nitrogen in soil (Kowalik, Kulbik 2002).

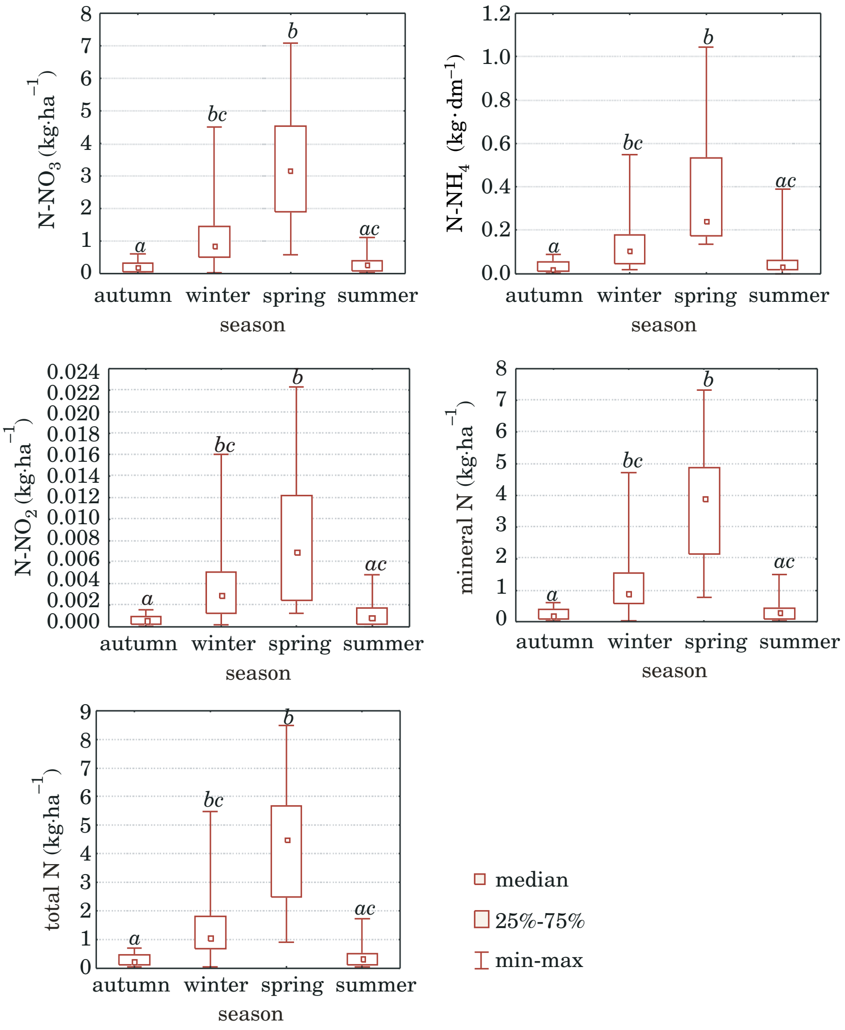

Fig. 11. Seasonal dynamics of loads of nitrogen in water discharged through a drainage system

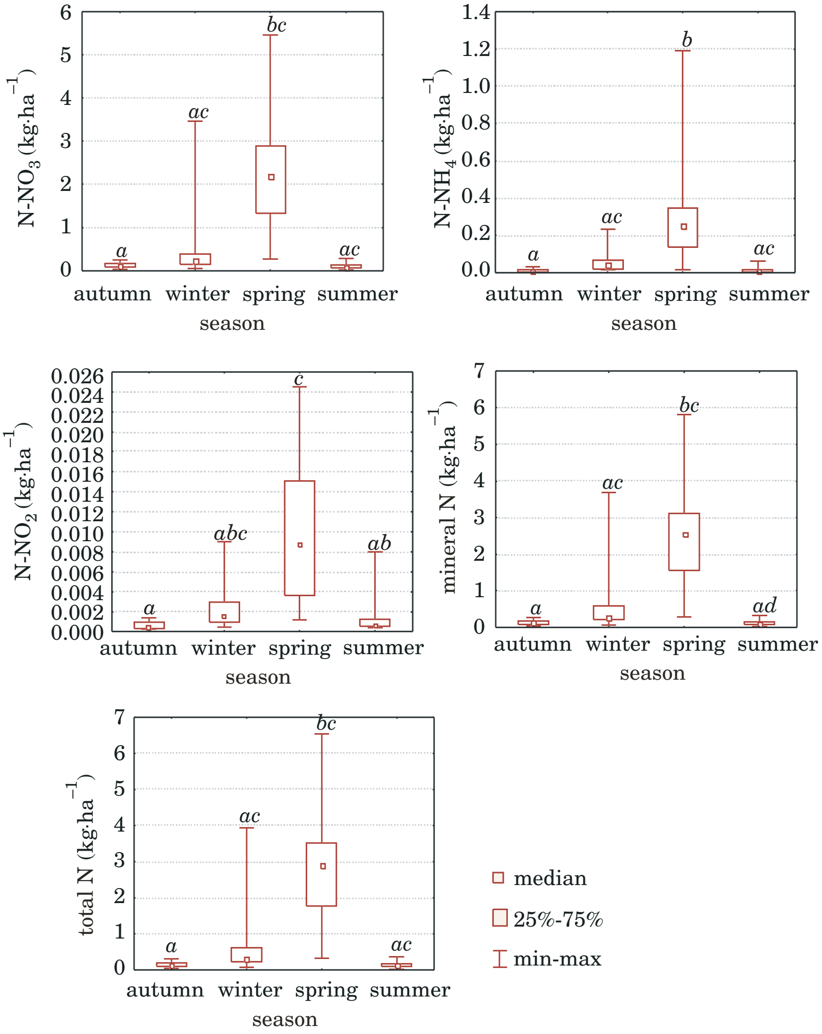

Fig. 12. Seasonal dynamics of loads of nitrogen in water discharged through a network of ditches and drains

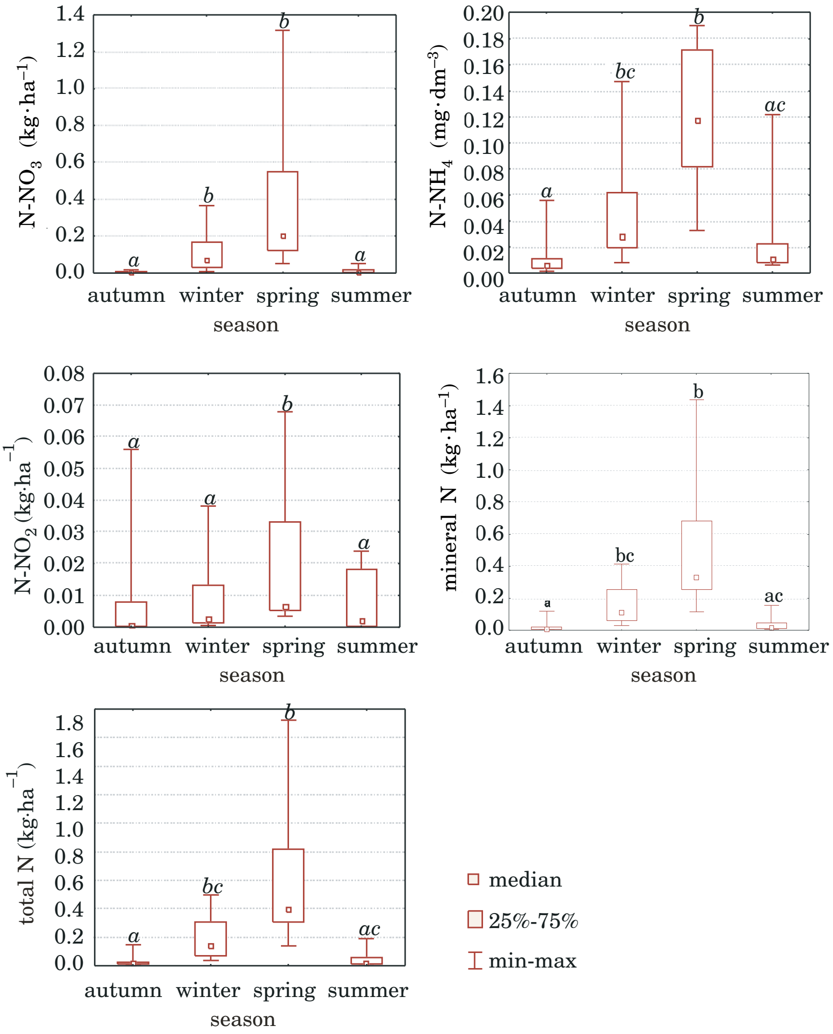

Fig. 13. Seasonal dynamics of loads of nitrogen in water discharged through draining ditches

CONCLUSIONS

1. The dynamics of water discharge from an agricultural basin depended on the amount and distribution of atmospheric precipitation as well as the type of a dewatering system, adjusted to the type of soils and type of land use. The volume of discharged water was mainly conditioned by atmospher-

ic precipitation in the winter half-year. Intensive rainfall occurring in summer would only slightly increase the outflow of water from the baic drained with a drainage system.

2. The dynamics of concentrations and loads of nitrogen in water discharged via draining systems varied with time and depended not only on the amount and distribution of precipitation over a year and in the multi-year period, but also on the type of land use in a given basin and a draining system. The largest variation in the concentrations of $N\text{-}NO_3$ (V=41%) and total nitrogen (V=33%) appeared in water drained with the drainage system, and the largest variation in the loads ($N\text{-}NO_3$ – V=87%, and total N – V=60%) was calculated for water carried away through draining ditches.

3. The largest concentration of total nitrogen (16.69 mg dm^{-3}) occurred in water carried away through the mixed system, and the lowest one – in water discharged through ditches. Within total nitrogen, mineral nitrogen reached from 84% in water flowing through ditches to 97% in water from drains. Within mineral nitrogen, the percentage of nitrates (V) ranged from 53% in water flowing through ditches to 95% in water from drains.

4. The annual outflow of total nitrogen per 1 ha was the highest from the agricultural basin dewatered by the drainage system, where it reached 13.13 kg. When it was dewatered through drains and ditches, the annual outflow of total nitrogen was 9.81 kg, falling down to 2.08 kg in the case of the basin drained through ditcches only. The percentage of nitrates (V) in the mineral nitrogen load was from 97% in drained water to 85% in water flowing through ditches and 83% in water discharged through drains and ditches.

5. The total nitrogen load carried away through the draining systems from agricultural basins was closely related to the amount of discharged water and water concentration of mineral nitrrogen compounds, especially $N\text{-}NO_3$. Due to the seasonal discharge of water, with the climax occurring in March-April, the largest loss of nitrogen from drained areas appears in spring.

6. Nitrates (V) made up the largest portion of total nitrogen polluting drained water, i.e. 92% of mineral N and 84% of total N in water flowing through drains, 88% of mineral N and 81% of total N in water drained trough ditches and drains and just 68% of mineral N and 59% of total N in water carried away through ditches.

7. The greatest threat of polluting surface water with nitrogen compound, due to the volume of its load discharged from sgricultural basins, occurred in spring, and the smallest one – in autumn and in summer.

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ACTIVITY OF β-GLUCOSIDASE, ARYLSULFATASE AND PHOSPHATASES IN SOIL CONTAMINATED WITH COPPER

Jadwiga Wyszkowska, Mirosław Kucharski, Jan Kucharski

Chair of Microbiology
University of Warmia and Mazury in Olsztyn

Abstract

A pot experiment was carried out to determine the effect of soil (loamy sand and sandy loam) contamination with copper doses of 0, 150, 450 mg $Cu \cdot kg^{-1}$ d.m. soil on the activity of β-glucosidase (EC 3.2.1.21), acid phosphatase (EC 3.1.3.2), alkaline phosphatase (EC 3.1.3.1) and arylsulfatase (EC 3.1.6.1) in soil. The resistance of these enzymes to copper pollution was also estimated. Soil samples were contaminated with copper chloride. The experiment was carried out in five replications, in two series. The first series was performed on uncropped soil and the second one – on cropped soil. The experimental plants were oat, spring rape and yellow lupine. The activity of soil enzymes was determined in the analyzed samples on the 25th and the 50th day of the experiment.

The results of the experiment showed that copper contamination in doses of 150 mg to 450 $mg \cdot kg^{-1}$ soil significantly inhibits soil's biochemical activity. The sensitivity of the tested enzymes to copper was determined in the following order: alkaline phosphatase > arylsulfatase > acid phosphatase > β-glucosidase.

The resistance of the above enzymes to copper depended on the cultivated plant species, soil type and the type of soil use and management. In samples of sandy loam, copper induced the smallest change in the activity of acid phosphatase and alkaline phosphatase, and in loamy sand – in the activity of arylsulfatase and acid phosphatase. In uncropped soil, copper was the least effective in changing the activity of arylsulfatase and acid phosphatase. All of the tested enzymes were less resistant to copper contamination in cropped than in uncropped soil. In soil planted with oat, β-glucosidase was the most resistant and arylsulfatase was the least resistant enzyme to copper contamination. In samples sown with spring rape, the analogous enzymes were arylsulfatase and alkaline phosphatase. In yellow lupine treatments, alkaline phosphatase was the most and β-glucosidase was the least resistant enzyme.

Key words: copper, β-glucosidase, phosphatase, arylsulfatase, resistance rate, soil contamination with copper.

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prof. dr hab. Jadwiga Wyszkowska, Chair of Microbiology, University of Warmia and Mazury, pl. Łódzki 3, 10-727 Olsztyn, Poland, e-mail: jadwiga.wyszkowska@uwm.edu.pl

AKTYWNOŚĆ β-GLUKOZYDAZY, ARYLOSULFATAZY I FOSFATAZ W GLEBACH ZANIECZYSZCZONYCH MIEDZIĄ

Abstrakt

W doświadczeniu wazonowym badano wpływ zanieczyszczenia gleby (piasku gliniastego oraz gliny piaszczystej) miedzią w dawkach: 0, 150, 450 mg $Cu \cdot kg^{-1}$ s.m. gleby na aktywność: β-glukozydazy (EC 3.2.1.21), fosfatazy kwaśnej (EC 3.1.3.2), fosfatazy alkalicznej (EC 3.1.3.1) i arylosulfatazy (EC 3.1.6.1) w glebie. Określono także odporność tych enzymów na zanieczyszczenie miedzią. Glebę zanieczyszczano chlorkiem miedzi. Badania prowadzono w 5 powtórzeniach, w dwóch seriach. W pierwszej serii doświadczenia gleba była nieobsiana roślinami, w drugiej – obsiana. Roślinami doświadczalnymi były: owies, rzepak jary i łubin żółty. W 25. i 50. dniu trwania eksperymentu oznaczono w próbkach glebowych aktywność enzymów glebowych.

Stwierdzono, że zanieczyszczenie gleby miedzią w zakresie od 150 mg do 450 $mg \cdot kg^{-1}$ gleby istotnie hamuje jej aktywność biochemiczną. Testowane enzymy, pod względem wrażliwości na miedź, można uszeregować następująco: fosfataza alkaliczna > arylosulfataza > fosfataza kwaśna > β-glukozydaza.

Odporność enzymów na działanie miedzi zależała od gatunku uprawianej rośliny, rodzaju gleby i sposobu jej użytkowania. W glinie piaszczystej miedź wywołała najmniejsze zmiany w aktywności fosfatazy kwaśnej i fosfatazy alkalicznej, natomiast w piasku gliniastym – β-glukozydazy oraz arylosulfatazy. W glebie nieobsianej roślinami miedź wywoływała najmniejsze zakłócenia w aktywności arylosulfatazy i fosfatazy kwaśnej. W glebie obsianej roślinami wszystkie testowane enzymy były mniej odporne na zanieczyszczenie miedzią niż w glebie obsianej. Najbardziej odpornym enzymem na działanie miedzi pod uprawą owsa była β-glukozydaza, a najmniej – arylosulfataza, pod uprawą rzepaku jarego, odpowiednio – arylosulfataza i fosfataza alkaliczna, natomiast pod uprawą łubinu żółtego najbardziej odporna była fosfataza alkaliczna, a najmniej β-glukozydaza.

Słowa kluczowe: miedź, β-glukozydazy, fosfataza, arylosulfataza, indeks odporności, zanieczyszczenie gleby miedzią.

INTRODUCTION

Copper is a biogenic element, whose small quantities are necessary for proper functions of live organisms, while high copper doses can be toxic. In Poland, soils containing excessive levels of this metal are rare and can be found mostly in southern parts of the country, especially in Silesia and Małopolska. In 2005, a monitoring study was completed, which revealed that the distribution of soil falling into different copper contamination categories had not changed significantly since the previous years, i.e. 0ş degree of contamination was determined in 95.9% soils, I^{o} – in 2.7%, II^{o} – in 0.5%, III^{o} – 0%, IV^{o} – 0.9% and V^{o} – 0% (Terelak et al. 2008). The highest level of copper pollution is noticed in the vicinity of copper plants, where soil is strongly degraded and difficult to reclaim. For this reason, the accumulation of heavy metals, including copper, in surface soil layers is highly dangerous because it disrupts the soil metabolism (De Brouwere et al. 2007, Mertens et al. 2007, Oliveira, Pampulha 2006, Wyszkowska et al. 2005b).

If excessive quantities of heavy metals reach the soil, they have a strongly toxic effect on soil microbes and inhibit the activity of soil enzymes (Renella et al. 2005, Mikanova et al. 2001, Kucharski, Wyszkowska 2004). Soil contamination with heavy metals slows down many biological processes. It affects populations and species diversity of macro- and microorganisms as well as soil's enzymatic activity (Bielińska 2005, Welp 1999, Wyszkowska et al. 2005b, Wyszkowska et al. 2005a, Zheng et al. 1999). The destructive effect of heavy metals on the microbiological and biochemical properties of soil is modified by the soil's granulometric composition, pH, organic content and sorptive capacity (Moreno et al. 2001).

The objective of this study was to determine the effect of copper contamination of soil characterized by different grain size distribution on the activity of b-glucosidase, acid phosphatase, alkaline phosphatase and arylsulfatase, and to estimate the resistance of these enzymes to excessive copper concentrations in soil. The study was carried out as part of research project No N N305 2258 33 supported by the Ministry for Science and Higher Education.

MATERIALS AND METHODS

The experiment was conducted in polyethylene pots (in five replications) in a greenhouse of the University of Warmia and Mazury in Olsztyn, Soil samples collected from the humus horizon were analyzed. In the natural state, they consisted of:

1) typical brown soil developed from loamy sand (pH in 1 mol $KCl \cdot dm^{-3}$ – 6.70; hydrolytic acidity – 7.8 mmol(+) kg^{-1}; total exchangeable cations – 98 mmol(+)$\cdot kg^{-1}$; exchange capacity of adsorption complex – 105.8 mmol(+) kg^{-1}; base saturation – 92.6%; content of: C_{org} – 11.0 $g \cdot kg^{-1}$, K – 180 $mg \cdot kg^{-1}$, Mg – 80 $mg \cdot kg^{-1}$, Ca – 1.43 $g \cdot kg^{-1}$, Na – 28 $mg \cdot kg^{-1}$ and N – 0.97 $g \cdot kg^{-1}$);

2) typical brown soil developed from sandy loam (pH in 1 mol $KCl \cdot dm^{-3}$ – 6.80; hydrolytic acidity – 5.2 mmol(+) kg^{-1}; total exchangeable cations – 80.0 mmol(+)$\cdot kg^{-1}$; exchange capacity of adsorption complex – 85.2 mmol(+) kg^{-1}; base saturation – 93.9 %; content of: C_{org} – 9.9 $g \cdot kg^{-1}$, K – 168 $mg \cdot kg^{-1}$, Mg – 50 $mg \cdot kg^{-1}$, Ca – 2.21 $g \cdot kg^{-1}$, Na – 57 $mg \cdot kg^{-1}$ and N – 1.14 $g \cdot kg^{-1}$);

The grain size composition of the above soils is presented in Table 1. Soil was contaminated with copper in the form of $CuCl_2 \cdot 2H_2O$ in the amount of 0, 150, 450 mg $Cu \cdot kg^{-1}$ d.m. soil. The first dose (150 $mg \cdot kg^{-1}$) was equivalent to the maximum admissible copper dose stated in the Regulation of the Minister for the Environment of 9 September 2002 (*Regulation of the Minister for the Environment,* 2002, Journal of Laws 02.165.1359). Soil samples were passed through through a 1 cm mesh sieve, mixed with mineral fertilizer and, in selected treatments, with copper chloride; afterwards, they were

Table 1

Granulometric composition of soil

Type of soil	Percentage of fractions (d)		
	sand $2.00 \geq d > 0.05$ mm	dust $0.05 \geq d > 0.002$ mm	clay $d \leq 0.002$ mm
Loamy sand	75.56	22.92	1.52
Sandy loam	47.92	48.71	3.37

placed in pots. Macronutrients were added to all pots in the following doses (as pure substance per $mg \cdot kg^{-1}$ soil): N – 100 (yellow lupine was not fertilized with nitrogen), P – 35, K – 100, Mg – 20. Nitrogen was applied in the form of $CO(NH_2)_2$, phosphorus – KH_2PO_4, potassium – KH_2PO_4 + KCl and magnesium – $MgSO_4 \cdot 7H_2O$. Soil samples weighing 3 kg each were placed in pots, and the moisture content of soil was brought to 60% capillary water capacity. The samples were planted with oat cv. Kasztan, spring rape cv. Huzar and yellow lupine cv. Mister. The plants were thinned after emergence, and the following number of plants were left in pots: oat – 12, spring rape – 8 and yellow lupine – 5. A control series of uncropped soil samples was established to support the determination of copper's effect on soil enzymes. The moisture content of soil was maintained at 60% capillary water capacity throughout the entire experiment (50 days).

The activity of the investigated enzymes: β-glucosidase (EC 3.2.1.21), acid phosphatase (EC 3.1.3.2), alkaline phosphatase (EC 3.1.3.1) and arylsulfatase (EC 3.1.6.1), was determined twice during the experiment (on day 25 and 50) in three successive replications. The above enzymes were determined in line with the procedure described by ALEF AND NANNPIERI (1998). Their resistance to soil contamination with copper was estimated by the method proposed by ORWIN and WARDLE (2004).

The results were processed statistically with the use of Duncan's multiple range test. Statistical analysis was performed with the Statistica application (StatSoft, Inc. 2006).

RESULTS AND DISCUSSION

The results of the study indicate that soil contamination with copper affected the soil's biological balance measured by the activity of β-glucosidase, acid phosphatase, alkaline phosphatase and arylsulfatase (Tables 2–9). Disturbances in the soil's homeostasis were dependent on several factors, including the applied metal dose and cultivated plant species (Table 2). In unpolluted soil samples, the highest levels of β-glucosidase activity were noted in treatments sown with oat, alkaline phosphatase – in treatments sown

Table 2

Effect of soil pollution with copper and crop species on activity of soil enzymes

Cu dose (mg kg^{-1} of soil)	Crop species		
	oats	spring oilseed rape	yellow lupine
β-glukosidase, mmol PNP kg^{-1} h^{-1}			
0	0.795 ± 0.127	0.739 ± 0.022	0.691 ± 0.037
150	0.624 ± 0.023	0.597 ± 0.035	0.609 ± 0.015
450	0.673 ± 0.029	0.509 ± 0.030	0.570 ± 0.016
Average	0.697	0.615	0.624
LSD	a – 0.016; b – 0.016; $a \cdot b$ – 0.028		
Acid phosphatase, mmol PNP kg^{-1} h^{-1}			
0	2.072 ± 0.068	2.515 ± 0.061	2.577 ± 0.113
150	1.520 ± 0.088	1.677 ± 0.063	1.913 ± 0.101
450	0.963 ± 0.078	1.496 ± 0.082	1.547 ± 0.089
Average	1.519	1.896	2.012
LSD	a – 0.030; b – 0.030; $a \cdot b$ – 0.051		
Alkaline phosphatase, mmol PNP kg^{-1} h^{-1}			
0	3.684 ± 0.241	4.871 ± 0.175	4.480 ± 0.114
150	3.197 ± 0.073	3.489 ± 0.141	3.410 ± 0.148
450	1.657 ± 0.124	2.229 ± 0.115	2.382 ± 0.115
Average	2.846	3.529	3.424
LSD	a – 0.050; b – 0.050; $a \cdot b$ – 0.087		
Arylosulphatase, mmol PNP kg^{-1} h^{-1}			
0	0.284 ± 0.013	0.398 ± 0.086	0.464 ± 0.029
150	0.186 ± 0.020	0.287 ± 0.074	0.283 ± 0.009
450	0.113 ± 0.015	0.188 ± 0.059	0.156 ± 0.015
Average	0.194	0.291	0.301
LSD	a – 0.044; b – 0.044; $a \cdot b$ – 0.077		

LSD for: a – copper rate, b – crop species

with spring rape, acid phosphatase and arylsulfatase – in pots cropped with yellow lupine. The lowest activity of β-glucosidase was observed in soil samples sown with yellow lupine, acid phosphatase, alkaline phosphatase and arylsulfatase – in treatments sown with oat. Although β-glucosidase, acid phosphatase, alkaline phosphatase and arylsulfatase are members of the same enzyme group, they responded differently to copper pollution even in soil samples contaminated with the copper doses which are admissible by

Table 3

Index of resistance of enzymes to soil pollution with copper depending on crop species*

Cu dose (mg kg^{-1} of soil)	Crop species		
	oats	spring oilseed rape	yellow lupine
β-glukosidase			
150	0.653 *b*	0.508 *c*	0.476 *d*
450	0.715 *a*	0.463 *d*	0.398 *e*
Average	0.684 *x*	0.486 *y*	0.437 *z*
Acid phosphatase			
150	0.586 *a*	0.521 *c*	0.538 *b*
450	0.302 *f*	0.422 *e*	0.476 *d*
Average	0.444 *x*	0.471 *y*	0.507 *z*
Alkaline phosphatase			
150	0.572 *b*	0.531 *c*	0.614 *a*
450	0.255 *e*	0.269 *e*	0.346 *d*
Average	0.414 *x*	0.400 *y*	0.480 *z*
Arylosulphatase			
150	0.511 *c*	0.660 *a*	0.610 *b*
450	0.248 *f*	0.414 *d*	0.277 *e*
Average	0.380 *x*	0.537 *y*	0.443 *z*

* homogenous groups for the activity of each enzyme are marked with the same letter

the Resolution of the Minister of the Environment. Copper contamination affected the activity of β-glucosidase, acid phosphatase, alkaline phosphatase and arylsulfatase. The sensitivity of the tested enzymes to the highest copper dose (450 mg kg^{-1} d.m. soil) was determined in the following order: arylsulfatase (decrease in activity by 66% in pots sown with yellow lupine) > alkaline phosphatase (decrease of 55% in treatments planted with oat) > acid phosphatase (decrease of 53% in pots cropped with oat) > β-glucosidase (decrease of 31% in treatments sown with spring rape). The above enzymes showed different resistance to the inhibitory effect of copper, subject to the crop species (Table 3). β-glucosidase was most resistant in soil sown with oat, acid phosphatase and alkaline phosphatase – in soil sown with yellow lupine, and arylsulfatase – in soil planted with spring rape.

The strength and direction of copper's adverse effect on enzymes was determined not only by the level of contamination and the cultivated plant species, but also by the type of soil use and management (Table 4). Regardless of the degree of soil's contamination with copper, on average, higher levels of β-glucosidase, alkaline phosphatase and arylsulfatase were deter-

Table 4

Effect of soil pollution with copper and land use on activity of soil enzymes

Cu dose (mg kg^{-1} of soil)	Land use	
	unseeded soil	seeded soil
β-glukosidase, mmol PNP kg^{-1} h^{-1}		
0	0.709 ± 0.028	0.742 ± 0.062
150	0.665 ± 0.022	0.610 ± 0.024
450	0.443 ± 0.012	0.584 ± 0.025
Average	0.605	0.645
LSD	*a* – 0.016; *b* — 0.013; *a* · *b* – 0.023	
Acid phosphatase, mmol PNP kg^{-1} h^{-1}		
0	1.975 ± 0.088	2.388 ± 0.081
150	1.914 ± 0.065	1.703 ± 0.084
450	1.562 ± 0.052	1.335 ± 0.103
Average	1.817	1.809
LSD	*a* – 0.030; *b* – 0.025; *a* · *b* – 0.043	
Alkaline phosphatase, mmol PNP kg^{-1} h^{-1}		
0	3.283 ± 0.112	4.345 ± 0.176
150	1.931 ± 0.116	3.365 ± 0.121
450	1.680 ± 0.061	2.089 ± 0.118
Average	2.298	3.267
LSD	*a* – 0.050; *b* – 0.041; *a* · *b* – 0.071	
Arylosulphatase, mmol PNP kg^{-1} h^{-1}		
0	0.258 ± 0.015	0.382 ± 0.130
150	0.198 ± 0.011	0.252 ± 0.034
450	0.137 ± 0.009	0.152 ± 0.030
Average	0.198	0.262
LSD	*a* – 0.044; *b* – 0.036; *a* · *b* – 0.062	

LSD for: *a* – copper rate, *b* – land use

mined in cropped than in uncropped soil. The average activity of acid phosphatase was comparable in both series. β-glucosidase, acid phosphatase and arylsulfatase were more resistant to copper's inhibitory effect in uncropped than in cropped soil (Table 5), whereas the average resistance of alkaline phosphatase to copper was similar in both series.

Soil type was an important factor which modified soil's enzymatic activity (Table 6). All of the tested enzymes were marked by higher levels

Table 5

Index of resistance of enzymes to soil pollution with copper depending on land use*

Cu dose (mg kg^{-1} of soil)	Land use	
	unseeded soil	unseeded soil
β-glukosidase		
150	0.853 *a*	0.546 *b*
450	0.465 *d*	0.525 *c*
Average	0.659 *x*	0.536 *y*
Acid phosphatase		
150	0.750 *a*	0.548 *c*
450	0.591 *b*	0.400 *d*
Average	0.671 *x*	0.474 *y*
Alkaline phosphatase		
150	0.487 *b*	0.573 *a*
450	0.355 *c*	0.290 *d*
Average	0.421 *x*	0.431 *y*
Arylosulphatase		
150	0.620 *a*	0.594 *b*
450	0.396 *c*	0.313 *d*
Average	0.508 *x*	0.453 *y*

* homogenous groups for the activity of each enzyme are marked with the same letter

of activity in sandy loam than in loamy sand, but the presence of copper was more likely to affect the reactions catalyzed by the tested enzymes in loamy sand. In loamy sand samples, a copper dose of 450 mg kg^{-1} lowered the activity of alkaline phosphatase by 68%, arylsulfatase – by 60%, acid phosphatase by 50% and β-glucosidase by 33%. In sandy loam, the investigated pollutant lowered the activity of alkaline phosphatase by 41%, arylsulfatase – by 56%, acid phosphatase by 29% and β-glucosidase by 19%. In sandy loam samples, acid phosphatase, alkaline phosphatase and arylsulfatase were more resistant to copper's inhibitory effect, whereas in loamy sand, the above was observed for β-glucosidase (Table 7).

Our analysis of another experimental variable, i.e. copper persistence, revealed that the average activity of acid phosphatase and arylsulfatase was higher on experimental day 25, and the activity of β-glucosidase and alkaline phosphatase on the day 50 (Table 8). The highest difference was noted in respect of β-glucosidase, whose activity on day 50 was 66% higher than on day 25, as well as acid phosphatase, whose activity was 35% higher on day 25. On the 25th day of copper deposition in soil, the average resistance

Table 6

Effect of soil pollution with copper and soil type on soil enzymatic activity

Cu dose (mg kg^{-1} of soil)	Type of soil	
	loamy sand	loamy sand
β-glukosidase, mmol PNP kg^{-1} h^{-1}		
0	0.683 ± 0.027	0.784 ± 0.080
150	0.574 ± 0.024	0.673 ± 0.023
450	0.460 ± 0.014	0.637 ± 0.030
Average	0.573	0.698
LSD	a – 0.016; b – 0.013; $a \cdot b$ – 0.023	
Acid phosphatase, mmol PNP kg^{-1} h^{-1}		
0	2.267 ± 0.083	2.303 ± 0.081
150	1.494 ± 0.075	2.018 ± 0.083
450	1.143 ± 0.062	1.641 ± 0.117
Average	1.634	1.988
LSD	a – 0.030; b – 0.025; $a \cdot b$ – 0.043	
Alkaline phosphatase, mmol PNP kg^{-1} h^{-1}		
0	3.108 ± 0.105	5.051 ± 0.216
150	2.061 ± 0.057	3.953 ± 0.182
450	1.001 ± 0.067	2.973 ± 0.141
Average	2.056	3.993
LSD	a – 0.050; b – 0.041; $a \cdot b$ – 0.071	
Arylosulphatase, mmol PNP kg^{-1} h^{-1}		
0	0.317 ± 0.171	0.385 ± 0.031
150	0.178 ± 0.039	0.299 ± 0.018
450	0.127 ± 0.035	0.170 ± 0.014
Average	0.207	0.285
LSD	a – 0.044; b – 0.036; $a \cdot b$ – 0.062	

LSD for: a – copper rate, b – type of soil

rate of β-glucosidase was 0.440, acid phosphatase – 0.567, alkaline phosphatase – 0.405 and arylsulfatase – 0.501. On the 50th day of the experiment, the following resistance rates were noted: 0.693, 0.480, 0.453 and 0.433, respectively (Table 9).

Table 7

Index of resistance of enzymes to soil pollution with copper depending on type of soil*

Cu dose (mg kg^{-1} of soil)	Type of soil	
	loamy sand	loamy sand
β-glukosidase		
150	0.729 *a*	0.516 *c*
450	0.543 *b*	0.477 *d*
Average	0.636 *x*	0.497 *y*
Acid phosphatase		
150	0.529 *c*	0.668 *a*
450	0.342 *d*	0.553 *b*
Average	0.436 *y*	0.611 *x*
Alkaline phosphatase		
150	0.509 *b*	0.593 *a*
450	0.194 *d*	0.419 *c*
Average	0.352 *y*	0.506 *x*
Arylosulphatase		
150	0.543 *b*	0.657 *a*
450	0.353 *c*	0.315 *d*
Average	0.448 *y*	0.486 *x*

* homogenous groups for the activity of each enzyme are marked with the same letter

Copper's inhibitory effect on the activity of all the analyzed soil enzymes was also noted in our previous study (WYSZKOWSKA et al. 2005a,b) as well as in experiments performed by other authors (GILLER et al. 1998, GULSER et al. 2008, KARLEN et al. 2003, SCHOENHOLTZ et al. 2000). The inhibitory effect of soil contamination with copper on enzymatic activity could be due to copper's indirect toxic influence on microbial proliferation (GILLER et al. 1998, KUCHARSKI, WYSZKOWSKA 2004, OLIVEIRA, PAMPULHA 2006) as well as its direct, destructive impact on enzymes (KARLEN et al. 2003, MORENO et al. 2001, WYSZKOWSKA et al. 2005b) and the processes catalyzed by those enzymes (GILLER et al. 1998).

Table 8

Effect of soil pollution with copper and date of analysis on activity of soil enzymes

Cu dose (mg kg^{-1} of soil)	Time of analysis, days	
	25	50
β-glukosidase, mmol PNP kg^{-1} h^{-1}		
0	0.682 ± 0.077	0.785 ± 0.031
150	0.428 ± 0.022	0.819 ± 0.025
450	0.320 ± 0.021	0.777 ± 0.023
Average	0.477	0.794
LSD	a – 0.016; b – 0.013; $a \cdot b$ – 0.023	
Acid phosphatase, mmol PNP kg^{-1} h^{-1}		
0	2.688 ± 0.091	1.882 ± 0.074
150	2.121 ± 0.079	1.392 ± 0.079
450	1.804 ± 0.106	0.980 ± 0.074
Average	2.204	1.418
LSD	a – 0.030; b – 0.025; $a \cdot b$ – 0.043	
Alkaline phosphatase, mmol PNP kg^{-1} h^{-1}		
0	4.013 ± 0.180	4.146 ± 0.141
150	2.659 ± 0.111	3.355 ± 0.128
450	1.742 ± 0.096	2.232 ± 0.112
Average	2.804	3.244
LSD	a – 0.050; b – 0.041; $a \cdot b$ – 0.071	
Arylosulphatase, mmol PNP kg^{-1} h^{-1}		
0	0.372 ± 0.058	0.330 ± 0.144
150	0.273 ± 0.046	0.204 ± 0.010
450	0.172 ± 0.041	0.125 ± 0.009
Average	0.272	0.220
LSD	a – 0.044; b – 0.036; $a \cdot b$ – 0.062	

LSD for: a – copper rate, b – date of analysis

Table 9

Index of resistance of enzymes to copper pollution depending on the date of analysis*

Cu dose (mg kg^{-1} of soil)	Time of analysis, days	
	25	50
β-glukosidase		
150	0.524 *c*	0.722 *a*
450	0.356 *d*	0.664 *b*
Average	0.440 *y*	0.693 *x*
Acid phosphatase		
150	0.627 *a*	0.570 *b*
450	0.507 *c*	0.389 *d*
Average	0.567 *x*	0.480 *y*
Alkaline phosphatase		
150	0.529 *a*	0.574 *b*
450	0.281 *d*	0.332 *c*
Average	0.405 *y*	0.453 *x*
Arylosulphatase		
150	0.644 *a*	0.556 *b*
450	0.358 *c*	0.310 *d*
Average	0.501 *x*	0.433 *y*

* homogenous groups for the activity of each enzyme are marked with the same letter

CONCLUSIONS

1. Copper contamination in doses of 150 mg to 450 mg·kg^{-1} soil significantly inhibits soil's biochemical activity. The sensitivity of the tested enzymes to copper was determined in the following order: alkaline phosphatase > arylsulfatase > acid phosphatase > β-glucosidase.

2. The resistance of the enzymes to copper depended on the cultivated plant species, soil type and the type of soil use and management. In samples of sandy loam, copper induced the smallest change in the activity of acid phosphatase and alkaline phosphatase, and in loamy sand – β-glucosidase and arylsulfatase proved to be most resistant to the tested pollutant. In uncropped soil, copper least altered the activity of arylsulfatase and acid phosphatase. All the tested enzymes were less resistant to copper contamination in cropped than in uncropped soil. In soil planted with oat, the enzyme most resistant to copper contamination was β-glucosidase, while aryl-

sulfatase was the least resistant. In samples sown with spring rape, the analogous enzymes were arylsulfatase and alkaline phosphatase, and in yellow lupine treatments, alkaline phosphatase was the most resistant and β-glucosidase was the least resistant enzyme.

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