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Jacek ANTONKIEWICZ[1]

ASSESSMENT OF CHEMICAL COMPOSITION OF BUSHGRASS (*Calamagrostis epigejos* L.) OCCURRING ON FURNACE WASTE AND CARBIDE RESIDUE LIME LANDFILLS.
PART 1. CONTENTS OF N, P, K, Ca, Mg AND Na

OCENA SKŁADU CHEMICZNEGO TRZCINNIKA PIASKOWEGO (*Calamagrostis epigejos* L.) WYSTĘPUJĄCEGO NA SKŁADOWISKACH ODPADÓW PALENISKOWYCH I WAPNA POKARBIDOWEGO.
CZĘŚĆ 1. ZAWARTOŚĆ N, P, K, Mg, Ca I Na

Abstract: Furnace waste and carbide residue lime landfills are characterized by specific physicochemical properties and may be in some ways arduous for the environment due to among others dusting and element migration. In order to prevent these outcomes reclamation should be conducted, among others by turfing. Wastes deposited on landfills are a rich source of nutrients for plants occurring on incineration ashes and carbide residue lime. Because of rich source of nutrients in incineration ashes and carbide residue lime after suitable treatment these wastes may be also used, for reclamation of other wastes or postindustrial areas. Therefore the investigations aimed at an assessment of macroelement contents in bushgrass occurring on these wastes. The macroelement contents in bushgrass depended on the kind of deposited wastes and ranged widely from 4.84 to 20.89 g N; 0.40–3.00 g P; 2.33–13.35 g K; 0.32–1.97 g Mg; 0.22–11.26 g Ca and 0.02–1.20 g Na $\cdot$ kg^{-1} d.m. Presented investigations aimed among others to assess the contents of these macroelements with respect to fodder. The optimal macroelement contents in pasture sward are: 3.0 g P; 17.0–20.0 g K; 2.0 g Mg; 7.0 g Ca; 1.5–2.5 g Na $\cdot$ kg^{-1} d.m. An assessment of plants collected from the landfills according to this criteria revealed that the contents of phosphoprus, potassium, magnesium and sodium in the analyzed plants did not have optimal values. In case of carbide residue lime, calcium content in plants was above the optimal value, whereas in furnace ashes this macroelement in bushgrass was approximate to the optimal value. Among the investigated elements bigger quantities of nitrogen, phosphorus, sodium and potassium were found in plants collected from furnace ash than from carbide residue lime landfills. Therefore the obtained results point out higher fertiliser value of furnace ashes than carbide residue lime.

Keywords: *Calmagrostis epigejos* L. (bushgrass), landfills, incineration ashes, furnace ashes, carbide residue lime, N, P, K, Mg, Ca, Na

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Furnace waste and carbide residue lime landfills are characterized by specific physicochemical properties and may be in some ways arduous for the environment due to among others dusting and element migration. In order to prevent these outcomes, the Council of the European Union accepted the directive on waste deposition [1]. The overall objective of this document is "to provide for measures, procedures and guidance to prevent or reduce as far as possible negative effects on the environment, in particular the pollution of surface water, groundwater, soil and air, and on the global environment, including the greenhouse effect, as well as any resulting risk to human health, from landfilling of waste, during the whole life-cycle of the landfill". If furnace and carbide lime wastes are not recycled or disposed outside the existing installations and facilities [2], it is suggested that reclamation measures should be conducted on the above mentioned waste landfills among others by means of turfing [3–5]. Wastes deposited on landfills are a rich source of nutrients for plants occurring on incineration ashes and carbide residue lime [6]. Because of rich source of nutrients in incineration ashes and carbide residue lime, after suitable treatment these wastes may be also used, for reclamation of other wastes or postindustrial areas. Therefore the investigations aimed at an assessment of macroelement contents in bushgrass occurring on these wastes.

Material and methods

The subject of investigations was the substratum of bushgrass (*Calamagrostis epigejos* L.) occurring on furnace waste and carbide residue lime landfills and on their embankments. Material representing "Sedimentation pool 3" with the area of 23 ha was sampled for the analyses. The sedimentation pool comprised three sections: 1) inactive the incineration ash section LŁ, where 20 samples were collected including 10 from the landfill bowl and 10 from the embankment, 2) active incineration ash section, where 10 samples were taken, including 5 from the landfill bowl and 5 from the embankments, 3) lime section – repository of carbide residue lime obtained after acetylene production, where 10 samples represented the landfill bowl and 10 the embankments. A total of 50 samples collected from the depth of 0–20 cm were subjected to physicochemical analysis. In order to determine some physicochemical properties of the analyzed substratum, the following assessments were made using methods commonly applied in agricultural chemistry and soil science [7]: granulometric composition with Boyoucose-Casagrande method modified by Prószyński, soil pH in H_2O and 1 mol KCl $\cdot$ dm^{-3} using potentiometric method, organic carbon using Tiurin method, nitrogen content with Kjeldahl method on Kjeltec System 1026 apparatus (Tecator), the contents of bioavailable phosphorus and potassium using Egner-Riehm method, bioavailable magnesium content using Schachtschabel method, total contents of P, K, Ca, Mg and Na were determined after the sample digestion in HNO_3 and $HClO_4$ (3:2) acids and the contents of these metals soluble in 1 mol $\cdot$ dm^{-3} HCl solution with Rinkis method.

The analysis of plant material comprised determining total nitrogen content using Kjeldahl method and other macroelement content following the material "dry" mineralization. Concentrations of P, K, Ca, Mg and Na in the substratum and in bushgrass were assessed using ICP-AES method on sequential spectroscope Ultrace JY-238. Statistical computations were conducted using Statsoft 7.1. program. Basic statistical parameters were computed: minimum, maximum and medium values and variation coefficient. Correlation coefficients describing the relationship between the element contents in plants and physicochemical properties of the substratum were also computed.

Results

Detailed physical and chemical properties of incineration ashes and carbide residue lime were presented in a previous publication [8]. The granulometric composition of furnace ashes and carbide residue lime may be diversified, which is connected with the method of their transport to the landfill [9]. The analyzed wastes were transported by means of water which has a crucial influence on sedimentation processes in sedimentation pools. Results obtained in the analyses of granulometric composition place the analyzed samples from the bowl of furnace ashes landfill in the ordinary dust category. Also heavy, medium and light silt loams were present on the furnace ash landfills. Mean contents of the silt fraction in furnace crust was 37 %. Beside silt fraction also the share of sand fraction was considerable in the ash crust, its mean content reached 36 %, whereas the floatable particles constituted the remaining 28 % . Granulation of samples collected from the bowl of post carbide lime section was very similar to furnace ashes. The share of sand and silt fractions reached 37 %. A greater share of sand fractions but the smaller of floatable fraction were determined in the samples from the landfill embankments. Beside ordinary dusts also loamy sand presence was determined in the embankments of furnace ash landfills. On the other hand, loam presence was noted in the embankments of carbide residue lime section.

Reaction. The analyzed furnace wastes and carbide lime revealed alkaline reaction. pH value measured in 1 mol · dm^{-3} KCl solution on the furnace ashes depository ranged from 6.77 to 10.69, while in H_2O from 7.50 to 10.52. In the samples collected from the inactive section of ashes (LŁ) pH value was by approximately one unit lower in comparison with the operating section of ash landfill section. It may evidence occurrence of lime leaching processes among others from inactive section an therefore lowered pH value. The reaction of carbide residue lime samples was more alkaline in comparison with the furnace ash landfills; its mean value in 1 mol KCl was 11.24 and in H_2O – 11.52. On the other hand, the reaction of samples collected from the embankments of the analyzed sections was slightly lower in comparison with the landfill bowl.

Organic carbon. The content of organic carbon in the analyzed wastes ranged widely from 1.21 to 168.92 g · kg^{-1} d.m. Mean content of organic carbon in the furnace ashes landfill (60.85 g · kg^{-1} d.m.) was much higher than in the carbide residue lime depository (31.60 g · kg^{-1} d.m.). Much higher content of organic carbon was registered in the embankment of the analyzed landfill than in its bowls.

Biovailable phosphorus, potassium and magnesium. Furnace ashes contained much larger quantities of bioavailable macroelements in comparison with carbide residue lime. Moreover it was found that the embankments of the analyzed landfill sections were far more abundant in bioavailable macroelements. Mean contents of bioavailable phosphorus, potassium and magnesium in furnace ash samples were respectively: 149.3 mg K, 142.80 mg K and 350.35 mg Mg $\cdot$ kg^{-1} d.m. According to limit numbers for soil assessment [10] the contents for bioavailaible P, K and Mg were on very high, high and medium level, respectively. On the other hand, average content of bioavailable phosphorus, potassium and magnesium in the carbide residue lime were: 83.58 mg P; 71.55 mg K and 117.09 mg Mg $\cdot$ kg^{-1} d.m., respectively and in view of phosphorus concentration corresponded to low level, while concerning potassium and magnesium to very low one.

Macroelement content in the wastes. Total contents of macroelements in furnace ash and carbide residue lime samples were diversified and ranged from 0.14–9.72 g Mg; 0.37–65.56 g Ca; 0.01–1.63 g K; 0.08–1.96 g Na; 0.07–1.95 g P; 0.06–2.07 g N $\cdot$ kg^{-1} d.m. (Table 1). Author's own research has demonstrated that furnace ashes were more abundant in nitrogen, phosphorus and potassium in comparison with carbide residue lime. Magnesium content in the samples from the analyzed landfills was equalized. Almost ten-fold higher calcium concentrations were found in the landfill carbide residue lime section in comparison with furnace ashes. Carbide residue lime also revealed much higher sodium content.

In Poland amounts of many elements (P, K, Ca, Mg and Na) soluble in 1 mol HCl $\cdot$ dm^{-3} are considered bioavailable, therefore these are forms relatively easily accessible. They are formed mainly of ions present in the soil solution and adsorbed on mineral and organic colloids of arable soils [11, 12]. Solubility of the analyzed macroelements in relation to the total contents ranged from 0.72 to 92.25 % P; 1.30–70.06 % K; 19.75–97.44 % Mg; 20.0–87.49 % Ca and 4.54–99.9 % Na. From among the researched macroelements the highest percent of sodium and the lowest of potassium was extracted. Moreover, in four samples it was determined that 1 mol HCl $\cdot$ dm^{-3} extracted greater amount of sodium than a mixture of nitric(V) and chloric(VII) acids (3:2). The highest variability in extraction of the analyzed macroelements was noted for sodium ($V = 132.7$ %) and the lowest for magnesium ($V = 39.9$ %). In his own investigations the Author found that 1 mol HCl $\cdot$ dm^{-3} to a greater degree extracted phosphorus and calcium from furnace ashes and sodium from carbide residue lime. The power of potassium and magnesium extraction by 1 mol HCl $\cdot$ dm^{-3} from the samples of the analyzed landfills was generally unified.

Macroelement contents in bushgrass. The macroelement contents in the researched plant ranged from 4.84 to 20.89 g N; 0.40–3.00 g P; 2.33–13.35 g K; 0.32–1.97 g Mg; 0.22–11.26 g Ca and 0.02–1.20 g Na $\cdot$ kg^{-1} d.m. (Table 1). Presented results point to differences in the contents of the analyzed macroelements in bushgrass present on the analyzed landfills. Considering the studied macroelements larger quantities of nitrogen, phosphorus, sodium and potassium were noted in the plants collected from furnace ash landfills than carbide residue lime. However, mean contents of magnesium in bushgrass growing on furnace ashes and carbide residue lime landfills were equalized. Con-

Table 1

Macroelement contents in plants and samples of wastes from analyzed landfill sections

Parameter	Plant	Total*	Soluble**	Plant	Total*	Soluble**	Plant	Total*	Soluble**
	Mg			Ca			K		
	$[g \cdot kg^{-1}$ d.m.]								
Furnace ash sections									
Minimum	0.32	0.14	0.03	0.22	0.37	0.12	6.21	0.23	0.03
Maximum	1.97	9.23	1.95	8.73	8.69	5.96	13.35	1.63	0.28
Mean	1.26	4.37	1.76	2.84	5.87	4.02	10.09	0.98	0.11
V [%]	45.40	48.67	30.16	85.88	33.04	35.35	16.88	39.84	39.13
Carbide residue lime section									
Minimum	0.48	2.15	0.96	0.33	4.99	3.27	2.33	0.01	0.00
Maximum	1.95	9.72	1.93	11.26	65.56	13.11	8.70	1.63	0.12
Mean	1.25	4.97	1.79	4.08	41.15	10.09	5.70	0.74	0.07
V [%]	39.54	44.03	17.04	88.33	68.76	39.75	31.71	87.98	62.55

Table 1 contd.

Parameter	Plant	Total	Soluble	Plant	Total	Soluble	Plant	Total	Soluble
	Na			P			N		
	[g · kg^{-1} d.m.]								
Furnace ash sections									
Minimum	0.02	0.08	0.01	1.26	0.07	0.04	6.12	0.06	—
Maximum	1.20	1.34	0.32	3.00	1.57	1.22	20.89	2.07	—
Mean	0.19	0.67	0.08	2.02	1.01	0.72	11.74	0.81	
V [%]	140.67	38.17	98.20	22.72	35.10	37.03	40.22	56.02	—
Carbide residue lime section									
Minimum	0.02	0.17	0.05	0.40	0.09	0.00	4.84	0.16	—
Maxmum	0.12	1.96	1.49	2.52	1.95	1.34	15.62	0.73	—
Mean	0.05	0.66	0.25	1.49	0.88	0.49	8.56	0.38	
V [%]	50.14	66.19	139.35	40.34	75.95	110.70	31.28	39.90	—

* Total content in wastes; ** content of forms soluble in 1 mol HCl · dm^{-3} in wastes.

siderably higher contents of calcium were assessed in bushgrass present in the carbide residue lime landfill section in comparison with the plants occurring on ashes. The analysis of chemical composition revealed that the plants absorbed the greatest amounts of nitrogen, than potassium, calcium, phosphorus and the smallest amounts of sodium.

The optimal macroelement contents in pasture sward are: 3.0 g P; 17.0–20.0 g K; 7.0 g Ca; 2.0 g Mg; 1.5–2.5 g Na · kg^{-1} d.m. [13]. An assessment of the plants taken from the landfills according to this criterion shows that contents of phosphorus, potassium, magnesium and sodium in the collected plants do not correspond to the optimal values. In case of carbide residue lime calcium content in plants was over the optimal value, whereas in furnace ashes of the inactive section this macroelement concentration in bushgrass approximated the optimal value.

Correlation coefficients. Occurrence of statistically significant relationships between macroelement contents in bushgrass and their amounts in the wastes and its physicochemical composition was determined by computing simple correlation coefficients at significance level $p = 0.001$ (Tables 2 and 3). In the presented research a statistically significant correlation was established between the contents of Mg, Ca, K, Na and P in plants and the total and soluble form contents of these elements in ashes and carbide residue lime (Table 2). Mg concentration in bushgrass growing in ash landfill sections was strongly correlated with the total contents of Mg and Ca in the ashes and calcium content in the plant. On the other hand, calcium content in the plant was positively correlated also with K content in the plant and with total Mg content in ashes. Also significant relationships were determined between K content in bushgrass and total content of calcium in ashes. The content of 1 mol HCl · dm^{-3} soluble Na in ashes most strongly affected this element content in bushgrass.

Mg content in bushgrass occurring on carbide residue lime was significantly correlated with Ca content in this plant. Total content of calcium in the acetylene production waste (carbide residue lime) was determined by this element solubility in 1 mol HCl · dm^{-3} but negatively correlated with the total and soluble calcium content, as well as positively correlated with K content in this waste.

Table 3 shows correlation relationships between the macroelement content in plants and wastes and physicochemical properties of the wastes. C and K contents in bushgrass growing in the ash landfill sections were most strongly affected by bioavailable magnesium, whereas sodium contents in bushgrass was determined by the reaction. Nitrogen content in bushgrass present on the ashes was negatively correlated with sand content but positively correlated with coarse grained clay, reaction and organic carbon. However, Ca content in bushgrass growing in the carbide residue lime landfill section was most strongly affected by sand and colloidal clay, whereas phosphorus content was affected by the substratum pH.

Values of simple correlation coefficients compiled in Table 3 point to a most significant relationship between total contents of Mg and Ca in incineration ashes and bioavailable magnesium content. The content of total and 1 mol HCl · dm^{-3} soluble Na in furnace ashes was most determined by the presence of colloidal clay and the substratum pH. On the other hand the total and 1 mol HCl · dm^{-3} soluble P content in

Table 2

Simple (linear) correlation coefficients (r) between macroelement content in plant and wastes

Specification		Plant	Total*	Plant	Total*	Soluble**	Total*	Soluble**	Plant	Total*	Total*
		Mg		Ca			K		Na		P
Quarters incineration ash (N = 30)											
Total*	Mg	0.70									
Plant		0.75	0.62								
Total*	Ca	0.63	0.85								
Soluble**			0.68								
Plant	K			0.81							
Total*					–0.61						
Soluble**	Na								0.73		
Soluble**	P									0.60	0.91
Quarters lime carbide (N = 20)											
Plant	Ca	0.85									
Soluble**					0.94						
Total*	K				–0.98	–0.90					
Soluble**					–0.81	–0.70	0.85				
Plant							0.76				
Total*	P				–0.93	–0.80	0.93	0.82			
Soluble**					–0.96	–0.84	0.94	0.80			0.98

r significant at: $p = 0.001$; * total content in wastes; ** content of soluble forms in 1 mol HCl · dm^{-3} in wastes.

Table 3

Simple (linear) correlation coefficients (r) between macroelement content in plant and substratum, and some its properties

Specification	Total*	Plant	Total*	Substr.**	Plant	Total*	Substr.**	Plant	Substr.**	Plant	Total*	Substr.**	Plant	Total*
	Mg	Ca			K			Na		P			N	
Quarters incineration ash (N = 30)														
Sand													–0.70	
Coarse silt clay													0.69	
Fine silt clay											0.63	0.68		
Colloidal clay									0.66					
pH_{KCl}								0.65	0.69				0.78	
pH_{H_2O}									0.67				0,65	0.63
Org. C													0.62	
Available P											0.60	0.69		
Available K							0.94							
Available Mg	0.70	0.64	0.60		0.69								0.66	

Table 3 contd.

Specification	Total*	Plant	Total*	Substr.**	Plant	Total*	Substr.**	Plant	Substr.**	Plant	Total*	Substr.**	Plant	Total*
	Mg	Ca			K			Na		P			N	
Quarters lime carbide (N = 20)														
Sand		–0.79												
Coarse silt clay														
Fine silt clay														
Colloidal clay		0.85												
pH_{KCl}			0.91	0.83		–0.90	–0.90			–0.83	–0.85	–0.87		
pH_{H_2O}			0.89	0.82		–0.88	–0.88			–0.80	–0.84	–0.85		
Available P			–0.95	–0.98		0.91					0.83	0.88		
Available K			–0.80	–0.80		0.82	0.91							
Available Mg			–0.91	–0.82		0.93	0.82				0.89	0.89		

r significant at: $p = 0.001$; * total content in wastes; ** content of soluble forms in 1 mol HCl · dm^{-3} in wastes.

the ashes depended on fine-grained silt clay and bioavailable phosphorus content. Total content of nitrogen in the ashes depended on the substratum pH.

In case of carbide residue lime the total and soluble Ca and K contents in this waste were determined by the reaction and the contents of bioavailable macroelements. The total and 1 mol HCl · dm^{-3} soluble P content in carbide residue lime were most strongly influenced by the reaction and the contents of bioavailable phosphorus and magnesium.

Because of many directions of changes occurring in the deposited wastes, their systematic control may be used for the proper assessment of the state of the natural environment.

Discussion

Granulometric composition and particularly considerable content of silt particles may be considered as an advantageous factor from the perspective of application for reclamation of other wastes or postindustrial areas. Moreover, a big share of the finest-grained fractions in these wastes favours processes of dusting, which poses a hazard to the environment, therefore a necessity of turfing to secure these areas [14]. Research conducted by Meller et al [15] corroborate the presented investigations, that fresh furnace ashes, which were subjected to washing processes to a smaller degree, are more alkaline than ashes deposited for a longer period of time. Higher pH value than in carbide residue lime was also corroborated by investigations conducted by Kac-Kacas and Drzas [16]. Depending on the kind of coal, incineration ashes contain diversified amounts on non-burned coal, which has swelling properties and therefore contributes to loosening the ash crust [6]. Higher contents of organic carbon in landfills and embankments of furnace wastes are explained by expanding vegetation [17]. High contents of bioavailable macroelement forms in ashes were also found by Meller et al [15] and Kac-Kacas and Drzas [16].

Mean content of nitrogen in mineral soils ranges from 0.2 to 4.0 g · kg^{-1} d.m. [18]. Nitrogen content in furnace ashes comparable to mineral soil concentrations have been explained by the amount of non-burned organic coal [17]. On the other hand, the total range of macroelement contents in mineral soils of Poland is: 0.1–2.0 g P; 3.0–21.0 g K; 0.2–8.0 g Mg; 0.5–49 g Ca; 1.00–10.0 g Na · kg^{-1} d.m. [19]. Total contents of phosphorus, magnesium and sodium in the samples from the analyzed landfills generally did not differ from these elements contents in arable soils. Potassium and sodium contents in furnace ashes and carbide residue lime were much lower in comparison with mineral soils. The tested ashes and particularly carbide residue lime were abundant in calcium, which was not registered in mineral soils [19]. The obtained results show that higher contents of macroelements in furnace ashes point to them as a macroelement source for plants [17]. Moreover, the analyzed furnace ashes, due to a source of macroelements may constitute a substrate for preparing ash-sludge mixtures for environmental management of post-industrial areas [20]. Identification of the properties of incineration ashes and carbide residue lime will make possible a selection of proper and efficient reclamation procedures [21].

Conclusions

1. Conducted analysis of granulometric composition showed silt granulation of incineration ashes and silt and loam granulation of carbide residue lime. The reaction of furnace ashes was neutral or alkaline, whereas carbide residue lime revealed exclusively alkaline reaction.

2. Much lower mean content of organic carbon and bioavailable macroelements were registered in carbide residue lime landfill in comparison with furnace ash repository.

3. Total contents of phosphorus, potassium and nitrogen in the analyzed samples of furnace ashes were much higher than in carbide residue lime samples. On the other hand calcium content in carbide residue lime landfill section was almost ten-fold higher in comparison with the analyzed ashes. Carbide residue lime was also more sodium abundant.

4. Higher contents of phosphorus, potassium, sodium and nitrogen were noted in bushgrass growing on furnace ash landfill in comparison with carbide residue lime. An opposite relationship was found for calcium, ie higher content in bushgrass occurring in carbide residue lime section.

5. According to fodder value criteria, the contents of phosphorus, potassium, magnesium and sodium in bushgrass did not correspond to optimal values.

6. Investigated furnace ashes and carbide residue lime were characterized by satisfactory physical and chemical properties allowing for their application for reclamation measured in the areas, mainly destined for non-agricultural purposes.

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OCENA SKŁADU CHEMICZNEGO TRZCINNIKA PIASKOWEGO WYSTĘPUJĄCEGO NA SKŁADOWISKACH ODPADÓW PALENISKOWYCH I WAPNA POKARBIDOWEGO

CZĘŚĆ 1. ZAWARTOŚĆ N, P, K, Ca, Mg I Na

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Abstrakt: Składowiska odpadów paleniskowych i wapna pokarbidowego charakteryzują się specyficznymi właściwościami fizykochemicznymi i mogą stwarzać określone uciążliwości dla otoczenia, do których można zaliczyć m.in. pylenie i migrację pierwiastków. Aby zapobiec tym skutkom, należy przeprowadzić zabiegi rekultywacyjne między innymi poprzez zadarnienie. Zdeponowane odpady na składowiskach stanowią bogate źródło składników pokarmowych dla roślin występujących na podłożach z popiołów paleniskowych i wapna pokarbidowego. Ze względu na bogate źródło składników pokarmowych w popiołach paleniskowych i wapnie pokarbidowym odpady te można także wykorzystać, po odpowiednim przetworzeniu, do rekultywacji terenów poprzemysłowych. Stąd celem badań była ocena zawartości makroskładników w trzcinniku piaskowym występującym na tych odpadach.

Zawartość makroelementów w trzcinniku piaskowym zależała od rodzaju składowanych odpadów i wahała się w szerokim zakresie: 4,84–20,89 g N; 0,40–3,00 g P; 2,33–13,35 g K; 0,32–1,97 g Mg; 0,22–11,26 g Ca; 0,02–1,20 g Na $\cdot$ kg^{-1} s.m. W niniejszych badaniach podjęto między innymi ocenę zawartości tych makroskładników według kryteriów jakości paszowej. Optymalna zawartość makroelementów w runi pastwiskowej wynosi: 3,0 g P; 17,0–20,0 g K; 2,0 g Mg; 7,0 g Ca; 1,5–2,5 g Na $\cdot$ kg^{-1} s.m. Oceniając rośliny pobrane ze składowisk według tego kryterium stwierdzono, że zawartość fosforu, potasu, magnezu i sodu w badanej roślinności nie odpowiadała wartościom optymalnym. W przypadku wapna pokarbidowego zawartość wapnia w roślinach była większa od wartości optymalnej, natomiast w popiołach paleniskowych zawartość tego makroelementu w trzcinniku była zbliżona do wartości optymalnej. Spośród badanych makroelementów większe ilości azotu, fosforu, sodu, potasu stwierdzono w roślinach zebranych ze składowisk popiołów paleniskowych aniżeli wapna pokarbidowego. Stąd uzyskane wyniki wskazują na większą wartość nawozową popiołów paleniskowych niż wapna pokarbidowego.

Słowa kluczowe: trzcinnik piaskowy (*Calmagrostis epigejos* L.), składowiska, popioły paleniskowe, wapno pokarbidowe, N, P, K, Ca, Mg, Na

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SULPHUR AND COPPER CONTENT IN OILSEED RAPE PLANTS AFTER THE APPLICATION OF AMMONIUM NITRATE-SULPHATE

ZAWARTOŚĆ SIARKI I MIEDZI W ROŚLINACH RZEPAKU PO ZASTOSOWANIU SALETROSIARCZANU AMONU

Abstract: Sulphur is an important nutrient for winter rape because its large requirement for this element. The influence of N-S fertilizer on the copper content in the plants of winter oilseed rape plants was studied in precise field experiment. The evaluation involved two treatments of a single rate for the first spring fertilizer application with 100 kg N · ha^{-1} in the AN treatment (nitro-chalk) and 100 kg N + 50 kg S · ha^{-1} in the ANS treatment (ammonium nitrate-sulphate). Its application to soil affected the content of total extractable and mineral sulphur in soil. Common application of N-S nutrients in ANS fertilizer had a positive effect on sulphur content in winter rape plants. Sulphur content in root ranged from 0.23 to 0.52 % and aboveground biomass from 0.33 to 0.79 %. The highest content was found in leaves (to 1.76 % of S in head leaves). A positive influence of the ANS fertilizer on the copper contents in different part of plants was determined. The highest Cu concentrations were determined in the leaves and in florescences, the lowest ones occurred in the stem. The concentration of Cu ranged within the interval of 1.56–8.75 mg Cu · kg^{-1} of dry matter depending on the growth period and the part of the plant. No differences in the copper content have been determined in the seeds of individual treatment. The highest uptake in the aboveground parts of the plants was recorded in the green pod period and amounted to 57.4 g Cu · ha^{-1} for the ANS treatment.

Keywords: copper, N-S fertilizer, sulphur, oilseed rape

Copper is taken up by plants mainly as the Cu^{2+} ion and also probably in the form of low molecular organic complexes. The copper content in plants usually lies in the range of 2–20 mg Cu · kg^{-1} of dry matter. A toxic effect of copper has been recorded in plants (in leaves) at the level of 20–35 mg Cu · kg^{-1} in dry matter [1]. However, Reuter and

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Robinson [2] indicated the toxicity level for oilseed rape plants as low as 15 mg Cu · kg^{-1}.

Copper shows similar properties as iron, ie it has highly stable complexes and the ability to transfer electrons. The copper activity is mainly involved in the enzyme redox reactions in plants. Copper has a high affinity to -SH groups, particularly to the proteins rich in cysteine and also to the carboxylic and phenolic groups. Therefore, in a xylem solution more than 98–99 % of copper is fixed in the form of complexes [3]. The same applies to cytoplasm and the cell organelles in which the concentration of Cu^{2+} and Cu^{+} ions is exceptionally low. As a rule, more than 50 % of copper in chloroplasts is fixed in plastocyanin. Copper is also a component of superoxide dismutase (CuZnSOD) and is very important in the activity of ascorbate oxidase. Furthermore, copper is a component of diamine oxidase and phenol oxidase. Phenol oxidase takes part in biosynthesis of lignin and alkaloids.

As Thiel and Finck show [4], the plants with higher nitrate nutrition also have a higher requirement for copper. Decreased lignification of cell walls is also a typical indication of insufficient copper nutrition in higher plants. It is known that copper deficiency affects the yield and seed formation more than vegetative growth. Deficient copper nutrition causes pollen sterility. Copper content in the vegetative parts of plants which occurs at the level of 1–5 mg Cu · kg^{-1} in dry matter is critical, but this depends on the plant species, plant organ, developmental period and nitrate nutrition [4].

The total copper content in non-contaminated soils usually fluctuates from 2 to 40 mg Cu · kg^{-1}. In contaminated soils more than 1000 mg Cu · kg^{-1} has been determined. Copper is predominantly absorbed by organic matter and also into iron and manganese hydrooxides. As Zeien states [5], the proportion of individual sorbents differs according to soil types and soil texture. The copper content in the soil solution of agricultural soils is usually less than 0.03 to 0.3 mg · dm^{-3}.

Sulphur is nowadays beginning to be a limiting element in the nutrition of winter oilseed rape. Application of sulphate fertilizer before rape sowing and N-S fertilizer application during growing period has become a common part of cultivation technology.

The aim of the presented experiment was to determine the influence of N-S fertilizer on the copper content and Cu removal during vegetation period of oilseed rape.

Material and methods

A precision field experiment was established in Prague – Uhříněves, at the experimental station of the Faculty of Agrobiology, Food and Natural Resources. The following treatments were followed in experiment: 1) 100 kg N · ha^{-1} (a single application of AN: nitro-chalk, 27 % N) – sidedress the first spring application, 2) 100 kg N + 50 kg S · ha^{-1} (a single application of ANS: ammonium nitrate-sulphate, 26 % N and 13 % S) – sidedress the first spring application.

The area of the trial plot was 20 m^2. There were 4 replicates of each variant. The experiment was established on Luvisol with saturated sorption complex. Within the framework of agricultural chemical analyses of soil the following contents of available nutrients were determined (Mehlich III): 220 mg of potassium, 119 mg of phosphorus and 123 mg of magnesium per 1 kg of soil. The total sulphur content before fertilizers application was 850 mg · kg^{-1}, mineral S content was 4–7 mg · kg^{-1}, and the total copper content amounted to 26 mg · kg^{-1}. Value of pH/$CaCl_2$ equaled 6.2. Winter oilseed rape ('Bristol' cv. – two zero variety) was used as the experimental crop.

The total contents of chemical elements in plant samples were determined in mineral extracts, which were obtained by the dry decomposition method. The content of copper was determined by the optical emission spectrometry with inductively coupled plasma (ICP-OES, Varian VistaPro, Australia). More information is available in the previous paper [6].

Results and discussion

The Uhříněves site is very fertile, with the appropriate higher average yields achieved over a period of three years. The seed yield in the unfertilized control object was 3.7 Mg · ha^{-1}, while in the AN treatment it was 49 % higher and in the ANS treatment was 60 % higher. After the application of ANS fertilizer the microelemenet concentrations, particularly Mn and Zn in the plants were conclusively increased [7]. The decrease in the molybdenum content was statistically significant [8].

There is no data on the Cu contents in the plants of winter oilseed rape for the current period in the Czech Republic. Neuberg et al [9] cite less than 3 mg Cu · kg^{-1} of the dry matter of leaves (with the vegetation height of 30–40 cm) as a very low content, 3–5 mg Cu · kg^{-1} as low, 5–20 mg Cu · kg^{-1} as medium, and above 20 mg Cu · kg^{-1} as high. Similarly, Bergmann [10] considers 5–12 mg Cu · kg^{-1} in the dry matter of the oilseed rape leaves (with the vegetation height of 30–50 cm) as an adequate content.

Khurana et al [11] carried out vegetation experiment with oilseed rape in sand cultures. They consider 3.8 mg Cu · kg^{-1} of the dry matter as a deficient content in young leaves. Finck [12] published the values of an optimum copper content in fully developed leaves at the budding period at the level of 3–5 mg Cu · kg^{-1} of dry matter. Subsequently, Finck [13] amended these values to 5–10 mg Cu · kg^{-1} of dry matter.

As is clear from the Fig. 1, the Cu contents in the leaves were higher than the critical values indicated in the literature. It is obvious that the Cu nutrition of the plants in presented experiment was sufficient. The Figs. 1–3 indicate that there is a noticeable tendency towards the higher copper contents in the plants of the ANS treatment. It must be emphasised that in the ANS treatment there was a steadily higher growth of the above-ground biomass and the influence of the diluting effect was higher here than in the AN treatment. This tendency towards the Cu increase (even though no significant differences have been determined) has to be subjected to a critical evaluation. The results show clearly that the highest contents were determined in the leaves, particularly the young ones. Inflorescence analysis also showed high copper contents. At all collection dates the lowest concentrations were determined in the stems, or branches. These results are in good correlation with those of Rossi et al [14], who showed the content in the oilseed rape at 10.5 mg Cu · kg^{-1} of dry matter in the leaves and 7.6 mg · kg^{-1} of dry matter in the stems. Similarly, Angelova et al [15] also found higher copper contents in the leaves than in the stems.

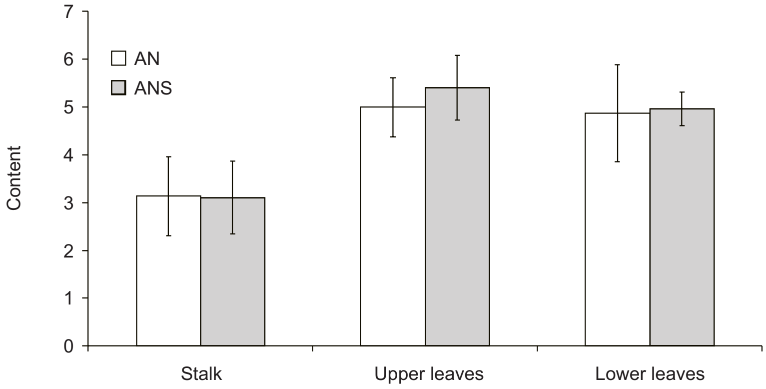

Fig. 1. Copper content in plants [mg Cu · kg^{-1}] – budding period

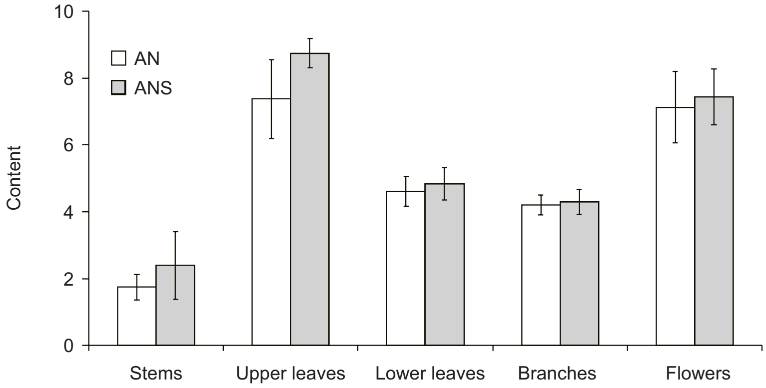

Fig. 2. Copper content in plants [mg Cu · kg^{-1}] – flowering period

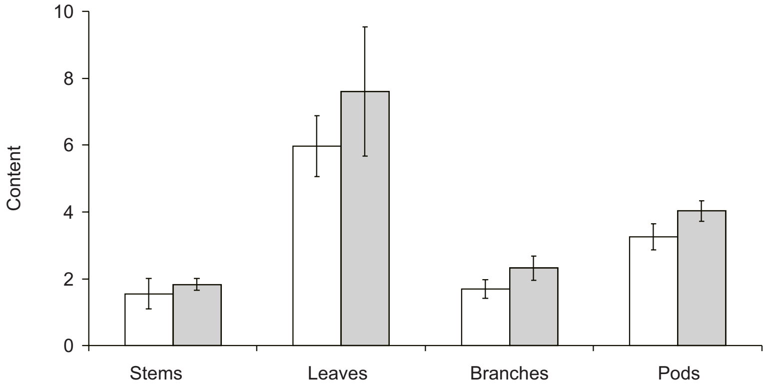

Fig. 3. Copper content in plants [mg Cu · kg^{-1}] – green pod period

The cause of the increased Cu content in the ANS treatment plants is related to the change of copper availability in the soil. Copper mobility in the soil depends on the pH value, redox status, the CEC value, the content and quality of organic matter, as well as on the content of clay minerals and Fe and Mn oxides [16]. It is well known that the copper mobility increases with the growing acidity of the soil environment, which is particularly obvious in the soils that are contaminated with copper [17]. Acidification effect can be expected for the ANS fertilizer as well as an associated increase in the Cu mobility. Indirect evidence is also provided by the results of the mineral sulphur content in the topsoil. The S content in the elongation growth period was 3.9 mg · kg^{-1} in the AN treatment, and 9.2 mg · kg^{-1} in the ANS treatment, in the flowering period it amounted to 6.3 mg S · kg^{-1} in the AN treatment and 15.2 mg · kg^{-1} in the ANS treatment, while in the green pod period it equaled 6.1 mg S · kg^{-1} in the AN treatment and 14.3 mg · kg^{-1} in the ANS treatment.

A number of authors [18, 19] emphasise, however, that the plant itself significantly influences the mobility of copper due to the changes in the rhizosphere (changes in ions concentrations, values of the redox potential, concentrations of the root exudates, etc). The changes in the rhizosphere pH values are also dependent on the form of nitrogen nutrition. The plants responded to nitrate nitrogen uptake by a relative increase in the rhizosphere pH value. In discussed experiment the different forms of nitrogen did not, apparently, have any influence on the uptake of copper. As determined by soil analyses, no significant differences in the NH_4^+ and NO_3^- contents in the topsoil and subsoil were found after 35 days of fertilizers application. It was also in good correspondence with an intensive microbiological activity of the Luvisol at the Uhříněves site.

As far as the maintenance of the cation-anion balance is concerned, the increased uptake of anions (particularly SO_4^{2-}) by the plants in the ANS treatment was equalised by an increased uptake of cations. It was, evidently, a very significant factor influencing the increased Cu concentration in these variants. Significantly higher sulphur content in individual parts of ANS treatment plants was determined during practically all growing period (Figs. 4–6). For example, during flowering the sulphur content in the AN treatment was 1.03 % (upper leaves) and 0.67 % (lower leaves), while the ANS treatment reached the values up to 1.18 % (upper leaves) and 0.97 % (lower leaves). The seed uptake of this element was 19 kg S · ha^{-1} (AN treatment) and 24 kg S · ha^{-1} (ANS treatment).

During its entire vegetation period the ANS treatment showed an increased formation of the biomass. It is known, that plants excrete a considerable proportion of the products of photosynthesis in the form of root exudates. It can therefore be expected that there was a greater quantity of exudates particularly in the ANS treatment. As Herms and Brümmer stated [20], these low molecular organic substances contribute to the increased Cu concentration in the soil solution.

The results of Hinsinger [18, 19] show that the root respiration and exudation of organic acids contribute to the acidification of the rhizosphere. An increased of exudates formation in the plants with ANS fertilization evidently contributed towards a higher mobility of copper in the soil environment.

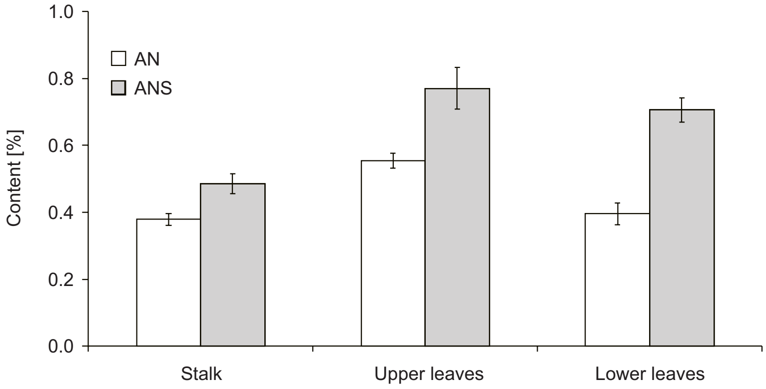

Fig. 4. Sulphur content in plants [% S] – budding period

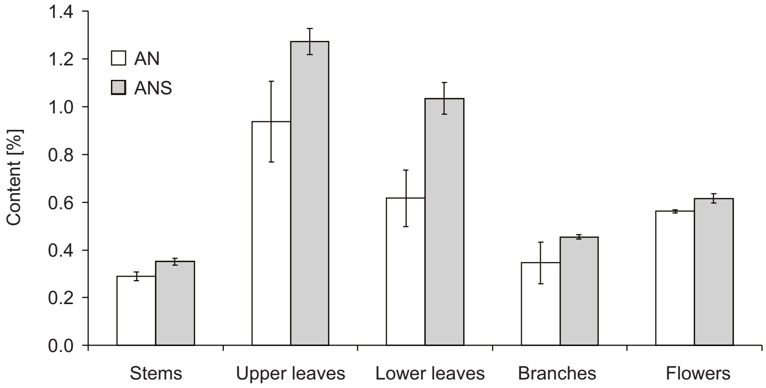

Fig. 5. Sulphur content in plants [% S] – flowering period

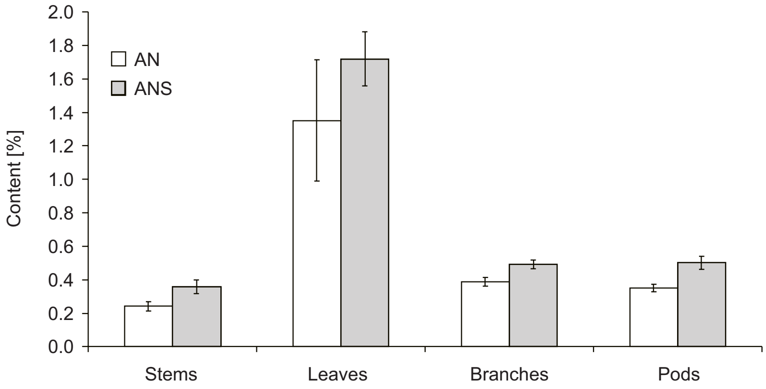

Fig. 6. Sulphur content in plants [% S] – green pod period

It is interesting that the differences in the Cu contents in the vegetative organs did not transform into the differences in the Cu contents in the seeds. An average content was 2.54 mg Cu · kg^{-1} of the seed dry matter in the AN treatment, and 2.55 mg Cu · kg^{-1} of the seed dry matter in the ANS treatment. The values which were determined are higher than the critical content of 2.2 mg Cu · kg^{-1}, by Khurana et al [11]. This, again, proves the fact that the oilseed rape plants in disscused experiment were adequately supplied with copper.

Higher Cu contents in the ANS treatment plants, together with the increase of biomass production resulted in significant differences in the uptake of this element by the plants' biomass. During the budding period the aboveground biomass accumulated 17.5 g · ha^{-1} in the AN treatment, and 18.9 g · ha^{-1} in the ANS treatment. During flowering period this difference increased further. The extraction was 38.9 g Cu · ha^{-1} in the AN treatment and 49.4 g · ha^{-1} in the ANS treatment. The values for the green pod period were determined as 38.9 g · ha^{-1} (AN) and 57.4 g · ha^{-1} (ANS).

Conclusions

A positive effect of the ANS fertilizer on the copper contents in different part of plants was determined. The highest Cu contents were determined in the leaves and in florescences, the lowest ones occurred in the stems. The copper content ranged within the limits of 1.56–8.75 mg Cu · kg^{-1} of dry matter depending on the growth period and the part of the plant. No differences in the copper content have been determined in the seeds of individual treatment. Significantly higher sulphur content in individual parts of ANS treatment plants was determined during practically all growing period.

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ZAWARTOŚĆ SIARKI I MIEDZI W ROŚLINACH RZEPAKU PO ZASTOSOWANIU SALETROSIARCZANU AMONU

Abstrakt: Siarka jest ważnym składnikiem dla rzepaku ozimego z powodu dużego zapotrzebowania na ten pierwiastek. W ścisłym doświadczeniu polowym badano wpływ nawozu azotowo-siarkowego (N–S) na zawartość miedzi w roślinach rzepaku ozimego. Ocena obejmowała dwie kombinacje pierwszego wiosennego nawożenia pojedynczą dawką: 100 kg N · ha^{-1} w formie saletrzaku (AN) i 100 kg N · ha^{-1} + 50 kg S · ha^{-1} w formie saletrosiarczanu amonu (ANS). Zastosowanie tego nawożenia do gleby oddziaływało na ogólną zawartość siarki oraz zawartość mineralnej siarki w glebie. Łączne stosowanie składników N-S w nawozie ANS miało dodatni wpływ na zawartość siarki w roślinach rzepaku. Zawartość siarki w korzeniach wahała się od 0,23 do 0,52 % a w nadziemnej biomasie od 0,33 do 0,79 % S. Najwięcej siarki zawierały liście (do 1,76 % S w liściach górnych). Stwierdzono dodatni wpływ nawozu ANS na zawartość miedzi w różnych częściach rośliny. Największą zawartość Cu zanotowano w liściach i kwiatostanach, a najmniejszą w łodygach. Zawartość miedzi mieściła się w zakresie 1,56–8,75 mg Cu · kg^{-1} suchej masy zależnie od fazy rozwojowej i części rośliny. Nie stwierdzono różnic w zawartości miedzi w nasionach z poszczególnych obiektów. Największe pobranie przez części nadziemne roślin, wynoszące do 57,4 g Cu · ha^{-1}, zanotowano w fazie zielonej łuszczyny w kombinacji ANS.

Słowa kluczowe: miedź, nawóz N-S (saletrosiarczan amonu), siarka, rzepak

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RESPONSE OF POTATO TO SOIL CONTAMINATION WITH MERCURY NEUTRALISED WITH SOIL IMPROVING SUBSTANCES

REAKCJA ZIEMNIAKA NA ZANIECZYSZCZENIE GLEBY RTĘCIĄ W WARUNKACH STOSOWANIA WYBRANYCH SUBSTANCJI NEUTRALIZUJĄCYCH

Abstract: Holding in mind the negative effect of mercury on environment, an experiment has been conducted in order to determine the influence of this metal on yield and chemical composition of potato grown on soil treated with various neutralizing substances.

The study consisted of a pot experiment, carried out in Kick-Brauckmann pots filled with 9 kg soil each. The soil material was subjected to simulated contamination with mercury sulphate added in the following doses: 0, 50, 100 and 150 mg Hg $\cdot$ kg^{-1}. Another experimental factor comprised application of several inactivating substances, such as zeolite, lime and bentonite. Zeolite and bentonite were added in the amounts equal 3 % of the whole soil mass in a pot whereas the dose of lime corresponded to 1 Hh of soil. All the pots received identical NPK fertilization. Edible potato of a moderately early, 'Zebra' cultivar, was the test plant.

Increasing soil contamination with mercury depressed the yield of tubers and mass of potato herbage. The largest yield decline was observed in the trials subjected to the most severe mercury contamination. As for potato tubers, a decline in their yield reached 63 % in the series with lime down to 31 % in the series with zeolite. The maximum potato herbage loss under the effect of mercury pollution was 48 % in the series with zeolite down to 23 % in the series with lime. Moreover presence of mercury in soil also affected the chemical composition of potato. Increasing rates of mercury in soil caused a rather regular depression in the content of potassium in potato tubers and herbage.

Keywords: mercury, lime, zeolite, bentonite, soil, contamination, neutralisation

Rapid technological progress alongside other transformations in the modern world are responsible for significant changes in natural environment. Heavy metals pose a particularly serious threat to nature [1–3]. Heavy metals are now found not only in heavily industrialised regions but also in natural and agricultural ecosystems [1].

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Mercury and its compounds are particularly harmful to humans. Owing to its high chemical and biological activity as well as a variety of forms it can occur in, mercury is easily involved in various chemical cycles in nature [4]. This is a dangerous phenomenon as once mercury enters a digestive system, it causes dangerous changes in living organisms [2]. More attention is now being paid to the presence of mercury in human foodstuff due to the actual risk of mercury poisoning in humans [5, 6]. It is assumed that presence of mercury should be carefully monitored in agricultural regions, where the metal can become a threat to the quality of farm produce [7]. Potatoes, next to cereals, are an important group of agricultural products in man's diet [8]. In Poland, consumption of potatoes is high, thus it is important to monitor their quality [9]. Quality control should first of all concern the presence of heavy metals in potato tubers. Another important aspect is the analysis of chemical composition of potato as a raw material.

Material and methods

The study consisted of a pot experiment carried out in a greenhouse at the University of Warmia and Mazury in Olsztyn in 2006. The experiment was established in Kick-Brauckmann pots, filled with 9 kg soil each. The soil material was collected from the arable and humus layer of soil and, regarding its grain-size distribution, represented light loamy sand. The experiment comprised two factors. Factor I consisted of substances which were to inactivate simulated mercury contamination. Depending on these neutralising substances, four series were created: without neutralising substances, with lime, bentonite or zeolite. Factor II involved increasing rates of mercury polluting the soil: 0, 50, 100 and 150 mg Hg $\cdot$ kg^{-1} of soil. Lime was introduced to soil in the amount corresponding to 1 hydrolytic acidity (Hh), whereas bentonite and zeolite were added in the quantity of 270 g per pot, which corresponded to 3 % of the soil mass per pot. Mercury was added to soil as mercury sulphate. In addition, mineral fertilizers were added: 1.2 g N, 1.2 g P_2O_5 and 1.4 g $K_2O \cdot$ pot^{-1}. Mineral fertilization was identical in all the pots. The above experimental trials were conducted with three replications. Potatoes, 'Zebra' cultivar, two tubers per pot, were planted on 28th June. They were harvested on 6th October. During the harvest, the yield of potato tubers and potato herbage was weighed, after which samples for chemical analyses were collected, dried, fragmented and mineralised in sulphuric acid. The plant material thus prepared was used for determination of: phosphorus with the vanadium-molybdenum method, potassium and calcium with the flame-photometric method and magnesium with the atomic absorption spectrophotometry.

Results

The experiment proved that both mercury as a soil contaminant and the inactivating substances added to soil (lime, bentonite, zeolite) had a large effect on the yield of potato tubers and herbage (Table 1).

Table 1

Yield of potato tubers and herbage [g f.m. · pot^{-1}]

Mercury dose [mg Hg · kg^{-1} of soil]	Neutralising substances							
	none		zeolite		lime		bentonite	
	tubers	herbage	tubers	herbage	tubers	herbage	tubers	herbage
0	378.8	124.4	325.0	129.4	218.5	94.8	263.7	93.0
50	363.9	84.2	245.6	119.4	185.2	71.7	229.3	74.9
100	230.8	83.5	128.9	117.5	117.4	72.2	185.9	61.6
150	149.1	75.6	121.0	99.1	114.3	58.8	181.3	48.2
Mean	280.7	91.9	205.1	116.3	158.9	74.4	215.1	69.4
LSD (p = 0.01)								
Tubers					Herbage			
48.13* 43.44** n.s.***					n.s.* 19.37** n.s.***			

* effect of mercury on yields, ** effect of neutralising substances on yields, *** interaction

In the control series (without the neutralising substances), the weakest mercury contamination dose depressed the tuber yield by 4 %, whereas the medium and highest rates of this heavy metal resulted in the tuber yield being 39 and 61 % lower, respectively, versus the control object, free of mercury. As for potato herbage, the three increasing rates of mercury introduced to soil depressed its yield by 32, 33 and 39 %, respectively. When comparing the control objects in each series, the yield of tubers and herbage, as a rule, decreased after introduction of the neutralising substances, except for the mass of potato herbage in the series with zeolite, where a 4 % increase occurred under the effect of the neutralising substance. Although the soil was improved with the neutralising substances, the yield of potato tubers and herbage tended to decrease as the rates of mercury in soil rose.

The highest decline in the tuber yield (63 %) was noticed in the series with zeolite, the lowest potato herbage yield, in turn, was obtained in the series with bentonite (48 %), both at the highest mercury soil pollution. Bentonite, on the other hand, had the strongest alleviating effect on the decrease in potato tuber yield caused by mercury (31 %), in the case of potato herbage yield, the most effective was zeolite (23 %). When lime was added to soil, the yield of tubers went down by 48 % and that of herbage by 38 %.

Apart from the effect produced by mercury contamination as well as the neutralising substances on yields of potato tubers and herbage, the two experimental factors also affected, in a variety of ways, the chemical composition of the plant material (Tables 2–5).

Increasing rates of mercury as a soil pollutant resulted in an increase in the content of phosphorus in potato tubers, which at the highest mercury rate reached 43 % relative to the control object in the same series. Regarding potassium and calcium, amounts of these two nutrients tended to decrease as the soil contamination with mercury became more severe. At the rate of 150 mg Hg, the respective losses were 15 and 9 %. In turn, the content of magnesium declined by an average of 39 % at 50 and 100 mg Hg per pot, but increased by 13 % versus the control when the soil was polluted with the highest mercury dose. In potato herbage, mercury contamination raised the content of phosphorus (36–86 %), potassium (21–22 %) and magnesium (25–30 %) but depressed that of calcium (40–52 %).

Table 2

Phosphorus (P) content in potato tubers and herbage [g · kg^{-1} d.m.]

Mercury dose [mg Hg · kg^{-1} of soil]	Neutralising substances							
	none		zeolite		lime		bentonite	
	tubers	herbage	tubers	herbage	tubers	herbage	tubers	herbage
0	3.23	2.11	3.47	2.24	3.65	2.32	3.78	2.22
50	4.35	3.62	2.84	1.90	3.10	1.74	3.55	2.63
100	3.67	2.86	3.69	2.25	2.84	2.29	3.66	2.72
150	4.61	3.93	3.10	2.51	2.88	1.95	3.80	2.59
Mean	3.97	3.13	3.28	2.23	3.12	2.08	3.70	2.54

Table 3

Potassium (K) content in potato tubers and herbage [g · kg^{-1} d.m.]

Mercury dose [mg Hg · kg^{-1} of soil]	Neutralising substances							
	none		zeolite		lime		bentonite	
	tubers	herbage	tubers	herbage	tubers	herbage	tubers	herbage
0	17.0	13.6	18.1	18.4	16.7	19.7	17.1	16.3
50	14.1	16.4	13.8	14.3	15.2	16.2	15.9	16.8
100	14.2	16.6	16.2	14.5	14.4	15.3	15.3	17.2
150	14.5	16.6	15.1	16.2	13.6	14.3	15.7	16.2
Mean	15.0	15.8	15.8	15.9	15.0	16.4	16.0	16.6

Table 4

Magnesium (Mg) content in potato tubers and herbage [g · kg^{-1} d.m.]

Mercury dose [mg Hg · kg^{-1} of soil]	Neutralising substances							
	none		zeolite		lime		bentonite	
	tubers	herbage	tubers	herbage	tubers	herbage	tubers	herbage
0	0.80	1.55	0.78	1.66	0.80	1.58	1.00	2.15
50	0.49	2.01	0.56	1.71	0.76	1.69	1.08	1.84
100	0.50	1.99	0.66	1.73	0.57	1.61	0.80	1.57
150	0.90	1.93	0.61	1.49	0.61	1.59	0.83	1.53
Mean	0.67	1.87	0.65	1.65	0.69	1.62	0.93	1.77

Table 5

Calcium (Ca) content in potato tubers and herbage [g · kg^{-1} d.m.]

Mercury dose [mg Hg · kg^{-1} of soil]	Neutralising substances							
	none		zeolite		lime		bentonite	
	tubers	herbage	tubers	herbage	tubers	herbage	tubers	herbage
0	0.64	5.56	0.53	7.24	0.56	6.05	0.59	5.67
50	0.63	3.35	0.58	4.05	0.43	4.93	0.48	4.40
100	0.63	2.93	0.64	3.27	0.53	3.47	0.49	3.82
150	0.58	2.69	0.38	3.93	0.37	3.70	0.54	3.62
Mean	0.62	3.63	0.53	4.62	0.47	4.54	0.52	4.38

Introduction of the neutralising substances to soil tended to mollify the above relationships. Besides, while analysing the mean contents of each macronutrient in all the series, it was found that all the neutralising substances had a positive effect on the accumulation of potassium, both in tubers (6–7 % increase) and herbage (4–5 % increase) as well as calcium (21–27 % increase) versus the control series (without the soil improving substances). A positive correlation was observed between the application of lime or bentonite and the content of magnesium in potato tubers (3–39 % increase). The average contents of the macronutrients indicate that the neutralising substances had a negative effect on the accumulation of phosphorus, both in tubers and in herbage, and calcium in tubers. Besides, addition of zeolite depressed the average content of magnesium in the whole potato plant while bentonite contributed to a decrease in the level of magnesium in potato herbage.

Discussion

The authors' own research has demonstrated that increasing rates of mercury have an adverse effect on yields of potato. Particularly harmful were the two highest rates of the heavy metal (100 and 150 mg Hg · kg^{-1}), which caused a 39 and 61 % decline in tuber yield, respectively. As regards the yield of potato herbage, the lowest mercury dose had a considerable effect, as it reduced the yield of herbage by 32 %. Higher doses of mercury caused a further albeit insignificantly higher depression in herbage yields. Similar results are reported by Wyszkowski and Wyszkowska [10], who showed a negative effect of mercury on yields of oats. They found out that the yield of oats was 44 % lower under the influence of 20 mg Hg · kg^{-1} of soil. The fact that mercury has a negative effect on crop yields finds further confirmation in an earlier study conducted by Ciećko et al [11], where lower yields of maize were observed as a result of soil contamination with mercury. In that case, the highest dose of mercury (150 mg Hg · kg^{-1}) caused a 27 % decrease in the yield of aerial maize organs. Also in that experiment, neutralising substances were tested, such as zeolite, lime and bentonite. They were all found to have a considerable alleviating effect on the negative influence caused by mercury on maize yields. In contrast, the present study failed to demonstrate an analogous effect of these soil improvers on potato yields, reduced by increasingly high rates of mercury in soil.

The study reported in this paper showed a considerable influence produced by the varied levels of soil contamination with mercury as well as the neutralising substances on the chemical composition of potato. Growing rates of mercury added to soil raised the content of phosphorus in potato tubers and herbage as well as potassium and magnesium in herbage but depressed the level of potassium in tubers as well as calcium in whole potato plants. The research conducted by Wyszkowski and Wyszkowska [10] also suggested that high levels of mercury in soil affected the chemical composition of oats. In their experiment, mercury raised the content of macroelements in oats, particularly that of phosphorus and calcium and, to a lesser extent, potassium and magnesium. By comparison, in a study completed by Ciećko et al [11], application of mercury to soil increased the levels of phosphorus, potassium and magnesium in maize, although the actual increase was more evident in aerial parts rather than in roots of this cereal. Soil pollution with mercury did not produce any significant effect on the content of calcium, which remained on a stable level. In the same experiment, the chemical composition of maize plants additionally depended on the type of a neutralising substance added to soil. Zeolite contributed to an increase in the content of potassium in maize, while causing a decline in the content of magnesium. In turn, liming caused a decrease in the content of phosphorus, potassium and magnesium, with calcium being an exception. In the present study, the neutralising substances also modified the chemical composition of potato. In the series with zeolite, lime and bentonite more potassium was determined in tubers and herbage and more calcium was found in herbage. In addition, bentonite and calcium had a positive effect on the content of magnesium in potato tubers. However, all the inactivating substances had a negative effect on the content of phosphorus in potato plants.

Conclusions

1. Contamination of soil with mercury had a negative effect on the yield of tubers and herbage of potatoes. The largest decrease in the tuber (69 %) and herbage yield (39 %), relative to the control, was observed at the highest mercury dose (150 mg Hg · kg^{-1}).
2. Our analysis of the yields obtained from the control objects in the particular experimental series shows that the neutralising substances added to soil had a negative effect on the yield of potato, except zeolite, which contributed to a 4 % increase in the mass of potato herbage.
3. Application of the inactivating substances to soil did not have any larger effect on reducing the depression in potato yield caused by increasingly larger doses of mercury.
4. Potato tubers collected from mercury polluted objects contained more phosphorus but less potassium and calcium compared with the control object. On the other hand, potato herbage contained more phosphorus, potassium and magnesium but less calcium under the effect of mercury contamination of soil.

5. The neutralising substances tested in the experiment caused an increase in the concentration of potassium in tubers and herbage and that of magnesium in potato tubers. The neutralising substances had a negative effect on the accumulation of phosphorus in the whole potato plant and that of calcium in potato tubers.

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REAKCJA ZIEMNIAKA NA ZANIECZYSZCZENIE GLEBY RTĘCIĄ W WARUNKACH STOSOWANIA WYBRANYCH SUBSTANCJI NEUTRALIZUJĄCYCH

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Abstrakt: Mając na uwadze negatywne oddziaływanie rtęci na środowisko przyrodnicze, wykonano doświadczenie, którego celem było określenie wpływu tego pierwiastka na plonowanie i skład chemiczny ziemniaka uprawianego w warunkach stosowania różnych substancji neutralizujących.

Za podstawę badań przyjęto doświadczenie wazonowe, które wykonano w wazonach Kick-Brauckmanna, mieszczących 9 kg gleby. Materiał glebowy został symulacyjnie zanieczyszczony siarczanem rtęci w dawkach: 0, 50, 100 i 150 mg Hg $\cdot$ kg^{-1}. W badaniach jednocześnie uwzględniono dodatek substancji inaktywujących rtęć, takich jak: zeolit, wapno i bentonit. Zeolit oraz bentonit zastosowano w ilości 3 % w stosunku do masy gleby w wazonie, a wapno w dawce odpowiadającej 1 Hh gleby. We wszystkich obiektach doświadczenia zastosowano jednakowe nawożenie mineralne (NPK). Rośliną testową był ziemniak jadalny odmiany średnio wczesnej ‘Zebra’.

Wzrastające zanieczyszczenie gleby rtęcią powodowało stopniowe zmniejszenie plonu bulw i masy łętów. Największy spadek plonu odnotowano w obiektach o największym zanieczyszczeniu gleby rtęcią. Zmniejszenie plonu bulw wynosiła od 63 % w serii z dodatkiem wapna do 31% w serii z zeolitem. Natomiast maksymalne obniżenie plonu łętów wynosiło od 48 % w serii z dodatkiem zeolitu do 23% w serii z wapnem. Ponadto rosnące zanieczyszczenie gleby rtęcią powodowało dość regularne zmniejszenie zawartości potasu w bulwach i łętach ziemniaka.

Słowa kluczowe: rtęć, wapno, zeolit, bentonit, gleba, ziemniak, zanieczyszczenie, neutralizacja

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ASSESSMENT OF CHEMICAL DEGRADATION OF SELECTED SOIL PROPERTIES AS INDUCED BY COPPER, ZINC AND HYDROGEN

OCENA CHEMICZNEJ DEGRADACJI WYBRANYCH WŁAŚCIWOŚCI GLEB POD WPŁYWEM MIEDZI, CYNKU I WODORU

Abstract: The concept of this study was based on the evaluation of the impact of copper (Cu), zinc (Zn) and hydrogen protons (H^+) on selected chemical characteristics of two arable soils: light loamy sand (No. 1) and silty soil (No. 2). The targeted chemical alterations of the soils concerned pH changes, water soluble forms of Cu and Zn and displaced alkaline cations as induced by Cu, Zn and H incorporation. Preliminarily, the amounts of exchangeable alkaline cations ($ExAC = Ca_{ex} + Mg_{ex} + K_{ex} + Na_{ex}$) were extracted by using 1 M CH_3COONH_4, pH 7.0 and these amounts were used for calculating amounts of Cu, Zn and H to be incorporated as follows: copper as $CuSO_4 \cdot 5H_2O$, zinc as $ZnSO_4 \cdot 7H_2O$ and hydrogen in the form of HCl at spiking rates corresponding to 0, 5, 10, 30, 60, 100, 150, 200, 300, 400, 500 and 1000 % of ExAC. Treatments were incubated for 16 weeks at 18 °C and kept at 75–85 % WHC (water holding capacity).

Copper, zinc and hydrogen effects on soil chemical properties can be first observed throughout pH changes, which were more pronounced for H treatments as compared with Cu and Zn ones. The amounts of water extractable Ca (ie displaced) at Cu and Zn spiking rates of 1000 % ExAC are 25 and 21 times higher, respectively, for the soil No. 1 (light loamy sand*),* whereas for the soil No. 2 (silty soil), 44 and 38 times higher, as compared with the control treatment. The impact of H is intermediate and represents 24 and 35 times, for the soils No. 1 and No. 2, respectively. Results reported in the paper drive specific attention on the destructive effects of Cu, Zn and H, separately. The joint impact of these metals along with protons is the core of agroenvironmental concern. The chemical degradation established on the basis of water extractable alkaline cations (WEAC) follows the range: H > Cu > Zn.

Keywords: copper, zinc, hydrogen cations, alkaline cations, arable soils, chemical degradation

The chemical degradation of soils is induced by several chemical compounds of which organic and mineral are most frequently reported. Their negative impacts ie bio(phyto)toxicity, depend on the chemical forms and persistence [1]. If organic

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contaminants are recognized to be biodegradable [2] unfortunately this can not account for mineral ones, such as trace elements, which persist in the environment. Among these, copper (Cu) and zinc (Zn) draw specific attention due to their dualistic classification: firstly as micronutrients (agricultural environments) and, secondly, toxic metals (severely contaminated soil environments). The most frequently encountered classification in terms of environmental concern is the second one, which intrinsically focuses on the occurrence of potential toxicity of metals due both to their elevated content in the soil and the strengthened mobility [3, 4].

Since most Cu and Zn geochemical processes proceed *via* exchange reactions, therefore it should be expected the occurrence of direct exchange mechanisms between Cu and Zn ions and alkaline cations (Ca^{2+}, Mg^{2+}, K^+ and Na^+) within soils sorptive complex [5, 6]. The magnitude of this phenomenon is closely related to the level of Cu and Zn incorporation into soils in one hand, and to the *"stability"* of soil fertility expressed by the bulk content of exchangeable alkaline elements on the other hand [7].

Throughout exchange mechanisms, hydrogen cations (H^+) may potentially displace alkaline cations from soil colloids, a process increasing their concentrations in the soil solution [8]. This process is boosted by the specifically high affinity of H^+ for phenolic and carboxylic groups of humic acids, by forming strong chemical bonds [9]. Under such conditions alkaline cations undergo mostly leaching, which consequently enhances the negative impact of protons over the soil sorptive complex [7].

The purpose of the study was to evaluate the impact of copper, zinc and hydrogen ions induced chemical changes in two arable soils and the resulting consequences for soil quality.

Materials and methods

Origin of soil samples

Soils used in the study were composite samples made by 20 subsamples collected in 2005 at the depth 0–30 cm from two sites: Swadzim (52°26′ N and 16°44′ E, Poland), an Experimental Station belonging to the University of Life Sciences in Poznan, designated here and after as soil No. 1, and Wrzesnia (52°19′ N and 17°35′ E, Poland), an agricultural farm leading intensive crop production (winter wheat, sugar beet and oilseed rape), as soil No. 2.

Physical and chemical soil analyses

Prior to chemical analyses, both soils were air-dried and crushed to pass a 1.0 mm screen. Particle size composition was determined according to the aerometric method of Bouyoucos-Casagrande [10] and organic carbon (organic C) by the Walkley-Black method as reported by Nelson and Sommers [11]. The pH was determined potentiometrically (*w/v*, 1:5) according to Polish Standard [12] in 0.010 M $CaCl_2$. Exchangeable alkaline cations ($ExAC = Ca_{ex} + Mg_{ex} + K_{ex} + Na_{ex}$) were extracted by using 1 M CH_3COONH_4, pH 7.0 according to Thomas [13] and the electrical conductivity (EC) was determined conductimetrically as described by Jackson [14]. Selected soil analytical data are reported in Table 1.

Table 1

Selected physical and chemical characteristics of soils used in the study

Soil	Silt 0.05–0.002	Clay < 0.002	organic C	EC	pH^c	Ca_{ex}	Mg_{ex}	K_{ex}	Na_{ex}	$ExAC^d$
	$[g \cdot kg^{-1}]$			$[\mu S \cdot cm^{-1}]$		$[mg \cdot kg^{-1}]$				
No. 1[a]	332	160	7.6	99.3	5.6	380.8	62.7	216.6	22.7	682.8
No. 2[b]	412	480	6.2	77.5	4.9	612.9	107.3	182.0	23.3	925.5

[a] light loamy sand; [b] silty soil; [c] in 0.010 M $CaCl_2$; [d] $Ca_{ex} + Mg_{ex} + K_{ex} + Na_{ex}$

Experimental design

Soils No. 1 and No. 2 were spiked with copper ($CuSO_4 \cdot 5H_2O$), zinc ($ZnSO_4 \cdot 7H_2O$) and hydrogen ions (HCl) at rates corresponding to 0, 5, 10, 30, 60, 100, 150, 200, 300, 400, 500 and 1000 % of ExAC (ie the sum of exchangeable alkaline cations) as listed in Table 2.

Table 2

Set of volumes of HCl and bidistilled water elaborated for H treatments

% of ExAC[a]	No. 1			No. 2		
	mmol $H^+ \cdot kg^{-1}$	5 mmol HCl $[cm^3]$	bidist. H_2O $[cm^3]$	mmol $H^+ \cdot kg^{-1}$	5 mmol HCl $[cm^3]$	bidist. H_2O $[cm^3]$
5	1.6	0.31	99.69	2.2	0.45	99.55
10	3.2	0.62	99.38	4.2	0.90	99.10
30	9.6	1.85	98.16	13.2	2.70	97.30
......	......	......	......	......	......	......

[a] $Ca_{ex} + Mg_{ex} + K_{ex} + Na_{ex}$

In the case of H-treatments, H^+ was added as described in Table 1: ExAC were converted to $mmol(+) \cdot kg^{-1}$ and a solution of 5.0 mmol $HCl \cdot cm^{-3}$ (ie 1 cm^3 contains 5 mmol H^+) was diluted with adequate amounts of bidistilled water to give a final solution of 100 cm^3 (Table 2), which were added to air-dried soils (1.0 kg).

Copper, zinc and H-spiked soils were incubated for 16 weeks at 18 ^oC and kept at 75–85 % WHC (water holding capacity). All treatments were triplicated and a composite sample (mean of three) was prepared at the end of the incubation, air-dried and analyzed in duplications for pH in 0.01 M $CaCl_2$, water soluble Cu and Zn and displaced alkaline cations (Ca^{2+}, Mg^{2+}, K^+ and Na^+). Graphs and computations were made by using Excel® Sheet.

Results and discussion

Soils pH changes

Soil physical and chemical characteristics reported in Table 1 show that both soils are acidic, mostly the silty one (No. 2) characterized by the highest content of silt and clay for 412 and 480 g $\cdot$ kg^{-1}, respectively. The soil No. 1 (light loamy sand) exhibits a higher salinity level (EC = 99.3 μS $\cdot$ cm^{-1}), as compared with the soil No. 2, but the organic carbon content of both soils was relatively low. One of the key characteristics of soils is the pool of exchangeable alkaline cations (ExAC) responsible for the regulation and maintenance of soil pH and other geochemical processes such as exchange mechanisms. As it can be observed, exchangeable calcium (Ca_{ex}) represented alone *ca* 56 and 66 % of all alkaline cations, respectively for soils No. 1 and No. 2. The same applies for exchangeable potassium (K_{ex}), with *ca* 32 and 20 %, respectively. Both Ca_{ex} and K_{ex} represent 88 and 86 % of the exchange potential of the studied soils, which implies that the lability of alkaline cations of these soils is significantly high. Acidic conditions could be partly responsible for this state.

The concept of this study is based on the evaluation of the impact of copper (Cu), zinc (Zn) and hydrogen ions (H^+) on selected soil chemical characteristics of two arable soils. The targeted chemical alterations concern pH changes, water soluble forms of Cu, Zn and displaced Ca^{2+}, Mg^{2+}, K^+ and Na^+ as induced by Cu^{2+}, Zn^{2+} and H^+ incorporation. As reported in Table 3, the amounts of Cu, Zn and H incorporated into soils were different and varied by a factor of 1.36 between soils No. 1 and No. 2 in the case of Cu^{2+}, Zn^{2+}, and by 1.47 for H^+, respectively.

Table 3

Amounts of Cu, Zn and H^+ incorporated into soils on the basis of the amounts of exchangeable alkaline cations (ExAC)

% of ExAC[a]	Cu[b], Zn[c] [mg $\cdot$ kg^{-1}]		[d]H^+ [mmol H^+ $\cdot$ kg^{-1}]	
	No. 1	No. 2	No. 1	No. 2
5	34.0	46.0	1.6	2.2
10	68.0	92.5	3.2	4.2
30	205.0	277.5	9.6	13.2
60	409.5	555.0	18.4	26.6
100	682.5	925.5	30.8	45.2
150	1024.0	1388.0	46.0	67.8
200	1365.5	1851.0	61.4	90.2
300	2048.0	2776.5	92.2	135.4
400	2731.0	3702.0	122.8	180.6
500	3413.5	4627.5	153.6	225.6
1000	6827.5	9255.0	307.0	451.4

[a] $Ca_{ex} + Mg_{ex} + K_{ex} + Na_{ex}$; [b] as $CuSO_4 \cdot 5H_2O$, [c] as $ZnSO_4 \cdot 7H_2O$; [d] as HCl

Copper, zinc and hydrogen effects on soil chemical properties can be first observed throughout pH changes (Fig. 1).

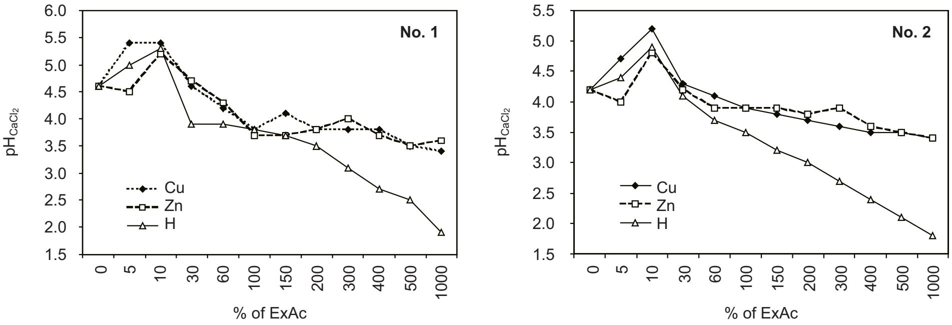

Fig. 1. Effect of Cu^{2+}, Zn^{2+} and H^+ spiking rates [% of ExAC] on pH changes for soils No. 1 and No. 2

The patterns of pH decrease are similar for both soils and are more pronounced for H ions treatments as compared to both Cu and Zn ones. Unexpectedly a raise of pH occurred at Cu, Zn and H ions spiking rates of 5 and 10 % ExAC. Processes involved in the chemical reactions of incorporated Cu, Zn and H concern preponderantly exchange mechanisms (retention and release). Therefore one may assume that the retention of Cu, Zn and H induced a simultaneous displacement and release into the ambient soil solution of equivalent units of Ca, Mg, K and Na, which in turn undergo hydrolysis reactions [6, 15]. The latter ones have been most specifically responsible for such unexpected pH raise at these two spiking rates.

$$CuSO_4 \cdot 5H_2O \longrightarrow Cu^{2+} + SO_4^{2-} + 5\ H_2O$$

$$ZnSO_4 \cdot 7H_2O \longrightarrow Zn^{2+} + SO_4^{2-} + 7\ H_2O$$

which means 1 eq Cu or Zn = SO_4^{2-} = H^+.

For the 5 % ExAC, 34.0 mg Cu have displaced 152.3 mg of all alkaline cations per kg of the soil No. 1. Therefore 34.0 mg Cu have induced 34.0 mg H^+ whose acidifying power was totally neutralized by 152.3 mg of all displaced alkaline cations. The same applies for the soil No. 2, where 46.0 mg Cu have displaced 151.0 mg of all displaced alkaline cations per kg of soil. This explained the gradual and progressive pH decrease along with increasing Cu spiking.

In the case of zinc, the exchange process at 5 % ExAC proceeded as follows:

a) 34.0 mg Zn have displaced 166.5 mg of all alkaline cations per kg of the soil No. 1;

b) 46.0 mg Zn have displaced 150.8 mg of all alkaline cations per kg of the soil No. 2.

A similar approach may be applied for H-spiked soils as below:

$$HCl \longrightarrow H^+ + Cl^-$$

Therefore 1 meq H^+ = 1 mmol H^+.

For the 5 % ExAC, 1.6 mmol H^+ have displaced 2.3 mmol(+) of Ca per kg of the soil No. 1. Therefore 1.6 mmol H^+ were totally neutralized by 2.3 mmol(+) of Ca, *alone*. The same applies for the 10 % ExAC, ie 3.2 mmol H^+ were neutralized by 3.67 mmol(+) of Ca, *alone*.

This geochemical evaluation elucidates the observed pH raise at the initial exchange process. Copper, zinc and H rates from 30 % ExAC and beyond, generated stronger acidification, which has not been neutralized by more and more displaced alkaline cations. In the current study, the highest amounts of incorporated H^+ amounted to 307.0 and 541.4 mmol $H^+ \cdot kg^{-1}$, respectively for soils No. 1 and No. 2 and were significantly lower as compared with *ca* 730.0 mmol $H^+ \cdot kg^{-1}$ incorporated yearly between 1975–1989 [16], as a result of ammonium sulphate, ammonium nitrate(V) and urea application to arable lands. The impact of such high fertilizers-generated H^+ may last for years and needs huge amounts of aglime or other neutralizing agents. Thus in most soils that are used in agriculture, acid rain does not appear to be a serious practical problem.

Water extractable Cu and Zn and their potential lability

Amounts of water extractable Cu and Zn (Fig. 2) are low as compared with those of Cu and Zn incorporated into soils and vary for the soil No. 1 within the ranges 0.6–52.1 % and 1.3–65.7 %, respectively for Cu and Zn. This corresponds to an extremely and relatively high amounts of Cu and Zn retained by the soil No. 1, ie from 99.4 to 47.9 % for Cu and from 98.3 to 34.3 % for Zn. Quite similar ranges were observed for the soil No. 2, where the amounts of Cu and Zn retained varied respectively from 99.2 to 58.5 % and 98.6 to 36.9 %. Such strong Cu and Zn partition to the solid phase may be attributed to three factors: Cu and Zn specific affinity to soil colloids [16], the relatively high level of silt and clay (Table 1), and the aqueous pH of Cu, Zn-spiked soils, as illustrated by the Fig. 2.

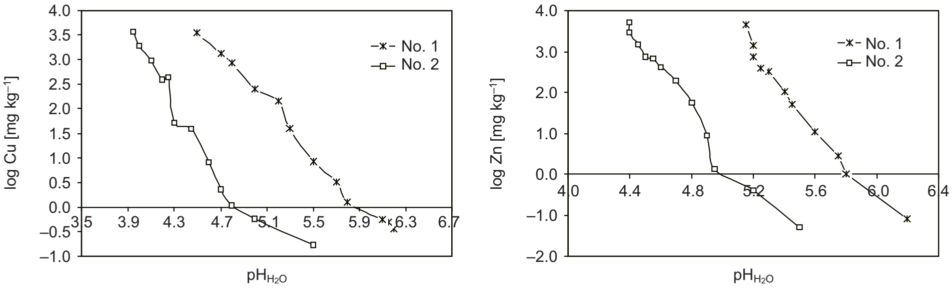

Fig. 2. Relationships between the amounts of water extractable Cu and Zn and aqueous pH of the investigated soils

According to Abd-Elfattach and Wada [18], the electronegativity values of Cu and Zn are 1.9 and 1.6, respectively. These physicochemical properties, greater for Cu as compared with Zn, express the affinity of both metals to soil colloids. The retention of both metals is in line with data obtained in the current work, ie, more copper was partitioned to both soils than did Zn. The role of silt and clay was decisive for such Cu and Zn behaviour, which may be explained by electrostatic and inner-sphere surface complexation reactions [19, 20].

Concentrations and the activity of Cu and Zn in the soil solution are controlled by dissolved organic and mineral substances, but most specifically by the pH of the solution. Trends illustrated in the Fig. 2 decidedly show, that both metals are very sensitive to pH changes and this sensitivity increases along with a raise of pH (steep slopes). Therefore, under such conditions, the emergence of easily hydrolyzed Cu and Zn forms takes place and this favors in turn the establishment of strong Cu and Zn partition to the solid phase. This effect induces chemical reactions, mostly exchange mechanisms leading to the displacement of significant amounts of alkaline cations.

Alkaline cations displacement and agroenvironmental consequences

Amounts of water extractable alkaline cations (WEAC) as influenced by Cu, Zn and H ions spiking to soils No. 1 (light loamy sand) and No. 2 (silty soil) are reported in Table 4 for selected spiking rates.

Table 4

Amounts of water extractable Ca, Mg, K and Na as influenced by Cu, Zn and H ions spiking rates (selected) for the soils No. 1 and No. 2

% of ExAC[a]	No. 1			No. 2		
	Cu	Zn	H	Cu	Zn	H
	WEAC (*Ca*)[b]			WEAC (*Ca*)		
	$[\text{Mg} \cdot \text{ha}^{-1}]$					
0	0.28 (*0.10*)			0.21 (*0.09*)		
5	0.46 (*0.20*)	0.50 (*0.20*)	0.35 (*0.14*)	0.45 (*0.25*)	0.45 (*0.22*)	0.75 (*0.49*)
10	0.42 (*0.20*)	0.50 (*0.23*)	0.46 (*0.22*)	0.37 (*0.21*)	0.33 (*0.19*)	0.60 (*0.39*)
60	0.98 (*0.57*)	1.12 (*0.68*)	2.02 (*1.43*)	1.25 (*0.91*)	1.20 (*0.91*)	2.05 (*1.52*)
100	1.50 (*0.99*)	1.29 (*0.89*)	2.26 (*1.48*)	1.80 (*1.42*)	1.86 (*1.44*)	3.20 (*2.49*)
150	1.90 (*1.34*)	2.01 (*1.35*)	2.95 (*2.05*)	2.67 (*2.20*)	2.62 (*2.05*)	4.36 (*3.50*)
500	3.18 (*2.45*)	2.60 (*1.96*)	4.57 (*3.19*)	4.60 (*3.83*)	4.50 (*3.71*)	5.01 (*3.38*)
1000	3.26 (*2.49*)	2.78 (*2.11*)	4.16 (*2.43*)	4.67 (*4.00*)	4.23 (*3.38*)	4.93 (*3.19*)

[a] Exchangeable alkaline cations (ExAC); [b] Amounts of Ca, only (in brackets)

Acidic soils are generally reported to exhibit low exchangeable calcium contents. This could be expectable, since hydrogen and aluminum ions promote a direct alkaline cations displacement and further their leaching, a process which in turn intensifies acidification. The amounts of ExAC listed in Table 1 are assessed for 2.05 $\text{Mg} \cdot \text{ha}^{-1}$, of which Ca_{ex} alone represents *ca* 56 % (ie, 1.14 $\text{Mg} \cdot \text{ha}^{-1}$) for the soil No. 1, whereas in the case of the soil No. 2, respectively ExAC = 2.78 $\text{Mg} \cdot \text{ha}^{-1}$ and *ca* 66 % of Ca_{ex} (ie, 1.84 $\text{Mg} \cdot \text{ha}^{-1}$). This implies that Ca is the main “stabilizing” factor of the exchange capacities of these soils as demonstrated by Grzebisz and Maciejewska [21] and Królak et al [22], who reported that exchangeable Ca was the first determinant of the cation exchange capacity at even 98 %, irrespective of soil pH.

Since alkaline cation displacement also took place in soil colloids, therefore exchangeability could be the intrinsic mechanisms. These reactions should operate throughout substitution or ionization of surface OH and COOH groups as suggested by Abd-Elfattah and Wada [18]. Interestingly, it should be mentioned that the direct impact of Cu, Zn and H ions over the soil buffering capacities was decidedly acute for H ion since just at 60 % (= 18.4 mmol $H^+ \cdot kg^{-1}$) and 100 (45.2 mmol $H^+ \cdot kg^{-1}$) % ExAC, respectively for soils No. 1 and No. 2, the amounts of water extractable Ca exceed those representing the ExAC (Table 1). In the case of Cu and Zn, the targeted spiking level for both soils is 150 % ExAC, which corresponds to 32.2 mmol $Cu^{2+} \cdot kg^{-1}$ and 43.8 mmol $Zn^{2+} \cdot kg^{-1}$.

Processes involved in the chemical reactions of incorporated Cu, Zn and H concern preponderantly exchange mechanisms (retention and release). Therefore one may assume that the retention of Cu, Zn and H ions induced the displacement and release of equivalent units of Ca, Mg, K and Na ions [6]. This process is the first step for soil chemical degradation by Cu, Zn and H ions and creates suitable, but only temporary, conditions for increased metals mobility and hence their potential uptake by crop plants [23] or leaching. Water extractable Ca, revealed that the amounts of Ca displaced at Cu and Zn spiking rates of 1000 % ExAC are 25 and 21 times higher, respectively, for the soil No. 1 (light loamy sand), whereas for the soil No. 2 (silty soil), 44 and 38 times higher, as compared with the control treatment. The impact of H ion was intermediate and represented 24.3 and 35 times, for the soils No. 1 and No. 2, respectively. Results reported in the paper drive specific attention on the destructive effects of Cu, Zn and H ions, separately. The joint impact of these metal ions along with hydrogen ions is the core of agroenvironmental concern. The chemical degradation established on the basis of water extractable alkaline cations (WEAC) follows the range: H > Cu > Zn.

Conclusions and statements

Investigated soils ie, light loamy sand (No. 1) and silty soil (No. 2), were characterized by an acidic pH. It was observed, that exchangeable calcium (Ca_{ex}) represented alone *ca* 56 and 66 % of all extractable alkaline cations (ExAC), respectively for soils No. 1 and No. 2. The same applies for exchangeable potassium (K_{ex}), with *ca* 32 and 20 %, respectively. Both Ca_{ex} and K_{ex} represent 88 and 86 % of the exchange potential of the studied soils, which implies that the lability of alkaline cations of these soils is significantly high.

Amounts of water extractable Cu and Zn (Fig. 2) are decidedly low as compared with those of Cu and Zn incorporated into soils and vary for the soil No. 1 within the ranges 0.6–52.1 % and 1.3–65.7 %, respectively for Cu and Zn. This corresponds to an extremely and relatively high amounts of Cu and Zn retained by the soil No. 1, ie, from 99.4 to 47.9 % for Cu and from 98.3 to 34.3 % for Zn. Quite similar ranges were observed for the soil No. 2, where the amounts of Cu and Zn retained varied respectively from 99.2 to 58.5 % and 98.6 to 36.9 %.

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OCENA CHEMICZNEJ DEGRADACJI WYBRANYCH WŁAŚCIWOŚCI GLEB POD WPŁYWEM MIEDZI, CYNKU I WODORU

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Abstrakt: Koncepcja przedstawionych badań dotyczy oceny oddziaływania miedzi (Cu), cynku (Zn) i protonów (jonów wodorowych H^+) na wybrane właściwości chemiczne dwóch gleb uprawnych: piasku gliniastego lekkiego (gleba Nr 1) i iłu pylastego (gleba Nr 2). Badane zmiany dotyczyły pH, wodnorozpuszczalnych form Cu, Zn oraz kationów alkalicznych wypartych z koloidów glebowych pod wpływem wniesienia do obu gleb jonów Cu, Zn i H. Wymienne kationy alkaliczne (ExAC = $Ca_{ex} + Mg_{ex} + K_{ex} + Na_{ex}$) ekstrahowano uprzednio przy użyciu roztworu 1 M CH_3COONH_4 przy pH 7.0, a uzyskane dane służyły do obliczenia ilości Cu, Zn i H do wniesienia do gleb w postaci: miedź jako $CuSO_4 \cdot 5H_2O$, cynk jako $ZnSO_4 \cdot 7H_2O$ i wodór w formie HCl. Dawki tych jonów odpowiadały 0, 5, 10, 30, 60, 100, 150, 200, 300, 400, 500 i 1000 % ExAC. Obiekty inkubowano przez 16 tygodni w temperaturze 18 ^{o}C oraz 75–85 % PPW (Polowej Pojemności Wodnej). Oddziaływania jonów Cu, Zn i H na chemiczne właściwości gleb ujawniły się najpierw poprzez zmiany odczynu, które były znaczące dla obiektów z jonem wodoru w porównaniu z obiektami z Cu i Zn. Wodnorozpuszczalne ilości Ca (tzn. wyparte) przy dawkach Cu i Zn wynoszących 1000 % ExAC są odpowiednio 25 i 21 razy większe dla gleby Nr 1 (piasku gliniastym lekkim), podczas gdy dla gleby Nr 2 (iłu pylastego), odpowiednio 44 i 38 w porównaniu z obiektem kontrolnym. Oddziaływanie H jest natomiast pośrednie, czyli 24 i 35 większe, odpowiednio dla gleby Nr 1 i Nr 2. Wyniki przedstawione w pracy zwracają szczególną uwagę na destrukcyjne działania jonów Cu, Zn i H stosowanych oddzielnie. Łączne oddziaływanie obu metali wraz z protonami wywołuje negatywne skutki w ekosystemach poddanych antropopresji. Szereg degradacji chemicznej oparty na ilościach wodnorozpuszczalnych kationów alkalicznych (WEAC) kształtował się następująco: H > Cu > Zn.

Słowa kluczowe: miedź, cynk, protony wodorowe, kationy alkaliczne, gleby uprawne, degradacja chemiczna

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EFFECT OF FARMYARD MANURE, SEWAGE SLUDGE AND ELEMENTARY SULPHUR APPLICATION ON THE CONTENT OF HEAVY METALS IN SOIL AND WHEAT GRAIN

WPŁYW NAWOŻENIA OBORNIKIEM, OSADEM ŚCIEKOWYM ORAZ SIARKĄ ELEMENTARNĄ NA ZAWARTOŚĆ METALI CIĘŻKICH W GLEBIE I ZIARNIE PSZENICY

Abstract: In a one-year pot experiment influence of fertilisation with elementary sulphur (0.42 g per pot containing 6 kg of soil), farmyard manure and sewage sludge (both in doses of 32 g dry matter per pot) on Fe, Cu, Zn, Mn and Cd content in soil and wheat grain was studied. The experiment comprised following variants: 1) control (not fertilised), 2) farmyard manure, 3) sewage sludge, 4) elementary sulphur, 5) farmyard manure and elementary sulphur, 6) sewage sludge and elementary sulphur.

Sulphur application did not lead to any statistical increase in the heavy metals content in soil, however it resulted in a higher content of manganese and a lower content of copper in the wheat grain.

Among studied soil characteristics only Fe content was lowered after farmyard manure fertilization while the content of Fe and Mn in grain increased. Application of sewage sludge resulted in significant increase in Fe, Zn and Cu in both soil and grain and in decrease in Mn content in grain.

The cadmium content in soil was affected by interaction of organic fertilizers and sulphur. Sulphur alone caused decrease in cadmium content in soil while together with organic fertilizing led to a significant increase.

Keywords: spring wheat (*Triticum aestivum* L.), heavy metals, elementary sulphur, farmyard manure, sewage sludge

Iron is concentrated in chloroplasts. Its complexes participate in electron transfer in the photosynthetic process. Thanks to ability to change the oxidation number iron takes part in many oxidation-reduction processes. Non-heme Fe proteins are involved in the reduction of nitrates and sulphates [1].

Zinc as a key component of many enzymes interferes in metabolism of carbohydrates, proteins, auxin, RNA and phosphate [1]. In wheat zinc can alter protein composition and lower negative effect of high temperatures during the grain filling stage [2].

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Thanks to the ability to change the oxidation number manganese plays an important role as a catalytic agent in many reactions. It is a specific component or can substitute for Mg in several enzymes. It is involved in oxygen-evolving system of photosynthesis and participates in the photosynthetic electron transport system [1].

Copper is involved in many physiological processes in plants including photosynthesis and respiration, carbohydrate distribution, reduction and fixation of nitrogen, protein metabolism. It controls water relationships as well as production of nucleic acids which reflects on reproduction ability of plants [1].

Plants supplied properly with zinc and copper show higher disease resistance, too [1]. Mn and Zn treated winter wheat plants were less attacked by *Drechslera tritici-repentis* (Died.) Shoem. causing the tan spot disease [3].

Mentioned elements are essential to plants. However, as heavy metals, in excessive amounts they can cause severe malfunctions and disturbances in many crucial processes [1].

Cadmium, on the other hand, is a typical non-essential element with negative effects on plants. Its fundamental characteristic is a strong affinity to sulfhydryl and phosphate groups, and ability of replacing zinc in some enzymes and consequently their inactivation [1].

Wheat does not belong among crops needing direct organic fertilising, effect differ from case to case. Some authors inform about significant increase in yield [4, 5], protein content and N, P, K, Fe, Zn, Mn uptake [5], whereas others observed significant yield decrease connected with organic fertilisers application [6, 7].

Organic fertilisers are used in crop systems to replace organic matter in soil which fell prey to mineralization. They preserve or even improve soil properties [7]. Besides, they serve as a source of microelements. In the case of sewage sludge, quite a large amount of different microelements and heavy metals is common which reflects in a higher total content of these metals in soil as well as in their higher bioavailability and consequently in an increasing content in plant tissues [4, 5, 8]. Plants supplied properly with sulphur can easily protect themselves from excessive metals using sulphur-containing phytochelatins. Therefore sulphur content in soil should be maintained at sufficient level. Especially under leaching conditions elementary sulphur is recommended as sulphur source [9]. However, it can significantly lower the soil pH value [10, 11] and thereby increase the heavy metals availability to plants [10–12].

Material and methods

In 2005 content of five metals in spring wheat grain was studied in a pot experiment carried out in Mitscherlich pots filled with 6 kg of sandy soil (Table 1) in the vegetation hall at Mendel University of Agriculture and Forestry in Brno.

Table 1

Soil properties prior to the experiment

C_{ox} [$g \cdot kg^{-1}$]	pH/$CaCl_2$	Content of available elements [$mg \cdot kg^{-1}$]									
		P	K	Ca	Mg	$S_{water\text{-}soluble}$	Fe	Zn	Cu	Mn	Cd
0.7	7.4	112	135	1990	113	9.5	2112	9.1	4.7	204	0.21

The experiment included the following variants: 1) control (not fertilised), 2) farmyard manure, 3) sewage sludge, 4) elementary sulphur, 5) farmyard manure + + elementary sulphur, 6) sewage sludge + elementary sulphur. The cattle manure was obtained from Agro Monet Moutnice, used sewage sludge originated from wastewater treatment plant Brno in Modřice. Each variant was established in 4 replications. The chemical composition of organic materials is shown in Table 2.

Table 2

Chemical composition of applied organic materials [$\mathrm{mg \cdot kg^{-1}}$ of fresh matter]

Organic material	Dry matter	N	P	K	Ca	Mg	S
	[$\mathrm{g \cdot kg^{-1}}$]	[$\mathrm{g \cdot kg^{-1}}$ of fresh matter]					
Sewage sludge	907	29.5	2.1	3.4	43.8	4.3	7.8
Farmyard manure	211	4.0	1.3	7.0	5.4	1.6	0.8
Organic material	OM	Fe	Zn	Mn	Cu	Cd	C:N
	[$\mathrm{g \cdot kg^{-1}}$ of fresh matter]	[$\mathrm{mg \cdot kg^{-1}}$ of fresh matter]					
Sewage sludge	565.0	51800	6810	452	347	4.1	11:1
Farmyard manure	111.0	17	189	119	19	0.6	16:1

OM – organic matter.

The pH value of used soil was determined in 0.01 mol · $\mathrm{dm^{-3}}$ $\mathrm{CaCl_2}$ solution, the P, K, Ca and Mg contents were determined according to the Mehlich III method, and the content of studied elements by means of AAS in a 2 mol · $\mathrm{dm^{-3}}$ $\mathrm{HNO_3}$ extract (soil/extract ratio was 1:10). The soil reaction was alkali, the supply of available P and Ca was good, the supply of available K and Mg was suitable and the content of water-soluble S was low. According to the Decree of the Ministry for the Environment No. 13/1994 Col. [13], the copper, zinc and cadmium contents were below the limit (the standard quotes the maximum as equal to 30 mg Cu · $\mathrm{kg^{-1}}$, 50 mg Zn · $\mathrm{kg^{-1}}$ and 0.4 mg Cd · $\mathrm{kg^{-1}}$ in light soils).

In late summer (24th August 2004), farmyard manure and sewage sludge were applied in dose of 32 g dry matter per pot (the dose was derived from 10 Mg per hectare) and elementary sulphur in a dose of 0.42 g per pot. All fertilisers were thoroughly mixed with the soil.

In spring (30th March 2005), 30 spring wheat grains, 'Vinjett' variety, were sown in each pot. One month later (29th April) the plants were thinned to 15 plants per pot. During vegetation the pots were watered at 60 % of the maximum water capacity, weeded and treated with the insecticide KARATE 2.5 WG and fungicide Falcon 460 EC.

The plants were harvested on 29th July in the stage of full maturity. After complete drying, the grain was threshed on the KMP 2 spike laboratory thresher. Contents of iron, zinc, manganese, copper and cadmium in grain (after mineralisation in $\mathrm{HNO_3}$) and soil (in 2 mol · $\mathrm{dm^{-3}}$ $\mathrm{HNO_3}$ extract) were determined by AAS method using the PHILIPS PU 9200 X apparatus.

Results were evaluated by multi-factor analysis of variance applying the STATISTICA version 7.1 software; successive tests were performed with Tukey's test of significance of differences.

Results

Elementary sulphur application alone had no impact on the heavy metals content in soil (in 2 mol · dm^{-3} HNO_3 extract) (Tables 3 and 4). However, the initial pH value of used soil was relatively high, so elementary sulphur could not change it to the degree that could increase the heavy metals availability.

Organic materials applied as fertilisers influenced statistically significant the content of Fe, Zn and Cu (Tables 3 and 4). The highest contents of mentioned elements showed variants fertilised with sewage sludge. That can be explained by higher contents of studied metals in the used sewage sludge which was applied in the same dose as farmyard manure. While in the case of Zn and Cu the lowest content was observed on controls, the lowest content of Fe showed plots fertilised with farmyard manure.

Elementary sulphur proved to affect the content of Cu and Mn in wheat grain; it led simultaneously to a Cu content decrease and a Mn content increase (Tables 5 and 6).

Different organic materials used for fertilisation influenced significantly content of Fe, Zn, Cu and Mn in wheat grain. The highest Fe content in grain showed variants fertilised with both organic materials and elementary sulphur as well as the variant fertilised with farmyard manure alone.

In variants fertilised with sewage sludge (no matter if with or without ES application) the significantly highest Zn content and the lowest Mn content in wheat grain were detetermined (Tables 5 and 6). The highest Mn content, statistically different from the Mn content in control variants, showed the variant fertilised with sewage sludge without elementary sulphur. The Cd content in grain did not vary neither according to organic fertilization nor sulphur addition.

Discussion

In their experiment Punia and Khaterpaul [14] found out that total copper and manganese contents in wheat grain were lower on variants fertilised with organic fertilisers than on variants fertilised with mineral fertilisers. In our experiment significant differences between used organic materials were noticed. Farmyard manure application did not influence Cu content and increased Mn content in wheat grain in comparison with control variants. Sewage sludge application resulted in a significant increase of Cu and decrease of Mn content.

Among variants fertilised with garbage and mushroom composts, cattle and chicken manures and municipal sludge the latter was most effective in increasing Fe, Zn and Mn content in wheat grain [5]. In our experiment similar results were noticed for Zn content. There was no statistical difference between the influence of farmyard manure and sewage sludge in the iron content in wheat grain from our experiment and in the

Table 3

Content of monitored heavy metals in soil after harvest

Variant	Fe		Zn		Cu		Mn		Cd	
	$[\mathrm{mg \cdot kg^{-1}}]$									
	$\bar{x} \pm s_x$		$\bar{x} \pm s_x$		$\bar{x} \pm s_x$		$\bar{x} \pm s_x$		$\bar{x} \pm s_x$	
Control	2239 ± 42	c	8.900 ± 0.389	a	4.228 ± 0.093	a	212.66 ± 4.12	a	0.086 ± 0.006	b
Manure	2073 ± 50	a	9.633 ± 1.199	a	4.176 ± 0.237	a	212.43 ± 8.73	a	0.071 ± 0.003	a
Sewage sludge	2394 ± 27	d	25.370 ± 2.673	b	4.934 ± 0.209	b	216.04 ± 5.29	a	0.082 ± 0.003	b
Control + ES	2174 ± 33	bc	9.960 ± 0.669	a	3.770 ± 0.218	a	203.71 ± 6.48	a	0.070 ± 0.003	a
Manure + ES	2110 ± 36	ab	10.645 ± 0.945	a	4.137 ± 0.122	a	216.27 ± 7.82	a	0.082 ± 0.005	b
Sewage sludge + ES	2396 ± 54	d	25.307 ± 1.736	b	5.027 ± 0.293	b	216.39 ± 12.73	a	0.082 ± 0.006	b

Variants marked with the same letter do not show statistically significant differences in the content of certain heavy metal ($\alpha \le 0.05$).

Table 4

Effect of studied factors on the metals content in soil

Factor	Level of factor	Fe		Zn		Cu		Mn		Cd	
		$[\mathrm{mg \cdot kg^{-1}}]$									
		$\bar{x} \pm s_x$		$\bar{x} \pm s_x$		$\bar{x} \pm s_x$		$\bar{x} \pm s_x$		$\bar{x} \pm s_x$	
Elementary sulphur	no	2236 ± 142	a	14.637 ± 8.082	a	4.446 ± 0.400	a	213.71 ± 6.00	a	0.079 ± 0.008	a
	yes	2227 ± 133	a	15.304 ± 7.473	a	4.311 ± 0.587	a	212.12 ± 10.53	a	0.078 ± 0.007	a
Organic materials	control	2207 ± 50	b	9.433 ± 0.757	a	3.999 ± 0.290	a	208.19 ± 6.94	a	0.078 ± 0.010	a
	manure	2092 ± 45	a	10.139 ± 1.136	a	4.157 ± 0.176	a	214.35 ± 7.94	a	0.076 ± 0.007	a
	sewage sludge	2395 ± 39	c	25.338 ± 2.087	b	4.981 ± 0.241	b	216.22 ± 9.03	a	0.082 ± 0.005	a

Variants marked with the same letter do not show statistically significant differences in the content of certain heavy metal ($\alpha \le 0.05$).

Table 5

Content of monitored heavy metals in wheat grain

Variant	Fe		Zn		Cu		Mn		Cd	
	[mg · kg^{-1}]									
	$\bar{x} \pm s_x$		$\bar{x} \pm s_x$		$\bar{x} \pm s_x$		$\bar{x} \pm s_x$		$\bar{x} \pm s_x$	
Control	16.534 ± 1.598	a	20.025 ± 0.660	a	1.938 ± 0.106	a	8.768 ± 1.009	a	0.013 ± 0.001	a
Manure	22.678 ± 3.309	c	18.719 ± 0.699	a	2.035 ± 0.268	ab	11.773 ± 1.079	b	0.013 ± 0.002	a
Sewage sludge	21.901 ± 1.424	bc	25.212 ± 1.583	b	2.354 ± 0.079	b	8.322 ± 0.731	a	0.009 ± 0.003	a
Control + ES	18.543 ± 0.721	ab	19.758 ± 1.090	a	1.890 ± 0.074	a	11.496 ± 0.717	b	0.014 ± 0.001	a
Manure + ES	22.887 ± 3.006	c	18.784 ± 1.168	a	1.828 ± 0.216	a	15.344 ± 2.531	c	0.010 ± 0.001	a
Sewage sludge + ES	22.514 ± 0.673	c	26.634 ± 1.475	b	2.125 ± 0.178	ab	8.649 ± 0.818	a	0.012 ± 0.006	a

Variants marked with the same letter do not show statistically significant differences in the content of certain heavy metal (α ≤ 0.05).

Table 6

Effect of studied factors on the metals content in wheat grain

Factor	Level of factor	Fe		Zn		Cu		Mn		Cd	
		[mg · kg^{-1}]									
		$\bar{x} \pm s_x$		$\bar{x} \pm s_x$		$\bar{x} \pm s_x$		$\bar{x} \pm s_x$		$\bar{x} \pm s_x$	
Elementary sulphur	no	20.076 ± 3.562	a	21.147 ± 2.981	a	2.096 ± 0.239	b	9.556 ± 1.780	a	0.012 ± 0.002	a
	yes	20.499 ± 2.575	a	21.219 ± 3.356	a	1.931 ± 0.174	a	11.732 ± 2.693	b	0.013 ± 0.003	a
Organic materials	control	17.826 ± 1.450	a	19.853 ± 0.940	a	1.907 ± 0.086	a	10.522 ± 1.571	b	0.013 ± 0.001	a
	manure	22.782 ± 2.929	b	18.751 ± 0.892	a	1.932 ± 0.251	a	13.559 ± 2.625	c	0.011 ± 0.002	a
	sewage sludge	22.208 ± 1.082	b	25.923 ± 1.607	b	2.240 ± 0.177	b	8.486 ± 0.739	a	0.011 ± 0.004	a

Variants marked with the same letter do not show statistically significant differences in the content of certain heavy metal (α ≤ 0.05).

case of manganese variants fertilised with farmyard manure showed even statistically higher content in grain than variants treated with sewage sludge.

We also did not confirm results of Cui and Wang [10] and Cui et al [11, 12] who observed significant increase in Zn availability and uptake by plants after sulphur addition.

Our results correspond to findings of Jamil Khan et al [4], since the sewage sludge application led to an increase of Fe, Zn, Cu and Mn content in soil, although the difference from the control variants was in the case of manganese not statistically significant.

Table 7

Correlation coefficients of the heavy metals content in the soil and wheat grain

Element		Grain				Soil					
		Fe	Zn	Cu	Mn	Fe	Zn	Cu	Mn	Cd	
Grain	Fe					0.23	0.49*	0.38	0.45*	0.05	Grain
	Zn	0.30				0.72*	0.70*	0.56*	0.22	0.12	
	Cu	0.48*	0.67*			0.56*	0.59*	0.43*	0.18	0.10	
	Mn	0.48*	–0.53*	–0.27		–0.45*	–0.29	–0.28	0.27	0.03	
	Cd	–0.09	–0.11	–0.11	0.02	–0.03	–0.12	–0.02	0.10	–0.22	
		Soil									
Soil	Zn	0.86*									
	Cu	0.81*	0.89*								
	Mn	0.38	0.32	0.54*							
	Cd	0.41*	0.28	0.45*	0.33						

* Cases showing statistically significant ($\alpha \leq 0.05$) correlation.

Morgounov et al [15] found a strongly positive correlation between Fe and Zn in spring wheat grain which is in contrast to our findings ($r = 0.30$) (Table 7). Chaudri et al [16] observed correlation between the total soil cadmium content and the Cd content in wheat grain. Neither this correlation was confirmed.

Conclusions

Elementary sulphur application did not influence the content of available forms of heavy metals in soil. The highest content of Fe, Zn and Cu in soil showed variants fertilised with sewage sludge. Different organic materials impact significantly on the Fe, Zn, Cu and Mn content in wheat grain whereas neither organic fertilization nor sulphur addition affected the Cd content in grain.

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WPŁYW NAWOŻENIA OBORNIKIEM, OSADEM ŚCIEKOWYM ORAZ SIARKĄ ELEMENTARNĄ NA ZAWARTOŚĆ METALI CIĘŻKICH W GLEBIE I ZIARNIE PSZENICY

Abstrakt: W jednorocznym doświadczeniu wazonowym badano wpływ nawożenia siarką elementarną (0,42 g na wazon, zawierający 6 kg gleby), obornikiem i osadem ściekowym (obydwa w dawkach 32 g s.m. na wazon) na zawartość Fe, Cu, Zn, Mn i Cd w glebie i ziarnie pszenicy. Doświadczenie obejmowało następujące warianty: 1) kontrola (bez nawożenia), 2) obornik, 3) osad ściekowy, 4) siarka elementarna, 5) obornik i siarka elementarna, 6) osad ściekowy i siarka elementarna.

Zastosowanie siarki nie doprowadziło do statystycznie znaczącego wzrostu zawartości metali ciężkich w glebie, jednak skutkowało większą zawartością manganu oraz mniejszą zawartością miedzi w ziarnie pszenicy.

Spośród badanych właściwości gleby tylko zawartość Fe obniżyła się po nawożeniu obornikiem, podczas gdy wzrastała zawartość Fe i Mn w ziarnie. Stosowanie osadu ściekowego skutkowało znacznym wzrostem zawartości Fe, Zn i Cu zarówno w glebie, jak i w ziarnie oraz zmiejszeniem zawartości Mn w ziarnie.

Zawartość kadmu w glebie była uzależniona od interakcji nawozów organicznych i siarki. Stosowanie samej siarki powodowało obniżenie zawartości kadmu w glebie, podczas gdy łączne jej stosowanie z materiałami organicznymi prowadziło do znacznego wzrostu zawartości Cd.

Słowa kluczowe: pszenica jara (*Triticum aestivum* L.), metale ciężkie, siarka elementarna, obornik, osad ściekowy

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EFFECT OF SULPHUR FERTILIZATION ON YIELDING AND TOTAL CONTENT OF NITROGEN, NITRATES(V) AND SULPHUR IN WHITE MUSTARD

WPŁYW NAWOŻENIA SIARKĄ NA PLONOWANIE ORAZ ZAWARTOŚĆ AZOTU OGÓLNEGO, AZOTANÓW(V) I SIARKI W GORCZYCY BIAŁEJ

Abstract: In 2-year pot experiments the direct and consequent effect of sulphur contained in the new fertilizer: ammonium nitrate sulphate designed for manufacturing, on yielding and the contents of nitrogen and sulphur in white mustard were analyzed. Effectiveness of the new fertilizer was compared with ammonium sulphate and NPK fertilization without sulphur. Two doses of sulphur fertilizers were applied in the 1st year of the experiment, while in the 2nd its consequent effect was researched at the identical NPK treatment. Mustard was harvested at flowering stage.

In the 1st year of the experiment increase in mustard yields depended on NPK fertilization without any significant sulphur effect. In the second year the consequent effect of sulphur led to an increase in yields by between 10 and 27 % and the greatest was registered in treatments where the new fertilizer was used. Sulphur fertilization had no significant effect on nitrogen content or the degree of this element utilization from fertilizers. In comparison with NPK treatment application of fertilizers containing sulphur caused a considerable increase in sulphur content in mustard.

Fertilization with sulphur caused an increase in nitrate(V) content in mustard shoots in the 1st year of the experiment, particularly in treatments where a double dose of this element was used. In the 2nd year of the experiment at absolutely higher $N-NO_3$ content a decline in the level of this nitrogen form was detected in plants under which sulphur was applied. The share of $N-NO_3$ in total nitrogen content in mustard ranged between 1.73 and 7.46 % in the 1st year of the experiment, whereas it was higher in the second year and ranged from 6.14 to 11.45 %.

Keywords: pot experiment, mustard, sulphur fertilization, yield, contents of sulphur, nitrogen, nitrates(V)

Considerable limiting industrial emissions over the last twenty years of the previous century in Poland and in other countries [1, 2] was particularly evident as a diminishing sulphur deposit in soils. Estimated SO_2 fall in 1980 in Poland was about 5 Tg (10^6 Mg), whereas currently registered values are over 5 times smaller [3, 4]. Under these con-

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ditions sulphur, as the basic element for plants became a deficit nutrient in soils [5–7], particularly in cultivation of plants with great demand for sulphur, often similar to the amount of absorbed phosphorus [1, 2, 5, 8]. Fertilization with this element became inevitable. It is possible owing to an extensive offer of industries manufacturing multicomponent fertilizers with various proportions of sulphur [2, 3].

The necessity of treatment with sulphur results mainly from the role this element plays in nitrogen metabolism [8, 9]. Intensive nitrogen fertilization is more efficiently utilized at proper plant supply with sulphur. It affects both increase in yield and the quantity of absorbed nitrogen [1, 7]. There are reports on the effect of sulphur fertilization on the content of nitrates(V) in plant [7, 10, 11].

Material and methods

The experiment was conducted for two years (2006 and 2007) in pots with 5 kg of air-dried, medium compact and slightly acid soil (pH in KCl = 6.1), containing 9.68 $g \cdot kg^{-1}$ of organic carbon, 0.73 $g \cdot kg^{-1}$ of total nitrogen and 0.19 $g \cdot kg^{-1}$ of total sulphur. Each year white mustard, ‘Barka’ c.v., was cultivated and harvested at the flowering stage. This vegetation stage allows for an optimal assessment of the degree of plant supply with nutrients [7, 12].

The research aimed to determine the effect of fertilization with sulphur applied in the form of ammonium sulphate and ammonium nitrate sulphate planned for manufacturing, in two doses: 0.15 and 0.30 $g S \cdot pot^{-1}$. The control was NPK treatment with chemically pure salts. Sulphur was applied in the 1st year of the experiment, whereas in the 2nd the consequent effect of sulphur was analyzed at equal NPK fertilization. Nutrient doses in both years were 1.12 g N; 0.3 g P and 1 $g K \cdot pot^{-1}$.

Each year of the experiment dry mass yield of white mustard roots and shoots was determined. This paper gives only the shoot yields.

In plant shoots total sulphur was assessed after wet mineralization in concentrated nitric(V) acid and after evaporation with HNO_3 the samples were treated with magnesium nitrate(V), evaporated again and incinerated at the temperature of 300 ^{o}C for 2 h and at 450 ^{o}C for 3 hours. After dissolving the remains in 25 % HNO_3 sulphur was determined with ICP-AES method on JY238 Ultrace apparatus. Total nitrogen was assessed with Kjeldahl method and nitrates(V) with colorimetry in phenololisulfonic acid.

The obtained results concerning yields were verified using analysis of variance and differences estimation using Duncan test at the significance level $p < 0.05$ [13].

Results and discussion

In the 1st year of the experiment yields of white mustard depended on NPK fertilization, without any significant effect of sulphur used in the fertilizers (Table 1).

Table 1

Yields of white mustard dry mass [g · pot^{-1}]

Treatment	2006 – I year	2007 – II year
	Top parts	
1. No fertilization – Control	23.85a*	5.02a
2. NPK	41.58b	19.79b
3. NPK + S1 – A	38.25b	21.70b
4. NPK + S2 – A	41.13b	21.76b
5. NPK + S1 – B	40.25b	24.91c
6. NPK + S2 – B	39.20b	25.16c

* Homogeneous groups according to the Duncan test, $p < 0.05$, A – $(NH_4)_2SO_4$; B – ammonium nitrate sulphate 26:13, S1 – 0.15 g S · pot^{-1}; S2 – 0.30 g S · pot^{-1}

This effect has been described also by other authors [7, 11, 14]. In comparison with the control treatment, without fertilizers, yields of shoots in the fertilized treatments increased by between 60 and 74 %, and most in NPK treatment.

In the 2nd year of the experiment, mustard yields were generally smaller than in the 1st year. In all treatments fertilized with sulphur a raise between 10 and 27 % in mustard yields was registered in comparison with yields obtained at exclusive application of NPK. In the second year fertilization with ammonium nitrate sulphate revealed a markedly better effect, in comparison with pure ammonium sulphate, particularly at higher sulphur dose. In their previous research conducted on the soil with similar content of sulphur the Authors did not note an increase in mustard yields in the 1st year, either. However, the consequent positive effect of these fertilizers on the amount of yields was statistically proved [11, 15]. Other authors also registered the consequent effect of sulphur fertilization as the increase in yields, particularly on heavy soils [14].

Table 2

Content of total nitrogen and N-NO$_3$ in white mustard dry mass [g · kg^{-1}]

Treatment*	Year 2006 – I year			Year 2007 – II year		
	total-N	N-NO$_3$	N-NO$_3$ in total-N [%]	total-N	N-NO$_3$	N-NO$_3$ in total-N [%]
1. No fertilization – Control	10.96	0.19	1.73	14.90	0.20	1.34
2. NPK	32.04	1.27	3.96	32.90	2.38	7.23
3. NPK + S1 – A	31.04	1.33	4.28	34.61	2.99	8.64
4. NPK + S2 – A	30.42	2.27	7.46	35.01	4.01	11.45
5. NPK + S1 – B	29.41	1.08	3.67	30.01	1.85	6.16
6. NPK + S2 – B	29.39	1.71	5.82	31.29	1.92	6.14

* See Table 1

Nitrogen content in mustard (Table 2) was affected by fertilization with this element, but sulphur addition did not significantly diversify its level in dry matter. In relation to the 1st year of the experiment, nitrogen content increased in plants cultivated in the 2nd year on treatments receiving sulphur fertilization, while the level in plants from NPK treatment remained the same. Nitrogen utilization from the applied dose was high in both years (74 % on average) and slightly diversified depending on sulphur fertilization. It confirms the results obtained by Fotyma [7] in the field experiment on crop rotation.

The content of N-NO_3 in mustard shoots in the treatments with sulphur was generally greater than when solely NPK was used in the 1st year of the experiment, particularly at the double dose of this element. In the experiments carried out by Brodowska and Kaczor [10] on wheat and rapeseed sulphur fertilization caused diminishing of nitrate(V) contents. The Authors obtained such result in their research while applying a single dose of sulphur as ammonium nitrate sulphate.

In the 2nd year of the experiment N-NO_3 content in mustard was higher than in the 1st year. In comparison with NPK treatment, in the ammonium nitrate sulphate treatments this nitrogen fraction diminished by between 19 and 22 %. In treatments fertilized with sulphur mustard contained the greatest quantities of N-NO_3 at the double ammonium sulphate dose and at the highest total N content in this treatment. It evidences nitrogen uptake stimulation by sulphur, whose considerable portion in this very treatment remained unused for organic compounds synthesis in the plant. In previous investigations sulphur fertilization caused a slight (ammonium form) or apparent (sodium salt) increase in nitrate(V) content in mustard siliques in the 1st year of the experiment. On the other hand in the 3rd year the effect of sulphur on a decrease in this nitrogen form in both plant fractions was visible [11].

The share of N-NO_3 in total nitrogen content in mustard ranged from 1.73 to 7.46 % in the 1st year of the experiment, while in the 2nd it was higher in fertilized treatments (6.14–11.45 %). The lowest share of nitrates(V) in total nitrogen was registered in mustard under which ammonium nitrate sulphate was applied. It evidences a beneficial

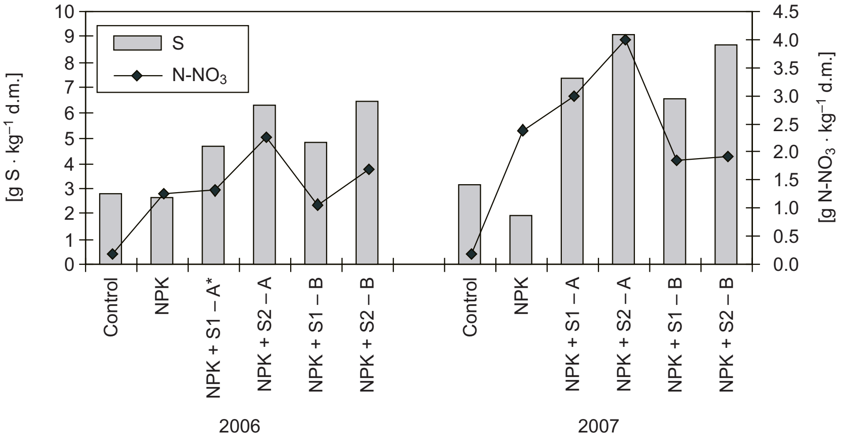

Fig. 1. Content of sulphur and nitrates(V) in white mustard
* See Table 1

effect of sulphur originating from this fertilizer in the process of nitrate reduction, also noted in other research [7, 11].

In comparison with NPK treatment, sulphur fertilization caused a considerable increase in sulphur content in mustard (Fig. 1).

Conclusions

1. In the two-year pot experiment yields of white mustard gathered at the flowering stage in the 1st year after sulphur fertilization depended on NPK treatment. Proven increase in yields in result of consequent effect of sulphur occurred only in the 2nd year of the experiment. New sulphur fertilizer – ammonium nitrate sulphate caused significantly more favourably increase in mustard yield in this year than ammonium sulphate and exclusive NPK fertilization.
2. Sulphur fertilization did not have any effect on nitrogen content in mustard or on the degree of nitrogen utilization from the applied fertilizers.
3. In the 1st year of the experiment sulphur fertilization led to an increase in nitrate(V) content in mustard shoots, particularly in treatments with a double dose of this element. In the 2nd year of the research, at absolutely higher content of $N\text{-}NO_3$ in comparison with plants fertilized exclusively with NPK a decline in this form level was observed in the plants fertilized with ammonium nitrate sulphate.
4. The share of $N\text{-}NO_3$ in the total nitrogen content in mustard ranged from 1.73 to 7.46 % in the 1st year of the experiment, it was higher in the 2nd year and ranged from 6.14 to 11.45 %.
5. Sulphur fertilization caused a considerable increase in sulphur content in shoots.

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WPŁYW NAWOŻENIA SIARKĄ NA PLONOWANIE ORAZ ZAWARTOŚĆ AZOTU OGÓLNEGO, AZOTANÓW(V) I SIARKI W GORCZYCY BIAŁEJ

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Abstrakt: W 2-letnim doświadczeniu wazonowym badano bezpośredni i następczy wpływ siarki zawartej w projektowanym do produkcji nowym nawozie saletrosiarczanie amonu na plonowanie oraz zawartość azotu i siarki w gorczycy białej. Efektywność nowego nawozu porównywano z siarczanem amonu i nawożeniem NPK bez siarki. Nawożenie siarką w dwóch dawkach stosowano w I roku doświadczenia, a w II roku badano następcze jej działanie przy jednakowym nawożeniu NPK. Gorczycę zbierano w fazie kwitnienia.

W I roku doświadczenia zwiększenie plonów gorczycy zależało od nawożenia NPK, bez znaczącego wpływu siarki. W drugim roku następcze działanie siarki spowodowało wzrost plonów o 10 do 27 %, największy w obiektach z nowym nawozem. Nawożenie siarką nie miało znacznego wpływu na zawartość azotu w gorczycy i stopień wykorzystania tego składnika z nawozów. W porównaniu z obiektem NPK stosowanie nawozów zawierających siarkę spowodowało znaczne zwiększenie zawartości siarki w gorczycy.

Nawożenie siarką powodowało w I roku doświadczenia wzrost zawartości azotanów(V) w częściach nadziemnych gorczycy, zwłaszcza w obiektach z podwójną dawką tego pierwiastka. W II roku badań, przy bezwzględnie większej zawartości $N\text{-}NO_3$, stwierdzono obniżenie poziomu tej formy azotu w roślinach, pod które stosowano siarkę. Udział $N\text{-}NO_3$ w całkowitej zawartości azotu w gorczycy mieścił się w zakresie od 1,73 do 7,46 % w I roku doświadczenia, a w II roku był większy i wynosił 6,14–11,45 %.

Słowa kluczowe: doświadczenie wazonowe, gorczyca, nawożenie siarką, plon, zawartość siarki, azotu, azotanów(V)

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NITROGEN CONTENT IN MAIZE AND SOILS FERTILIZED WITH ORGANIC MATERIALS

ZAWARTOŚĆ AZOTU W KUKURYDZY I GLEBACH NAWOŻONYCH MATERIAŁAMI ORGANICZNYMI

Abstract: With time and in result of sewage sludge application, soil physical, chemical and biological properties change which to a different degree (depending on the soil texture) affects bioavailability of nitrogen and diversifies the biological value of yield. The research aimed at an assessment of fertilization with sewage sludge and their mixtures with peat on the amount of maize biomass yield as well as contents of nitrogen in biomass and in soils with different texture. Aggregate effect in the yield of maize biomass fertilized with sewage sludge and its mixtures with peat was more advantageous than obtained in result of fertilizing with mineral salts. In comparison with organic materials and farmyard manure applied to the soil, mineral salts application notably increased nitrogen content in maize biomass. The total content of nitrogen in soils after the application of organic materials was bigger than the content assessed after the application of mineral salts.

Keywords: nitrogen, maize, soil, sewage sludge, peat

Fertilizer effectiveness of organic materials is conditioned by their nitrogen contents, particularly in mineral form [1]. Transformations in soil which nitrogen compounds undergo depend mainly on the soil pH and organic matter content. In soil characterized by a smaller acidification, the content of nitrate forms increases in relation to ammonia nitrogen content due to greater intensity of nitrification. On the other hand, in soils with higher content of organic matter not only concentrations of nitrogen ammonia forms should be higher but also nitrate content [2, 3].

Under conditions of balanced fertilization, nitrogen content in plants does not pose any greater hazard [4], however plant treatment with organic substances of waste origin may modify this relationship, therefore research in this area is necessary. Excess of mineral nitrogen, which may be released in result of waste organic substance mineralization poses a hazard not only for the environment but also causes its greater uptake which may disturb the balance in plant mineral economy.

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The use of sewage sludges causes changes of physical, chemical and biological properties of soils, which to a various degree (depending on soil texture) affects nitrogen bioavailability and may influence biological value of yield.

The investigations were conducted to assess the effect of treatment with sewage sludges and their mixtures with peat on the maize biomass yield amount and concentrations of nitrogen in the biomass and soils with different texture.

Materials and methods

The assessment of effect of fertilizing with organic materials on nitrogen content in maize and soil was conducted in a pot experiment in 2003–2005. The experimental design comprised the following treatments in four replications on three soils: object without fertilization – control (0), mineral fertilization – (NPK), farmyard manure – (OB), sewage sludge A (OŚA), sewage sludge B (OŚB) and mixtures of the sludges with peat (MOŚA, MOŚB). The "treatments" used in the research denote all experimental objects, except the one where no fertilizers were applied (0). The following soil material was used for the experiments: weakly loamy sand (GI), sandy silt loam (GII) and medium silt loam (GIII), which were collected from the arable layer (0–20 cm) of ploughlands near Krakow. The experiment made use of sewage sludges originating from two different mechanical-biological municipal treatment plants and their mixtures with peat (in weight ratio 1:1 per dry mass of organic materials). Peat with dry mass content of 408 $g \cdot kg^{-1}$ revealed the contents of 88 g ash, 34.4 g N, 0.91 g P and 1.14 $g\ K \cdot kg^{-1}$ d.m. The characteristics of chemical composition of the other organic materials and soil material (values per dry mass assessed at 105 ^{o}C) were given in Tables 1 and 2.

The pots used for the experiment contained 5.50 kg of air-dried soil material. Before the experiment outset the soils were gradually moistened to 30 % of maximum water capacity. After moistening sandy loam (GII) and medium silt loam (GIII) were limed to meet the requirement concerning pH stated by the decree on municipal sewage sludge [5]. The measure was applied separately in each pot. Chemically pure CaO was used in a dose calculated on the basis of soil hydrolytic acidity. Subsequently all soils were left for 4 weeks and water loses were supplemented occasionally. After this period organic materials were supplied in doses corresponding to 1.20 $g\ N \cdot pot^{-1}$. Phosphorus and potassium were supplemented to an equal level introduced with organic materials on all treatments (except the control) using 1.26 g P and 1.48 $g\ K \cdot pot^{-1}$ as water solutions of $Ca(H_2PO_4)_2 \cdot H_2O$ and KCl, respectively. On mineral treatment (NPK) the identical nitrogen dose was used as a water solution of NH_4NO_3, whereas equivalent doses of P and K as $Ca(H_2PO_4)_2 \cdot H_2O$ and KCl, respectively. In the second and third year of the experiment fertilizer components were used as solutions of chemically pure salts. The quantities of supplied components were 0.80 g N, 0.2 g P and 1.40 $g\ K \cdot pot^{-1} \cdot year^{-1}$.

In each year of the experiment maize (FAO 240), 'San' c.v., was cultivated and 5 plants per pot were left. Maize was harvested always at the 7–9 leaves stage. Growing period in the first year was 47 days, 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 ^{o}C) to constant weight and the yield of dry mass of aboveground parts and roots was determined. After crushing in a laboratory mill, the plant material was wet mineralized in a concentrated sulphuric(VI) acid and subsequently nitrogen was assessed with Kjeldahl method [6]. Soil material collected each year after completed growing season was analyzed with respect of changes of physicochemical properties occurring in result of applied fertilization. In dried soil material sifted through a sieve with 1 mm mesh total nitrogen content was determined after reduction of nitrate nitrogen to ammonia using the metallic zinc and iron mixture (9:1) and samples mineralization in a concentrated sulphuric(VI) acid with Kjeldahl method [6]. Assessments in the plant and soil material were made on Kjeltec II Plus apparatus (Tecator).The results were verified statistically using a fixed model, in which soil or fertilization was the factor. The statistical computations considered one-way ANOVA and the significance of differences was estimated using LAS Fisher test at significance level $p < 0.05$ [7].

Results and discussion

Organic materials used in the experiments differed with their chemical composition, including nitrogen content (Table 1). A peat supplement decreased the contents of most elements in the mixture in comparison with the content of components in sludges, except for nitrogen concentration in sewage sludge A mixture (MOŚA), which was due to a greater amount of this component in peat.

Table 1

Chemical composition of materials used in experiment

Determination		FYM (OB)	Sewage sludge A (OŚA)	Sewage sludge A + peat (MOŚA)	Sewage sludge B (OŚB)	Sewage sludge B + peat (MOŚB)
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 content						
N	[$g \cdot kg^{-1}$ d.m.]	21.6	17.0	24.7	37.4	35.1
S		7.24	8.81	6.23	14.62	7.85
P		22.60	5.48	3.00	19.32	7.64
K		26.69	2.71	1.88	2.81	1.64
Ca		4.83	15.66	13.31	9.22	11.95
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
Mn		379	129	102	277	163

The soil materials used in the experiment belonged to different texture groups, but also greatly differed with chemical properties, including total nitrogen content (Table 2).

Table 2

Some properties of soils before the establishment of the experiment

Determination				Soil		
				(GI)	(GII)	(GIII)
Granulometric composition	∅	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.]	0.96	1.25	1.72
Organic C				9.37	13.36	17.68
Total S				0.16	0.28	0.32
Available forms						
P			$[mg \cdot kg^{-1}$ d.m.]	79	217	29
K				166	359	138
Mg				134	154	126
$S\text{-}SO_4$				13.4	11.9	11.4

Average for three years maize yields (aboveground parts and roots) from treatments obtained on weakly loamy sand (GI) were apparently (over 20 %) smaller than the yields on the other soils, sandy silt loam (GII) and medium silt loam (GIII) (Fig. 1).

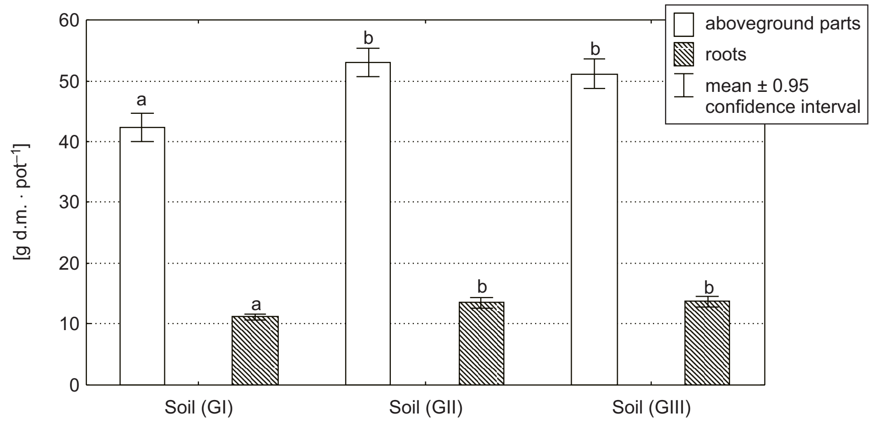

Fig. 1. Average (for 3 years) yields of maize aboveground parts and roots from individual treatments. Means followed by the same letters did not differ significantly at $p < 0.05$ according to the Fisher test

The differences in biomass yield obtained on sandy and medium loam were non significant.

Fertilization with sewage sludge or their mixtures with peat and farmyard manure allowed to obtain significantly bigger yields that on the treatment where exclusively mineral compounds were used (Table 3).

Table 3

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

Object	Yield of biomass		Nitrogen in the plant		Nitrogen in the soil
	Cz.n.*	K**	Cz.n.*	K**	
	[g d.m. · pot^{-1}]		[g N · kg^{-1} d.m.]		
No fertilization (0)	22.1 a	7.4 a	8.1 a	6.95 a	1.42 a
NPK (NPK)	42.5 b	10.1 b	19.2 d	15.68 b	1.41 a
FYM (OB)	48.0 c	13.1 cd	12.5 bc	7.81 a	1.53 a
Sewage sludge A (OŚA)	47.3 bc	11.8 c	13.1 c	7.83 a	1.49 a
Sewage sludge A + peat (MOŚA)	49.3 c	11.8 c	12.7 bc	7.88 a	1.62 a
Sewage sludge B (OŚB)	50.4 c	14.1 d	12.7 bc	7.21 a	1.43 a
Sewage sludge B + peat (MOŚB)	55.6 d	15.8 e	11.1 b	7.19 a	1.53 a

* Cz.n. – aboveground parts; ** K – roots; Means followed by the same letters in columns did not differ significantly at $p < 0.05$ according to the Fisher test.

Larger yields were gathered from mineral salt treatment (NPK) in the first year of the experiment (Fig. 2). In the subsequent years, maize response to fertilization with organic materials was positive, despite blurring the differences in soils with weakly sandy loam texture (GI) and sandy silt loam (GII). In both cases when sewage sludge with peat mixtures were used larger yields were obtained that when only the sludge was applied.

Fertilizer value of municipal sewage sludges was confirmed by numerous investigations [8, 9]. Also in the Author's own research fertilization with organic materials, including sewage sludge, produced better effects visible as greater yields of maize biomass than treatment with mineral salts. This effect cannot be wholly ascribed to the applied sewage sludges or their mixtures with peat. Apart from the after-effect of organic fertilizer activity influencing plant yields, another important factor affecting the crop yields was supplementary mineral fertilization used in the second and third year of the research. It may be supposed that on treatments where organic materials were used, the factor determining the crop yields might have been also other components supplied to the soil with organic materials, such as: sulphur, magnesium or microelements which were not balanced. Wiater et al [10] obtained worse direct effect of sewage sludge granulate on maize yield than under the influence of mineral treatment but the residual effect of granulate fertilizer activity was better. Drab and Derengowska [11] demonstrated an advantageous effect of fertilizing with sewage sludge on plant yielding,

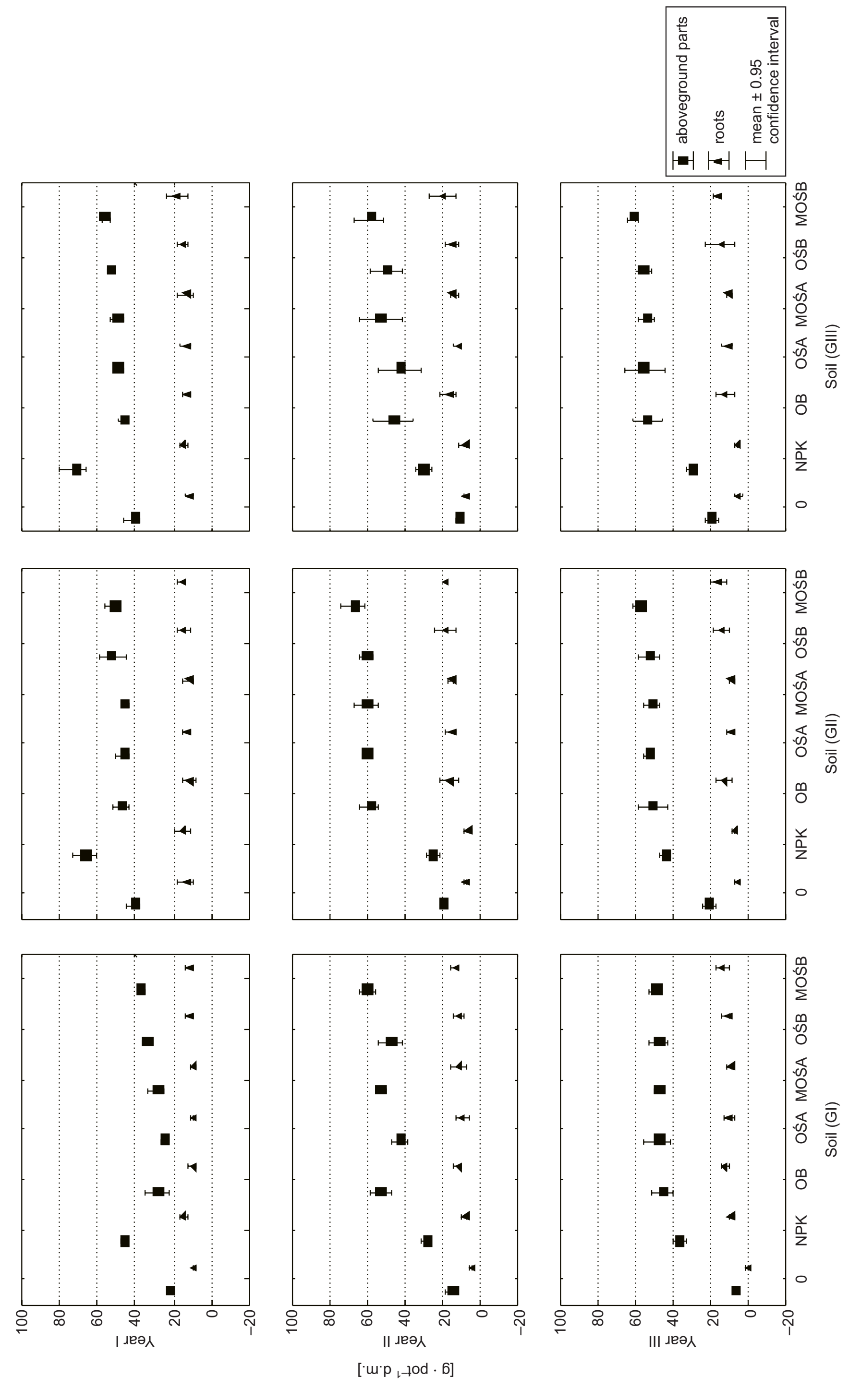

Fig. 2. Yields of maize aboveground parts and roots from three soils in each year of the experiments

at the same time showing that crop yield, irrespective of soil, was conditioned by the amount of applied sewage sludge.

The highest content of nitrogen was assessed in biomass of aboveground parts and roots from mineral salt treatment (NPK) (Table 3). Average nitrogen content for three years on this treatment was 19.2 g N · kg^{-1} d.m. and was between 46 % and 73 % bigger than the content assessed in maize shoots fertilized with organic materials. Opposite to mineral treatment, most of the nitrogen in the farmyard manure or sewage sludge occurs in organic combinations unavailable to plants. The rate of nitrogen mineral form release from organic materials is conditioned by the intensity of biological processes, which restricts, especially at the initial period, fertilizer effect of this component. Greater diversification of nitrogen content was observed in roots, where this component content on organic materials treatment was between 99 % and 118 % smaller in comparison with the content assessed in roots of plants on treatments receiving mineral fertilization with this component. No significant soil-dependent diversification in nitrogen contents in maize biomass were registered (Fig. 3) and the effect of fertilization with sewage sludge on nitrogen content in plant biomass was comparable with the influence of fertilization with mixtures of sewage sludge and peat (Fig. 4).

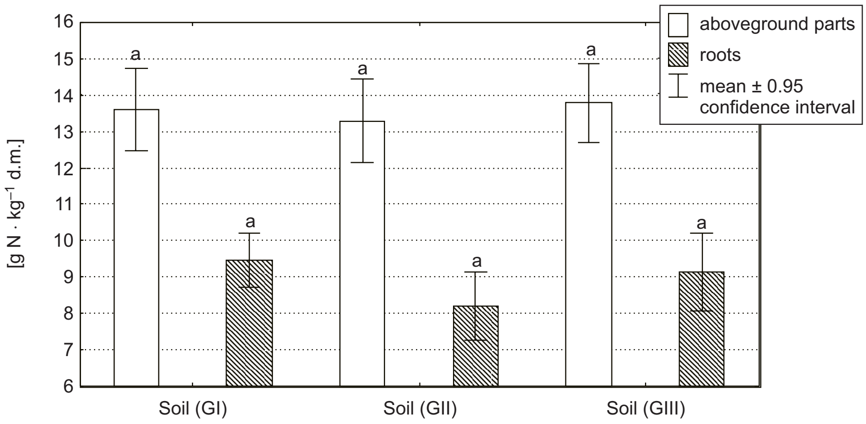

Fig. 3. Average (for 3 years) nitrogen content in maize aboveground parts and roots from treatments. Means followed by the same letters did not differ significantly at $p < 0.05$ according to the Fisher test

Former investigations conducted by Gondek and Filipek-Mazur [12] revealed that irrespective of the fertilization date or cultivated plant, or even plant part, the highest nitrogen contents were assessed in biomass of plants from treatments where mineral salts were used. Plant fertilizing with farmyard manure and sewage sludge at different dates did not apparently diversified this element content in plants. In the Author's own research, identical nitrogen dose was applied for all soils of organic and mineral salt treatments. Considering nitrogen amount which may be mobilized from the applied organic materials and its supplementing with the mineral form of this element or increasing organic material dose could have contributed to greater nitrogen uptake by plants. According to Wołoszyk and Krzywy [8] greater doses of sewage sludge lead to

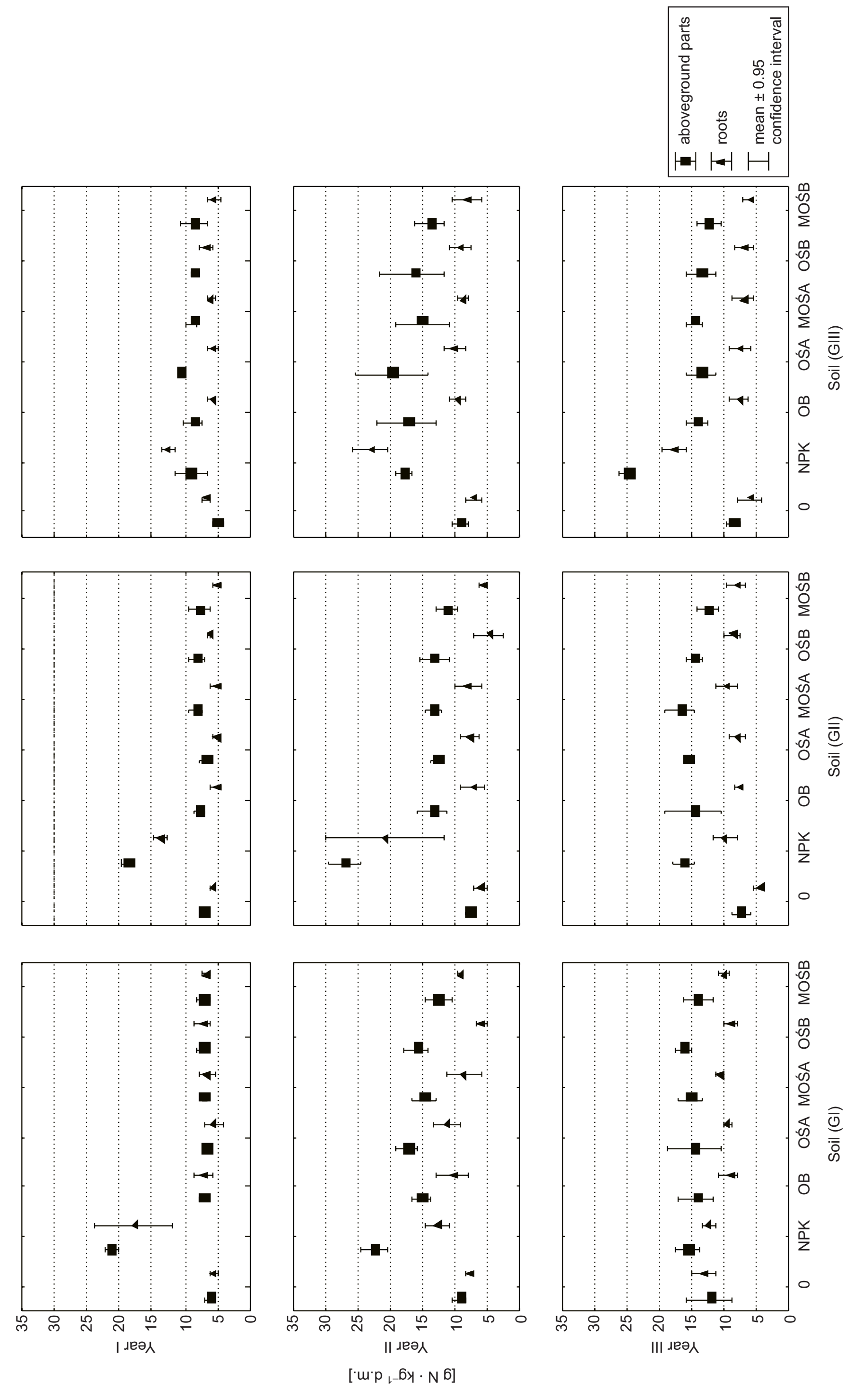

Fig. 4. Nitrogen content in maize aboveground parts and roots from three soils in each year of the experiment

increase in nitrogen content in plants. Jakubus [13] points to relatively weak effect of sewage sludge on plant chemical composition, however according to the author it depends on the kind of the sludge. In the opinion of Czuba [14] an assessment of the fertilization effect on fodder plants is difficult because of the proper selection of the right quality indicators. One of such indicators is total protein content, which increases in plants with increasing nitrogen fertilization. Analysis of maize aboveground parts biomass with respect to its feed nutritional value for ruminants showed that irrespective of the applied fertilization the analyzed biomass contained lesser amounts of crude protein per dry mass (nitrogen content multiplied by 6.25) in comparison with the value characteristic for maize for green forage at the stage of sixth leaf forming [15].

Average value of total nitrogen from the treatments was the highest in heavy soil (GIII) (Fig. 5).

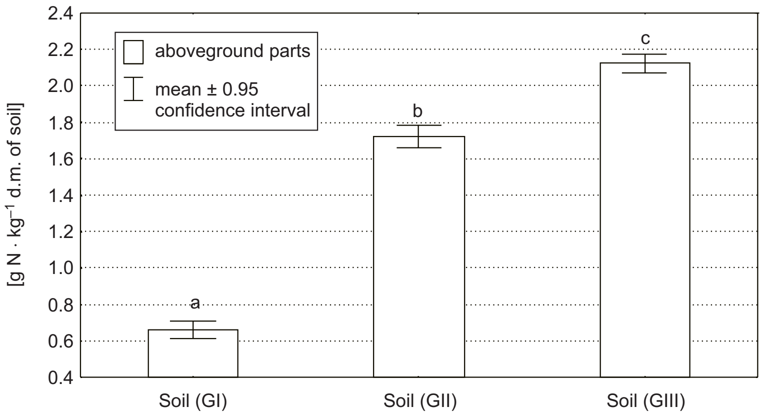

Fig. 5. Average (for 3 years) total nitrogen content in soils from analyzed treatments. Means followed by the same letters did not differ significantly at $p < 0.05$ according to the Fisher test

Mean value of total nitrogen in soils after the application of organic materials and farmyard manure was generally higher than the content assessed in soils of mineral treatments (NPK) (Table 3). The effect of fertilization with sewage sludges on the content of total nitrogen forms in soil was comparable with the effect of fertilization with mixtures of sewage sludge and peat (Fig. 6).

The soils applied for the experiment differed not only by their total nitrogen content but also by the content of organic matter. It is known that soil nitrogen content depends on organic matter content, however fertilization and the amounts of nitrogen absorbed by plants also significantly affect the quantity of this element [16–18]. Therefore, it is obvious that the applied organic materials contributed to an increase in this component quantities in soil. Also research by Wołoszyk and Krzywy [8] shows that fertilization with sewage sludge increases total nitrogen content in soil. Stępień and Adamiak [19] obtained similar results of soil fertilization with organic fertilizers.

Fig. 6. Nitrogen content in three analyzed soils in each year of the experiment

Conclusions

1. Fertilization with sewage sludge and sludge mixtures with peat significantly better affected maize yields (over the three year period of research) than treatment with mineral salts.

2. In comparison with organic materials and farmyard manure supplied to the soil, fertilization with mineral salts notably increased nitrogen content in maize biomass.

3. Mixtures of sludge and peat, as compared with the sewage sludge separately, slightly more beneficently affected maize biomass yield but did not diversify nitrogen content in the plant biomass.

4. Despite lack of significance of differences it was found that total nitrogen content in soils after the application of organic materials was greater than the quantity assessed after mineral salt use.

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ZAWARTOŚĆ AZOTU W KUKURYDZY I GLEBACH NAWOŻONYCH MATERIAŁAMI ORGANICZNYMI

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Abstrakt: Z upływem czasu w wyniku stosowania osadów ściekowych następują zmiany właściwości fizycznych, chemicznych i biologicznych gleby, co w różnym stopniu (w zależności od składu granulometrycznego gleby) wpływa na dostępność azotu dla roślin, różnicując wartość biologiczną plonu. Celem podjętych badań była ocena wpływu nawożenia osadami ściekowymi i ich mieszaninami z torfem na ilość plonu biomasy kukurydzy oraz zawartość azotu w biomasie i glebach o zróżnicowanym składzie granulometrycznym. Sumaryczny efekt w plonie kukurydzy nawożonej osadami ściekowymi i mieszaninami osadów z torfem był korzystniejszy niż uzyskany w wyniku nawożenia solami mineralnymi. W porównaniu z zastosowanymi doglebowo materiałami organicznymi i obornikiem, nawożenie solami mineralnymi znacznie zwiększyło zawartość azotu w biomasie kukurydzy. Ogólna zawartość azotu w glebach, po zastosowaniu materiałów organicznych, była większa od zawartości oznaczonej po zastosowaniu soli mineralnych.

Słowa kluczowe: azot, kukurydza gleba, osady ściekowe, torf

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BEHAVIOUR OF Cd, Cr, Cu, Pb, AND Zn IN FLUVISOL AND CAMBISOL FERTILIZED WITH COMPOSTS

ZACHOWANIE Cd, Cr, Cu, Pb I Zn W GLEBACH NAPŁYWOWEJ I BRUNATNEJ NAWOŻONYCH KOMPOSTAMI

Abstract: Risk elements contents play important role in compost quality determination. The aim of the work was to evaluate main risk elements availability in two soils with various parameters after composts application. The changes of risk elements mobility in soils fertilized with composts differed in dependence on soil parameters (risk elements contamination of soil, clay particles content, organic matter content and pH value) and on used extraction agent. Cadmium, zinc and partly chromium extracted in 0.01 mol · dm^{-3} $CaCl_2$ solution were immobilized and lead was released to soil solution of both soils. The remediation ability of composts in cadmium and lead contaminated soil was found only for cadmium.

Keywords: heavy metals, compost, soil, availability

Council Directive 1999/31/EC on the landfill of waste enacts that member states of European Union are obliged to reduce the amount of biodegradable municipal waste placing on the dumps. Composting and the application of compost to the soil follow the principle of recycling and sustainability. The major limitation of soil application of compost can be the total heavy metal contents and their bioavailability within the soil-plant system.

Because mineralization results in a reduction in organic matter content, the actual amounts of heavy metals in the finished compost usually increased during composting. This means that although the original feedstock may have acceptable heavy metal levels, the concentration in the final compost may exceed regulatory levels [1]. Total content of risk elements and especially their bioavailability in soil-plant system can be limiting factor for compost application into agricultural soil. Cadmium, zinc, and in

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lesser extent copper belong to the most mobile risk elements, respectively. On the other hand, lead and chromium are ranked among the least mobile elements [2]. The mobility of risk elements is affected especially by pH value, clay mineral content and organic matter content and its quality [3–6]. The organic matter contained in composts may provide additional sites for metal adsorption to take place, which can increase metals retention and the cation-exchange capacity in the soils and therefore composts can be convenient application for remediation of contaminated soils [7]. Knowledge of risk elements mobility in composts and subsequently in soil is very important for decision about suitability of compost application into soils of various agrochemical parameters.

Our study was implemented to assess behaviour of cadmium, chromium, copper, lead and zinc in two different soil types after 2 doses of 6 various composts application.

Material and methods

The experimental composts consisted from: 1) anaerobically stabilized sewage sludge (I and II) from two different wastewater treatment plants; 2) wood chips I obtained from leafy trees and were finely cut (< 10 mm); 3) wood chips II obtained from coniferous trees which were cut on larger pieces (< 50 mm); 4) grass originated from intensively cultivated lawn. The individual components differed in content of studied risk elements (Table 1).

Table 1

Total content of risk elements in components [mg · kg^{-1} of dry matter]

Component	Cd	Cr	Cu	Pb	Zn
Sewage sludge I	4	65	165	80	784
Sewage sludge II	7	220	173	137	1824
Wood chips I	0.1	8	7.5	3	18
Wood chips II	0.5	56	7.3	8	75
Grass	0.2	4	7.9	5	64

Thoroughly mixed materials were composted in aerated fermenters with air supply in the rate of 40 dm^3 per hour for 12 weeks. The composition of individual composts was mainly optimised according to the C:N ratio. The composition is shown in Table 2.

Table 2

Design of the experiment

Treatment	Weight portion of components in raw compost (in fresh matter)
1	Sewage sludge I (1/3) + wood chips I (1/3) + grass (1/3)
2	Sewage sludge I (1/3) + wood chips II (1/3) + grass (1/3)
3	Sewage sludge II (1/2) + wood chips I (1/4) + grass (1/4)
4	Sewage sludge II (1/2) + wood chips II (1/4) + grass (1/4)
5	Sewage sludge I (1/2) + wood chips I (1/2)
6	Grass (6/7) + wood chips I (1/7)

The effect of composts treatments was assessed in pot experiment with oat (*Avena sativa* L., ‘Atego’ cv.). Soil was passed through a 5 mm sieve, air-dried, and 5 kg of each soil (based on dry weight) was thoroughly mixed with N, P, K applied in ammonium nitrate (1 g N per pot) and potassium hydrogen phosphate (0.16 g P and 0.4 g K) in control treatments and with the same amount of nutrients plus composts from experiment described above in the rate of 48 g and triple rate of 144 g per pot. The composts for the pot experiment were sampled from the fermenters at the end of the incubation experiment. All treatments were set up in three replications. Agrochemical parameters of used soils are described in Table 3.

Table 3

Agrochemical parameters of used soil

Soil	pH $CaCl_2$	$C_{org.}$	P_{MIII*}	K_{MIII*}	Cd_{tot}	Cr_{tot}	Cu_{tot}	Pb_{tot}	Zn_{tot}
		$[g \cdot kg^{-1}]$	$[mg \cdot kg^{-1}]$						
Fluvisol	5.5	5.7	384	158	0.255	8.6	5.62	10.8	50.0
Cambisol	6.2	17.0	51.3	188	6.16	50.8	35.7	1022	228

* MIII = Mehlich III extraction.

Available portion of risk elements in compost and soil was determined in the extract of 0.01 mol · dm^{-3} $CaCl_2$ [8] and 0.11 mol · dm^{-3} CH_3COOH solutions [9]. Contents of risk elements in soils, plants, and soil extracts were determined by inductively coupled plasma optical emission spectrometry (ICP-OES, VARIAN VistaPro, Varian, Australia).

Data were evaluated by one-way analyses of variance. Tukey’s test was used for means comparison and statistical significance of hypotheses was assessed at $\alpha = 0.05$. All statistical analyses were performed using Statgraphics programme, version 5.1 [10].

Results and discussion

The changes of risk elements bioavailability in Fluvisol and Cambisol after application of composts (average of 6 treatments) in 2 doses determined in the extract of 0.01 mol $CaCl_2 \cdot dm^{-3}$ and 0.11 mol $CH_3COOH \cdot dm^{-3}$ are shown in Figures from 1 to 4.

Cadmium is ranked among the most dangerous risk element in food chain [11]. In presented study case, the total cadmium content in Cambisol exceeded significantly the threshold limits allowed in notice number 13/1994 of Ministry of Environment of the Czech Republic [12]. Soil sorption capacity for Cd was enhanced by addition of composts into Fluvisol (Fig. 1) and immobilization of cadmium extractable with 0.01 mol $CaCl_2 \cdot dm^{-3}$ was observed. The differences caused by various rates of composts were not significant. In the opposite, the increasing compost rate in Cambisol resulted in more apparent decrease of extractable cadmium content. Application of triple rate of compost with sewage sludge I and wood chips I significantly increased available Cd content in soil compared with single rate of the same compost. Higher sewage sludge sorption capacity obviously took effect. Different behavior of cadmium extracted with 0.11 mol $CH_3COOH \cdot dm^{-3}$ was found. Cadmium was released from the oxidizable

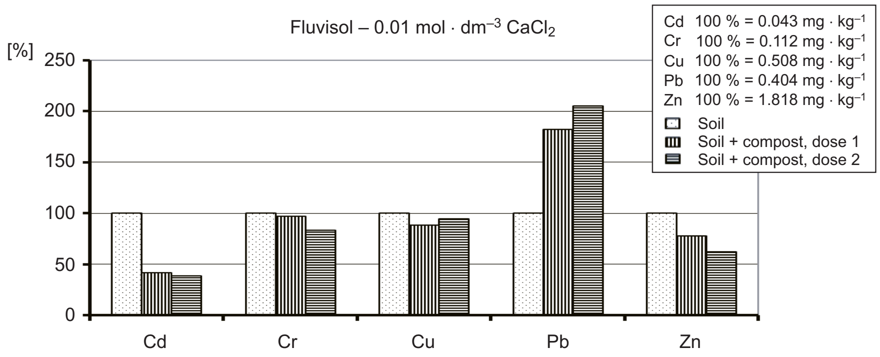

Fig. 1. Relative content of risk elements [%] determined in the extract of 0.01 mol $CaCl_2 \cdot dm^{-3}$ in Fluvisol after composts application

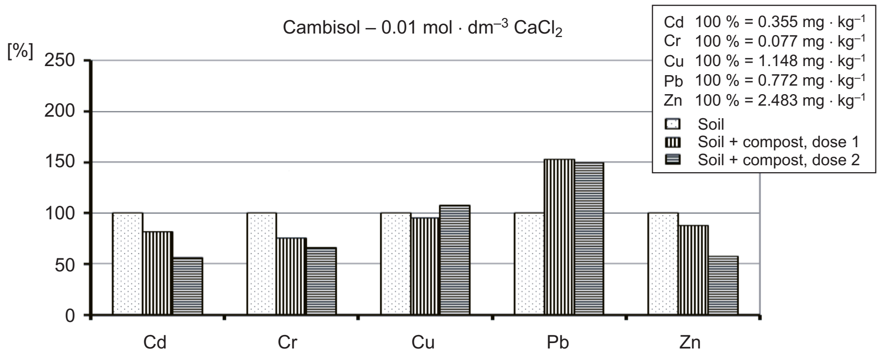

Fig. 2. Relative content of risk elements [%] determined in the extract of 0.01 mol $CaCl_2 \cdot dm^{-3}$ in Cambisol after composts application

fraction after triple rate of compost application. The effect was caused by higher mineralization of composts in the sandy Fluvisol. Decrease of Cd extracted with 0.01 mol $CaCl_2 \cdot dm^{-3}$ was found in Cambisol (Fig. 2) after triple rate of compost in relation to control soil (by 40–50 %) and simple compost rate (by 20–30 %). It is consistent with Perez-de-Mora et al [13] who found the decline of Cd released with the same extracting agent in their experiment with heavy metals contaminated soil, especially in the first two years after compost application. No statistical differences were found in Cd extracted with 0.11 mol $CH_3COOH \cdot dm^{-3}$ among treatments in Cambisol containing higher amount of organic matter and higher pH value (Table 3) compared with Fluvisol. Compost addition to the soil decreased the Cd content by 6 % compared with control.

Bioavailability of **chromium** in both soil and compost is low [14] and the effects of individual treatments on its mobility were expectably lower compared with more mobile elements. Although total contents of Cr were 6 times higher in Cambisol, the differences in extraction efficiency of used agents were not found in control treatment. Chromium extracted with 0.11 mol $CH_3COOH \cdot dm^{-3}$ was released into Fluvisol from compost by mineralization process. Relative high heterogeneity of chromium in treated soils together with low levels of extractable portions of chromium resulted in non-significant differences among the groups of experimental data.

Copper is bound predominantly in organic fraction of soil [15]. Relative contents of Cu strong fluctuated in individual treatments. However, copper was released markedly from compost containing sewage sludge I (1/2 of weight) and wood chips I (treatment 5). It was probably caused by the highest content of Cu in sewage sludge from used components. Moreover, the sewage sludge I was stabilized for shorter time compared with sewage sludge II and Cu bonds with organic matter were less stable. Significant differences in Cu content were not found between two rates of compost. Available Cu content (0.11 mol $CH_3COOH \cdot dm^{-3}$) decreased by 14 % in Cambisol fertilized with composts compared with control. It was apparently caused by higher portion of organic matter and clay particles [16].

Cambisol contained 7 times more total **lead** than is allowed in notice number 13/1994 of Ministry of Environment of the Czech Republic [12]. The contamination was mainly of anthropogenic origin. Portion of Pb extracted with 0.01 mol $CaCl_2 \cdot dm^{-3}$

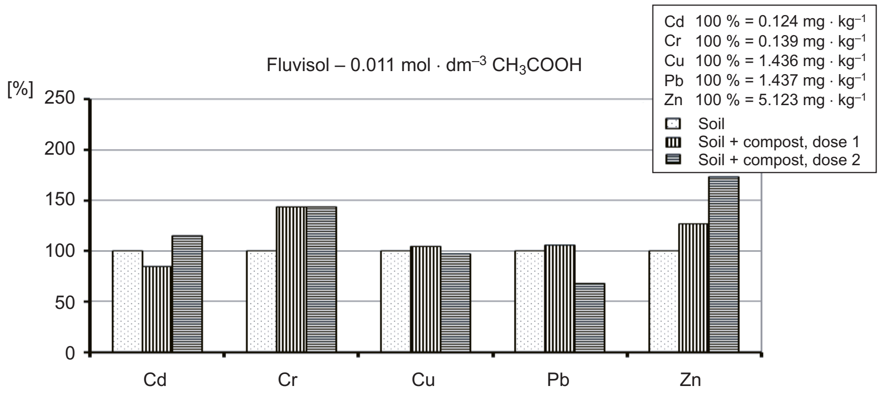

Fig. 3. Relative content of risk elements [%] determined in the extract of 0.11 mol $CH_3COOH \cdot dm^{-3}$ in Fluvisol after composts application

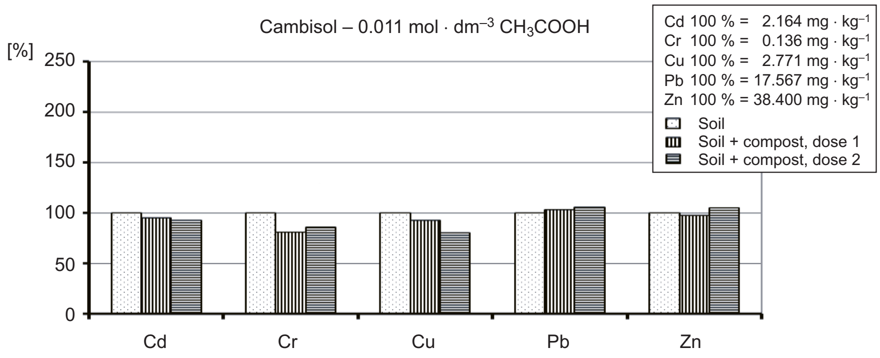

Fig. 4. Relative content of risk elements [%] determined in the extract of 0.11 mol $CH_3COOH \cdot dm^{-3}$ in Cambisol after composts application

and 0.11 mol $CH_3COOH \cdot dm^{-3}$ from this soil only 0.08 % and 1.7 %, respectively, of total content. Compost application released the weakest bound Pb. The Pb content extracted with 0.01 mol $CaCl_2 \cdot dm^{-3}$ was increased by 93 % in Fluvisol fertilized with compost. Compost application decreased Cd amount extracted with 0.11 mol $CH_3COOH \cdot dm^{-3}$ in this soil by 13 % compared with unfertilized soil. Composts applicated in triple rate (144 g per pot) participated predominantly in the decrease Cd extract concentration thanks to sufficiency of organic matter.

The availability of **zinc** was affected by individual compost rates. Zinc content extracted with 0.01 mol $CaCl_2 \cdot dm^{-3}$ was regularly decreased in both soils after application of higher rate of compost. Available Zn contents in soils fertilized with composts correlated with zinc content in used composts. It was seen eg in treatment 3 and 4 fertilized with sewage sludge II with high content of zinc. Opposite situation was observed in the case of 0.11 mol $CH_3COOH \cdot dm^{-3}$ extractable portion of zinc. Available Zn extracted with that agent was released into solution in proportion to compost rate due to convenient conditions for mineralization. Significant differences among treatments and rates were found out in light Fluvisol uncontaminated with risk elements. In regard of total Zn content was only 40 % of permissible limit given by above-mentioned notice, compost fertilization can be convenient way for supply of this important micronutrient into the soil.

Conclusions

The change of elements mobility in soil fertilized with composts differed in dependence on soil properties (risk elements soil contamination, clay particles content, amount and quality of organic matter and pH value) and used extraction agents. Application of composts decreased plant-available portions (extracted with 0.01 mol $CaCl_2 \cdot dm^{-3}$) of cadmium, zinc and partly chromium in soil. On the contrary, the application of compost resulted in more intensive release of available lead from compost to soil solution. Therefore, remediation effect of compost treatment in Cd and Pb contaminated soil was determined only for cadmium.

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ZACHOWANIE Cd, Cr, Cu, Pb I Zn W GLEBACH NAPŁYWOWEJ I BRUNATNEJ NAWOŻONYCH KOMPOSTAMI

Abstrakt: Zawartość pierwiastków szkodliwych odgrywa ważną rolę w wyznaczaniu jakości kompostu. Celem badań była ocena przyswajalności głównych pierwiastków szkodliwych w dwóch glebach o różnych parametrach po zastosowaniu kompostów. Zmiany mobilności tych pierwiastków w glebach nawożonych kompostami różniły się w zależności od właściwości gleby (zanieczyszczenia gleb pierwiastkami szkodliwymi, zawartości frakcji iłu, zawartości materii organicznej i wartości pH) i od użytego odczynnika ekstrakcyjnego. Kadm, cynk i częściowo chrom ekstrahowane roztworem 0,01 mol $CaCl_2 \cdot dm^{-3}$ były unieruchamiane, a ołów był uwalniany do roztworu glebowego obydwu gleb. Zdolność remediacyjną kompostu w glebach zanieczyszczonych kadmem i ołowiem stwierdzono tylko w przypadku kadmu.

Sowa kluczowe: metale ciężkie, kompost, gleba, przyswajalność

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SEASONAL CHANGES IN MICROBIAL ACTIVITY OF BROWN SOIL FERTILISED WITH DAIRY SEWAGE SLUDGE

SEZONOWE ZMIANY AKTYWNOŚCI MIKROBIOLOGICZNEJ GLEBY BRUNATNEJ NAWOŻONEJ OSADEM ŚCIEKÓW MLECZARSKICH

Abstract: Respiration activity and numbers of chosen group of microorganisms were determined in brown soil fertilised with dairy sewage sludge. Research were carried out in field experiment. The study comparing the effect of fertilisation with dairy sewage sludge and of mineral fertilisation on the populations of selected microbial groups and on soil respiratory activity were made. The study showed that dairy sewage sludge introduced in the soil caused similar effect on microbiological characteristics as mineral fertilization.

Keywords: brown soil, dairy sewage sludge, field experiment, mineral fertilisation

In Poland, the problem of sewage treatment, including also industrial sewage, is growing steadily, as with the increasing number of new sewage treatment plants proper wastes management is becoming an increasingly important issue, and the final utilisation of sewage sludge in particular. Due to its properties, sewage sludge is a waste that is difficult to process and utilize in a manner friendly to the environment [1, 2]. Organic and mineral compounds introduced in soil together with sewage sludge significantly affect the numbers of microorganisms whose activity in soils subjected to the effect of such wastes changes [3, 4]. Determination of the numbers of microbial groups and of the intensity of processes related with cycles with fundamental importance for soil fertility, ie carbon and nitrogen circulation, is an element included in the monitoring of the environment [5, 6]. Such tests are used in the estimation of soil

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fertility and productivity, and permit the acquisition of comprehensive knowledge on changes taking place in the soil environment [7–10].

One of the methods of determination of microbial activity of soil and changes that take place in it under the effect of natural and anthropogenic environmental factors, is the measurement of respiratory intensity [11]. Microbial activity in soil involves decomposition of organic matter and its oxidation, accompanied by emission of CO_2 [12]. The degree of microbial growth in soil depends on its physical and chemical properties, fertilisation, climatic conditions, as well as on tillage and cultivation factors, and especially on soil content of organic matter which is a source of energy and nutrients for microorganisms [10, 12]. Quantitative-qualitative studies on microorganisms are among the basic indices for the estimation of the degree of soil degradation or for improvement of its quality, and are also used for the monitoring of soils fertilised with sewage sludge [9].

The objective of the study reported herein was to make a comparison of the effect of dairy sewage sludge and mineral fertilisation on the populations of selected microbial groups and on the respiratory activity of a brown soil.

Material and method

The study comparing the effect of fertilisation with dairy sewage sludge and of mineral fertilisation on the populations of selected microbial groups and on soil respiratory activity were conducted on the basis of a field experiment. The experiment was set up on a brown soil, developed from a silty clay formation, that was characterised by the following grain size composition: 8 % of sand fraction (1.0–0.1 mm), 47 % of silt fraction (0.1–0.02 mm), and 45 % of washable particles (< 0.02 mm). The sludge used in the experiment originated from the sewage treatment plant of the Regional Dairy Cooperative in Krasnystaw. The basic characteristics of the soil and the sludge are given in Table 1.

Table 1

Characteristics of soil and dairy sewage sludge

Measurements	Unit	Brown soil	Dairy sewage sludge
pH	[-]	5.95	8.4
C	[g · kg^{-1} d.m.]	8.2	397
N	[g · kg^{-1} d.m.]	1.2	33.1
C/N	[-]	6.8	12.0
P	[g · kg^{-1} d.m.]	17.9	11.4
K	[g · kg^{-1} d.m.]	25.8	2.3

The design of the experiment included two objects – soil amended with dairy sewage sludge (1) and soil with mineral fertilisation (2). In autumn of 2004, object 1 was fertilised with dairy sewage sludge at the dose of 35 Mg · ha^{-1} (10.5 Mg d.m. · ha^{-1}), and in spring ammonium nitrate fertilisation was applied, at the dose of 200 kg · ha^{-1}, to complement of level of potassium in the soil. Mineral fertilisation in object 2 was applied as follows: in autumn – 300 kg · ha^{-1} of "polifoska" (multicomponent NPK fertiliser – 6 % N, 20 % P_2O_5, 30 % K_2O), and in spring – 150 kg · ha^{-1} of ammonium nitrate (34 % N). Both objects were sown with winter wheat. Soil samples for analyses were taken at 3 phases of plant vegetation, ie in the phases of heading, milk ripeness, and full ripeness.

Microbiological analyses included determinations of respiratory activity [13], of so-called total microbial number [14] and filamentous fungi [15], of the numbers of cellulolytic bacteria, "proteolytic" bacteria and fungi, and of ammonifying and nitrifying bacteria [22]. To investigate the effect of the experimental treatments and the times of analyses on the values of the studied microbiological features of the soils, two-factor analyses of variance were performed. Mean values of the features under analysis, for the experimental treatments and for the times of analyses, were compared by means of 95 % Tukey intervals of confidence, at the level of significance $\alpha = 0.05$. Statistical processing of results was made using the Statistica 7.1 software package. No analysis of variance was performed for the numbers of cellulolytic, ammonifying and nitrifying bacteria, as the numbers of those microbial groups were determined using McCrady tables, based on the principles of mathematical statistics.

Results

The study did not reveal any significant differences between the experimental objects in terms of respiratory activity of the soil. Significant differences were found only between the particular times of analyses. The highest respiratory activity was noted, in both experimental objects, in the phase of wheat heading, and the lowest in milk ripeness (Fig. 1).

The seasonal changes of bacteria number in both experimental objects are presented in Fig. 1. Both in the soil with the dairy sewage sludge and in that with mineral fertilisation seasonal variations were observed in the populations of the studied microbial groups. In the soil with mineral fertilisation an increasing trend was observable in the total bacterial numbers, while in the soil with the sludge an increase was noted in the numbers of those microorganisms in the milk ripeness phase, and a decrease in the full ripeness phase of winter wheat.

The presented results (Fig. 1) indicate that the numbers of fungi, throughout the period of the study, were significantly higher in the soil with the dairy sewage sludge than in that with mineral fertilisation, and displayed seasonal variations. The highest number of fungi was observed in the phases of wheat heading and full ripeness, both in the soil with the sludge and in that with mineral fertilisation. The lowest numbers of fungi, in both experimental objects, were recorded for the 2nd date of analyses, ie in the milk ripeness phase of winter wheat.

Analysis of the results presented in Fig. 1 indicates notable differences in the numbers of cellulolytic bacteria in the experimental objects. Analysis of the mean numbers of celulolytic bacteria shows that application of dairy sewage sludge caused stronger stimulation of growth of the microbial group in question compared to the soil with mineral fertilisation.

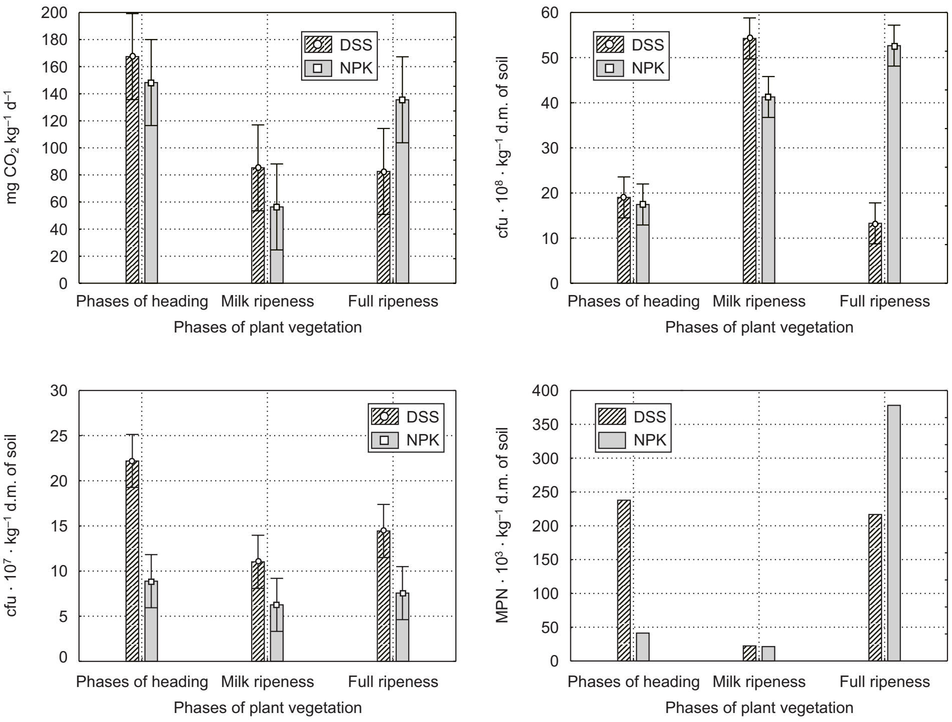

Fig. 1. Respiratory activity, total numbers of bacteria, total numbers of cellulolytic fungi and bacteria in soil; DSS – dairy sewage sludge, NPK – mineral fertilisation

Data concerning the numbers of proteolytic bacteria in the experimental objects are given in Fig. 2. The study shows that in the soil with mineral fertilisation there was a tendency towards increase in the number of proteolytic bacteria during the period of the study. On the other hand, in the object with dairy sewage sludge a decrease was observed in the numbers of those microorganisms with the progressing duration of the experiment.

Throughout the whole period of the study the numbers of the microbial groups under examination were on a somewhat higher level in the soil amended with dairy sewage sludge than in the soil with mineral fertilisation. However, seasonal changes were observed in the numbers of those microbial groups (Fig. 2). Analysis of variance did not reveal any significant differences in the numbers of the studied microbial groups, the only such differences being those between the dates of successive analyses.

Data presented in Fig. 2 indicate that on the 1st and 2nd dates of analyses (phases of heading and of milk ripeness) the number of ammonifiers was notably greater in the soil amended with dairy sewage sludge than in that with mineral fertilisation. On the other hand, on the 3rd date of analyses, ie in the phase of full ripeness of winter wheat, a greater number of the studied microorganisms was recorded in the object with mineral fertilisation. Noteworthy is the considerable drop in the numbers of the studied

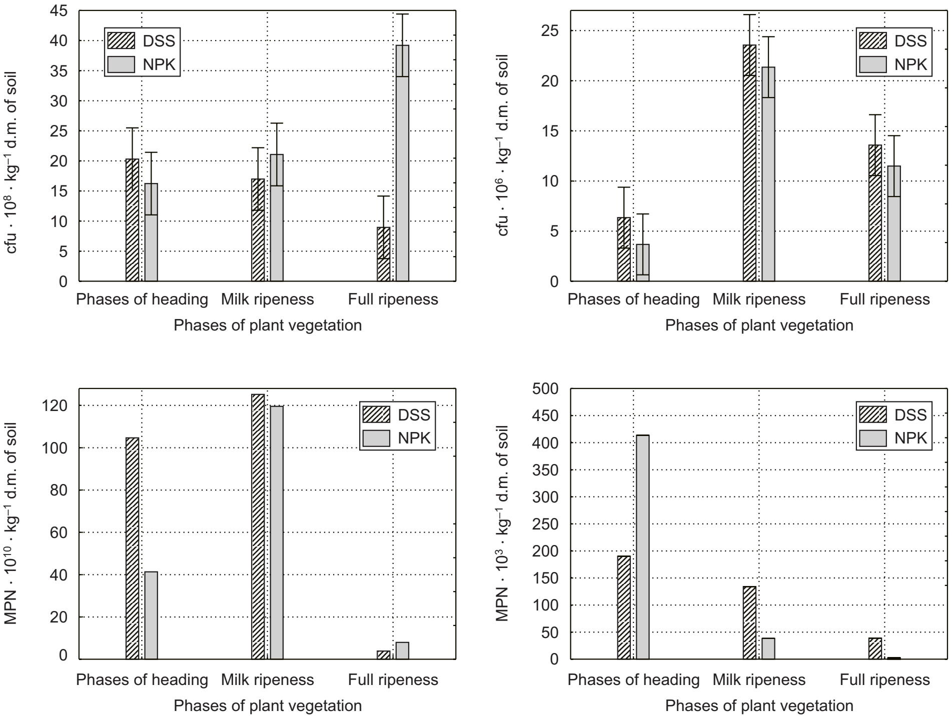

Fig. 2. Numbers of proteolytic bacteria, numbers of "proteolytic" fungi, numbers of ammonifying bacteria and numbers of nitrifying bacteria in soil; DSS – dairy sewage sludge, NPK – mineral fertilisation

microbial groups on the 3rd date of analyses, in both experimental objects. Analysis of mean numbers of the studied microorganisms for the experimental objects indicates that the number of ammonifiers in the soil amended with dairy sewage sludge was on a notably higher level compared to that in the object with mineral fertilisation.

The number of nitrifying bacteria in both experimental treatments decreased considerably with the progressing duration of the experiment, reaching the lowest level in the final stage of the study, ie in full ripeness phase of winter wheat (Fig. 2). Analysis of mean numbers of nitrifiers for the experimental objects indicates that the soil with mineral fertilisation was characterised by a greater number of nitrifying bacteria compared to the soil with the sludge. That result was largely affected by the significantly greater number of the studied microorganisms in the soil with mineral fertilisation than in that with the sludge on the 1st date of analyses, ie in the heading phase of winter wheat.

Discussion

Under conditions of improper management, organic wastes, included sewage sludge, contribute to degradation of the environment [1, 16]. Therefore, it is necessary to develop methods of utilisation of such wastes that would be safe for the environment, eg through their application in agriculture [16, 17]. From the viewpoint of ecology, an important issue is to acquire knowledge on the effect of dairy sewage sludge on microorganisms and on their activity in soils. Therefore, in the study presented herein, an attempt was undertaken at determining the effect of such wastes on the microbiological parameters of soils.

Our own studies have shown that the respiratory activity and the numbers of microbial groups in the studied soils were significantly dependent on the experimental factors applied, ie on the fertilisation and the time of its effect in the soil. The results of studies indicate stimulating effects of dairy sewage sludge with relation to soil microorganisms. That effect should be attributed to soil enrichment with organic matter, total nitrogen and mineral components, which has been indicated by numerous authors [10, 18–21] who observed stimulation of microbial growth in soils amended with sewage sludge. The significant increase in the numbers of bacteria in the treatment with sewage sludge could have been a result of selection of bacterial populations utilising the available nutrients occurring in the sludge. Stimulation of so-called total bacterial population by sewage sludge has been observed by Furczak and Joniec [12] in a laboratory experiment. Increase of bacterial and fungal populations in soil under the effect of sewage sludge has been recorded in a study by Gostkowska et al [23] under field conditions. In the course of discussed studies was also demonstrated statistically confirmed increase of populations of the microbial groups under study in soil amended with dairy sewage sludge. However, the acquired data indicate that the changes in the bacterial and fungal populations were also affected by the development phase of the crop plant. The obtained results in the study indicate that the introduction of dairy sewage sludge had a positive effect on the populations of soil bacteria and fungi in comparison with the mineral fertilisation applied.

A distinctly stimulating effect of dairy sewage sludge on populations of cellulolytic bacteria in spring, and weakening in subsequent months of analyses, was observed in a study by Jezierska-Tys et al [24]. Comparing the numbers of cellulolytic bacteria in a soil amended with dairy sewage sludge at the dose of 22 $\mathrm{Mg \cdot ha^{-1}}$ to those in a treatment with mineral fertilisation, they found that the growth of those microorganisms was stronger in the treatment with dairy sewage sludge. The results obtained in our study indicate participation of cellulolytic bacteria in breaking up carbon complexes occurring in dairy sewage sludge introduced in the soil.

Our own studies indicate that dairy sewage sludge had a stimulating effect on the growth of “proteolytic” microorganisms. Dairy sewage sludge is a rich source of organic nitrogen compounds [25], hence the stimulation of growth of “proteolytic” bacteria and fungi, observed in this study, was likely due to soil enrichment in organic nitrogen compounds of sludge origin, constituting a source of nutrients for that microbial group. The acquired data indicate that dairy sewage sludge had more favourable effect on the growth of “proteolytic” bacteria and fungi compared with mineral fertilisation. The weakening effect of the applied waste on the numbers of soil microorganisms with progressing duration of the experiment should be probably attributed to exhaustion of nutrient substrates available to those microbial groups.

Another microbial group whose population increased following the introduction of dairy sewage sludge was that of nitrifying bacteria. It was demonstrated that the sludge introduced in the soil had more favourable effect on the population of nitrifying bacteria compared with mineral fertilisation. In the course of the vegetation season a decrease was observed in the numbers of those microorganisms in the soil amended with dairy sewage sludge, which was probably related with depletion of substrates for that microbial group. Mazur [26] emphasizes that the presence of nitrifier activity in itself is an obvious indicator of soil properties favourable to crop plants, due to the high requirements of that microbial group with respect to available nutrients, its sensitivity to soil acidity and insufficient aeration. It can also be accepted that the mobility of nitrates(V), uninhibited by processes of sorption, is conducive to the uptake of that form of nitrogen by plants as compared with the ammonium form. It appears, therefore, that stimulation of the growth of nitrifying bacteria as a result of soil amendment with dairy sewage sludge indicates also a favourable effect of that waste on soil properties.

Conclusions

Dairy sewage sludge effected the seasonal changes of microbial activity of brown soil. The presented study showed that dairy sewage sludge had a favourable effect on the microbiological properties of brown soil. The study showed that soil amendment with dairy sewage sludge had a favourable effect on the microbiological equilibrium of the soil compared with the soil with mineral fertilisation, which is evidenced by more intensive growth of most of the analysed microbial groups in the soil with sludge than in the soil with mineral fertilisation.The microbiological and biochemical tests applied proved to be sensitive indicators of the biological properties of soil amended with dairy sewage sludge.

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SEZONOWE ZMIANY AKTYWNOŚCI MIKROBIOLOGICZNEJ GLEBY BRUNATNEJ NAWOŻONEJ OSADEM ŚCIEKÓW MLECZARSKICH

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Abstrakt: Celem przeprowadzonych badań było porównanie oddziaływania osadu ścieków mleczarskich i nawożenia mineralnego na liczebność wybranych grup mikroorganizmów i aktywność respiracyjną gleby brunatnej. Wzbogacenie gleby osadem ścieków mleczarskich wywarło korzystny wpływ na jej równowagę mikrobiologiczną w porównaniu z glebą nawożoną mineralnie, o czym świadczy bardziej intensywny rozwój większości analizowanych grup drobnoustrojów w glebie z osadem niż nawożonej mineralnie.

Słowa kluczowe: doświadczenie polowe, gleba brunatna, nawożenie mineralne, osad ścieków mleczarskich

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CHANGES IN VALUES OF BASIC INDICATORS OF SOIL ACIDIFICATION AS THE EFFECT OF APPLICATION OF SEWAGE SLUDGE AND FLOTATION LIME

ZMIANY WARTOŚCI PODSTAWOWYCH WSKAŹNIKÓW ZAKWASZENIA GLEBY W EFEKCIE STOSOWANIA OSADU ŚCIEKOWEGO I WAPNA POFLOTACYJNEGO

Abstract: The variability of selected factors of soil acidification: soil reaction (pH_{KCl}), exchange acidity and mobile aluminium were analyzed under the conditions of sewage sludge and flotation lime influence. The research was based on a two-year strict pot experiment, which was set up using complete randomization method, on soil material obtained from the vicinity of a sulphur mine. Prior to the experiment the soil was characterized by a very acidic reaction, an exchange acidity (Hex) value of 19.92 mmol(+) $\cdot$ kg^{-1}, mobile aluminium content of 8.60 mmol(+) $\cdot$ kg^{-1}, and moreover, by a low content of available phosphorus and potassium, very low content of available magnesium and high content of $S\text{-}SO_4$. The results obtained show that applied experimental factors (sewage sludge, flotation lime) positively influenced the changes in the analyzed soil properties. Under the influence of liming, the value of soil pH was changed distinctly and, in consequence, the soil reaction was changed from very acidic to acidic or slightly acidic. Moreover, soil liming caused a decrease in the values of exchange acidity and mobile aluminium in the examined soil. The influence of sewage sludge on the analyzed soil properties, in most objects, was also beneficial but less distinct than in the case of flotation lime.

Keywords: soil acidification, sewage sludge, flotation lime, soil reaction, exchange acidity, mobile aluminium

The extraction of sulphur by underground melting method causes the considerable changes in the environment, including chemical degradation of soils situated around

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sulphur mines [1–3]. One of the many negative effects of environment transformation is undoubtedly strong acidification of soils from these territories and that is why the basic task to improve soil environment is neutralization of acidic reaction [4]. Because of that fact waste substances, including flotation lime, found application in the restoration of the territories of former mines [5]. Moreover, organic sewage sludge has been proposed as useful in the process of soil structure restoration [6, 7]. The additional advantage of this method is high fertilization value [8].

The performed studies were aimed to analyse the effect of the flotation lime and sewage sludge on the selected indicators of acidification of soil, reaction (pH), exchange acidity (Hex) and mobile aluminium, obtained from the vicinity of a former sulphur mine 'Jeziórko'.

Material and methods

The experiments were conducted based on the results of a two-year strict pot experiment carried out in the period 2004–2005. The experiment was performed using complete randomization method on soil material obtained from a vicinity of a former sulphur mine. Prior to the experiment the soil with the granulometric composition of light loamy sand was characterized by the following properties: pH_{KCl} – 3.57, Hex – 19.92 mmol(+) $\cdot$ kg^{-1}, mobile aluminium content – 8.60 mmol(+) $\cdot$ kg^{-1}, as well as by a low content of available phosphorus and potassium, a very low content of available magnesium and high content of $S\text{-}SO_4$. Two variable factors were used in the experiment: a dose of sewage sludge and a dose of flotation lime. Both factors were applied on three levels, according to the following pattern:

1. Ss_0Ca_0 4. Ss_1Ca_0 7. Ss_2Ca_0
2. Ss_0Ca_1 5. Ss_1Ca_1 8. Ss_2Ca_1
3. Ss_0Ca_2 6. Ss_1Ca_2 9. Ss_2Ca_2,

where:

Ss_0 – without sewage sludge application;
Ss_1 – sewage sludge applied in the amount of 10 g $\cdot$ kg^{-1} soil;
Ss_2 – sewage sludge applied in the amount of 20 g $\cdot$ kg^{-1} soil;
Ca_0 – no liming;
Ca_1 – flotation lime in the amount of 3.096 g $\cdot$ kg^{-1} soil;
Ca_2 – flotation lime in the amount of 6.192 g $\cdot$ kg^{-1} soil.

The lower sewage sludge dose constituted 1 % of soil mass in the pot, whereas the higher one – 2 %. Under the field conditions, taking into considerations the mass of 20 cm of soil layer in the area of 1 ha, these doses corresponded to 30 Mg (Ss_1) and 60 Mg $\cdot$ ha^{-1} (Ss_2). The lime doses were calculated on the base of hydrolytic acidity (Hh), taking 0.75 Hh for the lower dose and 1.5 for the higher one.

Sewage sludge used in the experiment had been hygienized and stabilized and had a pH_{KCl} value of 8.30. The flotation lime applied in the experiment contained 429.14 g CaO $\cdot$ kg^{-1}.

In the first year of the experiment barley, 'Cwał' variety, was cultivated on the analysed soil and in the second year – spring rape, 'Mozart 00' variety. The plants were cultivated in 8 replications. The harvesting of overground parts of plants took place in the phase of heading (oat) or flowering (rape) and in the phase of full maturity (oat, rape). In all the phases the harvesting of plants was carried out in four replications. All experiment objects were also constantly fertilized with NPK and a microelement solution, in the amounts adjusted to the plants' nutritional needs. The following parameters were determined in the soil material obtained prior to the experiment and in the second year of the experiment, after the rape harvest in the flowering and full maturity phase: pH in 1 mol · dm^{-3} KCl solution determined potentiometrically with a glass electrode and exchange acidity and mobile aluminium – determined using Sokolow method in the extract of 1 mol KCl · dm^{-3}.

Results and discussion

The sewage sludge and flotation lime used in the experiment favourably affected the changes in the values of analysed indicators of soil acidification (Table 1).

Table 1

The effect of sewage sludge and flotation lime application on the value of selected indicators of soil acidification

Determination / Object	Reaction	Exchange acidity (Hex)	Mobile aluminium
	pH_{KCl}	[mmol(+) · kg^{-1}]	
Soil after rape harvest in the flowering phase			
Ss_0Ca_0	3.62	14.70	4.70
Ss_0Ca_1	4.66	3.10	0.80
Ss_0Ca_2	5.62	1.90	0.50
Ss_1Ca_0	3.72	11.90	4.80
Ss_1Ca_1	4.60	2.70	0.70
Ss_1Ca_2	5.78	2.20	0.60
Ss_2Ca_0	3.81	10.80	4.10
Ss_2Ca_1	4.68	3.10	0.90
Ss_2Ca_2	5.65	1.40	0.10
Soil after rape harvest in the full maturity phase			
Ss_0Ca_0	3.68	13.70	5.60
Ss_0Ca_1	4.35	3.70	1.30
Ss_0Ca_2	5.46	1.40	0.40
Ss_1Ca_0	3.77	12.50	5.50
Ss_1Ca_1	4.51	2.60	0.80
Ss_1Ca_2	5.54	1.60	0.70
Ss_2Ca_0	3.82	10.50	4.20
Ss_2Ca_1	4.60	2.20	0.40
Ss_2Ca_2	5.63	1.60	0.40
Before the experiment	3.57	19.92	8.60

Explanation: Ss – sewage sludge; Ca – liming.

The soil reaction is an indicator which correlates with the content of basic cations in the soil, thus supplying of them with the flotation lime aims to stabilization and optimization of soil reaction. As the effect of many-year sulphur contamination of soil, unfavourable changes in the saturation of sorption complex occur [9], which leads to the decrease of soil pH value. The procedure of soil liming affects its reaction and leads to the increase of pH value [10, 11]. In the performed experiment de-acidifying properties of liming were observed in the all experimental objects, in which this procedure was applied. The values of pH_{KCl} of soil in liming objects place in the range of 4.35–5.65 in comparison with the values in the objects without liming, which fluctuated from 3.62 to 3.82. The lower dose of liming caused the change of soil reaction from very acidic to acidic, whereas the higher one to slightly acidic. It was reflected in the pH values, which amounted to 4.35–4.68 in the objects with the lower dose of liming and 5.46–5.78 with the higher one.

Accompanying the change in soil reaction, the values of exchange soil acidity and mobile aluminium were also improved in the effect of liming. In both cases a considerable decrease of values of analysed acidification indicators was observed. Similarly as in the case of pH value, the favourable effect took place in all the experimental objects. The liming applied in the lower dose caused on the average four-times decrease of exchange acidity in comparison with the values from control objects. The higher dose of flotation lime resulted in 7.5-time decrease in exchange soil acidity value when compared with not-limed objects.

Studies by other authors [11, 12] indicate that liming is the most effective method to lower the concentration of mobile aluminium ions. In the performed experiment, in the effect of application of this procedure, the content of mobile aluminium decreased to trace amounts, similarly as reported by other authors [13–15]. In the objects where lime was applied in the amount of $3.096 \text{ g} \cdot \text{kg}^{-1}$ of soil, these values ranged from 0.4 to 1.3 $\text{mmol(+)} \cdot \text{kg}^{-1}$ and decreased 6.5-times on average in comparison with values from the objects with no liming. The soil which was limed with the higher dose of flotation lime was characterized with the content of mobile aluminium in the range of 0.1–0.7 $\text{mmol(+)} \cdot \text{kg}^{-1}$. In this case the average decrease was 15-times when compared with the values from Ca_0 objects. The highest, 41-times decrease in the content of mobile aluminium was observed in the objects Os_2Ca_2, in the soil samples, taken in the phase of rape flowering.

The effect of sewage sludge, applied in the experiment, on the change of analysed soil acidification indicators was less explicit and less unambiguous than in the case of flotation lime. Supplying organic matter to the soil does not usually cause considerable changes in soil reaction [16]. In the performed experiment soil pH values from the objects where sewage sludge was applied insignificantly increased. The average increase of pH values caused by application of sewage sludge was about 1.5 % in comparison with control objects. This tendency occurred both for lower and higher doses of sewage sludge. The increase of soil pH value in the effect of application of sewage sludge was also observed by other authors [17, 18].

The values of exchange acidity were favourably affected by application of sewage sludge. In most of objects where sewage sludge was applied in lower dose, the values of exchange acidity were lower of about 17 % in comparison with control objects and of about 29 % when higher dose of sewage sludge was applied. The inconsiderable increase of exchange acidity value in comparison with the values form objects with no sewage sludge was observed in the objects Ss_1Ca_2, in the soil samples taken from under plants harvested both in the phase of flowering and full maturity and in the objects Ss_2Ca_2 in the soil samples taken from under plants harvested in the phase of full maturity. The increase of exchange acidity value caused by the application of living-farming-industrial sewage sludge was also observed by Grzywnowicz and Strutyński [19].

The sewage sludge caused the favorable change of the content of mobile aluminium in soil to a lesser degree than liming. The highest 5-times decrease of the content of mobile aluminium in the test soil in comparison with the control objects was observed in the combination Ss_2Ca_2, in the soil samples taken from under plants harvested in the phase of flowering and about 3-times in the objects Ss_2Ca_1 with the single dose of lime. In the other objects lower decrease in the content of mobile aluminium in soil was observed and in some objects – even insignificant increase in the value of this indicator. Generally, it may be concluded, that the lowest content of mobile aluminium was observed in soil samples in the series both limed and fertilized with sewage sludge. It is an evidence for the favorable cooperation of both experimental factors on the content of mobile aluminium in soil.

Conclusions

1. The applied waste substances, both flotation lime and sewage sludge, have positively affected the analysed indicators of soil acidification.
2. The application of flotation lime has contributed to a change of soil reaction from very acidic to acidic and slightly acidic which is optimal for the growth of some cultivated plants.
3. The effect of flotation lime has also resulted in a substantial decrease in the exchange acidity of the analysed soil and in the soil content of mobile aluminium.
4. The sewage sludge has affected the changes in the values of analysed soil acidification indicators to a lesser extent than flotation lime.

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ZMIANY WARTOŚCI PODSTAWOWYCH WSKAŹNIKÓW ZAKWASZENIA GLEBY W EFEKCIE STOSOWANIA OSADU ŚCIEKOWEGO I WAPNA POFLOTACYJNEGO

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Abstrakt: Przeanalizowano wpływ osadu ściekowego oraz wapna poflotacyjnego na zmiany w wartościach wybranych wskaźników zakwaszenia gleby: odczynu (pH), kwasowości wymiennej (w) i glinu ruchomego. Badania prowadzono w ścisłym dwuletnim doświadczeniu wazonowym, które założono metodą kompletnej randomizacji, na glebie pochodzącej z okolic kopalni siarki. Gleba przed doświadczeniem charakteryzowała się bardzo kwaśnym odczynem (pH_{KCl}), wartością kwasowości wymiennej wynoszącej 19,92 mmol(+) $\cdot$ kg^{-1} oraz zawartością glinu ruchomego – 8,60 mmol(+) $\cdot$ kg^{-1}, a ponadto małą zawartością przyswajalnego fosforu i potasu, bardzo małą zawartością przyswajalnego magnezu oraz dużą zawartością S-SO_4. Uzyskane wyniki wskazują, że zastosowane w doświadczeniu czynniki zmienne (wapno poflotacyjne, osad ściekowy) korzystnie wpłynęły na zmiany analizowanych właściwości gleby. Pod wpływem wapnowania wyraźnemu podwyższeniu uległa wartość pH gleby, w wyniku czego odczyn z bardzo kwaśnego zmienił się w kwaśny lub lekko kwaśny. Ponadto wapnowanie spowodowało zmniejszenie wartości kwasowości wymiennej i zawartości glinu ruchomego w badanej glebie. Wpływ osadu ściekowego na analizowane właściwości glebowe był również w większości obiektów korzystny, jednakże zmiany ich wartości wywołane tym czynnikiem były mniej widoczne.

Słowa kluczowe: zakwaszenie gleby, osad ściekowy, wapno poflotacyjne, odczyn gleby, kwasowość wymienna, glin ruchomy

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EFFECT OF WASTE ROCK WOOLS ON THE SPRING BARLEY (*Hordeum vulgare* L.) YIELD AND SOME SOIL PARAMETERS

ODDZIAŁYWANIE ODPADÓW WEŁNY MINERALNEJ NA PLONOWANIE JĘCZMIENIA JAREGO (*Hordeum vulgare* L.) I NIEKTÓRE WŁAŚCIWOŚCI GLEBY

Abstract: The effect of two recycled waste rock wools (Nobasyp and Agrodrap) on the yield parameters of spring barley and some agrochemical soil parameters has been studied in a pot trial conducted in a vegetation cage located at the SAU in Nitra (48°18′ N, 18°05′ E) on the Haplic Luvisol (25 kg soil per pot).

Achieved results have shown that the application of both kinds of rock wool had a positive but not statistically significant effect on the grain and straw yield of spring barley. The Nobasyp effect on the yield was more positive than the one of Agrodrap. Combined application of Nobasyp (20 Mg · ha^{-1}) and NPK fertilizers resulted in the highest grain and straw yields. On the other hand, combined application of Agrodrap and NPK fertilizers (under the use of both doses – 10 and 20 Mg · ha^{-1}) was not well-founded, because the yields achieved by interactive effect of NPK fertilizers and Agrodrap were lower than the ones achieved by solely use of NPK fertilizers. The rock wool + NPK fertilizers application inhibited the negative effect of nitrogen on increase of crude protein content and the decrease of starch in the barley grain.

Both kinds of rock wool significantly increased the cation exchange capacity, made the soil lighter, moderately increased pH of the soil and have had a positive effect on the total carbon content and the organic matter quality. The positive effect of Nobasyp on quantitative and qualitative parameters of spring barley and some soil parameters enables Nobasyp to be accepted not as a waste product but as a soil remediate substance, or an indirect fertilizer.

Keywords: rock(basalt) wools, soil amendment, indirect fertilizers (soil amended materials), spring barley (*Hordeum vulgare* L.)

Information about the application of rock wools in the field production is very rare [1, 2] even though they are marked out by high porosity – more than 90 %, good water capacity which never decreases below 80 %, elasticity of 95 % as minimum, 200 % and

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more absorbability. Their use has been connected mainly with the hydroponic plants growing [3, 4]. Kováčik [5] introduces their application as components of substrate for green roofs, sports and recreation areas, or for growing the carpet grass.

The goal of presented research was to learn the effect of two nearly identical recycled, not used basalt wools (Agroban and Nobasyp) – produced by the company of Izomat Nová Baňa for the use in hydroponic plant growing and for construction industry – on yield parameters of model crop (spring barley) and on some agrochemical soil parameters.

Material and methods

The effect of two recycled waste rock wools (Nobasyp and Agrodrap) on the yield parameters of spring barley and some agrochemical soil parameters has been studied in a pot trial conducted in a vegetation cage located at the Slovak Agricultural University in Nitra (48°18′ N, 18°05′ E) on the modal brown soil (Haplic Luvisol).

Nobasyp is a commercial name for loose thermoinsulating material sold as Nobasil. Nobasyp means loose Nobasil. It is produced as a result of milling (recycling) of Nobasil which has not met the requirements of the consumer (shape, thickness, colour, etc.). Agrodrap can be obtained by scrapping the pieces of garden rock wool sold as Agroban. Agroban means scrapped Agrodrap. Agrodrap is produced with the aim to evaluate Agroban which is made with different parameters from the ones the buyer requires.

There have been weighed out 23.5 kg of Haplic Luvisol into containers of 30 kg capacity. The agrochemical and hygienic-toxicological parameters of the soil are given in the Table 1.

Soil pH was measured in H_2O and 1 mol · dm^{-3} KCl solution (exchangeable soil reaction). Ammonium nitrogen and nitrate nitrogen were analysed colorimetrically: NH_4^+-N after reaction with Nessler agent and NO_3^--N after reaction with phenol--2,4-disulphonic acid. Inorganic nitrogen N_{an} was calculated as sum of N-NH_4^+ + + N-NO_3^-. Available phosphorus, potassium and magnesium were extracted according to Mehlich II method and next P was determined colorimetrically, K using flame photometry and Mg was determined with atomic absorption spectrophotometry. Total carbon content C_{ox} was analysed by Tiurin method, and carbonates $CaCO_3$ – volumetrically. Heavy metals Cd, Pb, Hg, As, Cr and Ni were determined with atomic absorption spectrophotometry after mineralization by acids mixture (HF + $HClO_4$).

100 spring barley (*Hordeum vulgare* L.) seeds, ‘Express’ var., have been sown into each container and the soil surface has been sandblasted by sterile sand (1.5 kg). The total weight of the soil was 25 kg. After the seeds have germinated, the number of plants per a container has been reduced to 75. The level of soil humidity has been kept by regular watering on the value of 60 % of full water capacity.

There have been 8 variants: 0 – control; NS_1 – Nobasyp dose of 20 Mg · ha^{-1}, AD_1 – Agrodrap dose of 20 Mg · ha^{-1}; NPK – the dose of NPK fertilizers consisting of 140 kg N · ha^{-1}, 50 kg P · ha^{-1} and 40 kg K · ha^{-1}; NPK + NS_1 – fertilizers + the basic dose of Nobasyp 20 Mg · ha^{-1}; NPK + $NS_{1/2}$ – half a dose of Nobasyp 10 Mg · ha^{-1},

Table 1

Agrochemical parameters of soil and tested materials used in pot experiment and limiting values of heavy metals for soil additives [6]

Material	NH_4^+-N	NO_3^--N	N_{an}	P	K	Ca	Mg	Cd	Pb	Hg	As	Cr	Ni	pH in		EC	$CaCO_3$	C_{ox}
	[mg · kg^{-1}]													H_2O	KCl	[mS · cm^{-1}]	[g · kg^{-1}]	
Soil	6.6	2.2	8.8	31.9	339	2050	331	1.2	38	0.073	9.58	48	40	6.19	5.66	0.03	19.6	18.8
Agrodrap[1]	10.1	0.5	10.6	34.5	602	3850	331	< 1	< 5	0.002	< 0.04	< 80	< 45	9.71	10.10	0.19	18.9	36.5
Nobasyp[2]	7.7	3.2	10.9	30.5	618	9100	361	< 1	< 5	0.002	< 0.04	< 80	< 49	10.4	10.08	0.19	32.9	33.4
Limiting values of heavy metals for soil additives								2.0	50	1.0	10	100.0	50	—	—	—	—	—

Explanations: [1] rock wool – recycled Agroban; [2] rock wool – recycled Nobasil; EC – electrical conductivity.

NPK + AD_1 – fertilizers + the basic dose of Agrodrap 20 Mg · ha^{-1}; NPK + $AD_{1/2}$ – fertilizers + half a dose of Agrodrap. All variants were repeated four times. The doses of NPK nutrients have been calculated taking into account the N_{an} and available P and K contents in the modal brown soil as well as the requirements of the nutrients for planned yield. N has been applied with the DAM 390 fertilizer, P in the form of plain superphosphate and K in the form of KCl as 60 % potash salt. The amount of applied doses of Nobasyp and Agrodrap were chosen in accordance with the earlier experiments of Kováčik [7].

The harvest of spring barley (DC 91) was realized in its growing phase. The grain and straw yields, N-substances (% N × 6.25) and starch (method of Ewers) contents in the grain have been evaluated. After harvest a soil sample was taken from each pot and some agrochemical and pedological parameters were determined. The cation exchange capacity was detected as a sum of the base of saturation and the total acidity of soil, while composition of humic substances was determined by Kononova-Beltchikova method [8].

Results and discussion

The factor of trial variant statistically significantly affected all investigated quantitative and qualitative yield parameters of spring barley (Table 2).

Table 2

Effect of variability sources on yielding parameters of spring barley

Source of variation	d.f.	F-calculated			
		grain	straw	crude protein	starch
Treatment	7	84.046^{++}	71.763^{++}	199.37^{++}	12.562^{++}
Replication	3	1.423	0.375	0.303	0.243
Residual	21				
Total	23				

d.f. – degree of freedom.

The application of both rock wools (Nobasyp and Agrodrap) affected the grain and straw yields of spring barley in a positive but not statistically significant way. At the same time there was found moderately negative affect on starch content (var. 2 and 3 versus var. 1 – Table 3).

Nobasyp affected the yield more positively than Agrodrap. The affect of the wools on the level of N-substances was insignificant, however, the combined application of rock wools and NPK fertilizers inhibited the negative effect of nitrogen on the growth of N-substance content in the barley grain. This fact does not corresponds to the results of Orlik and Marzec [1] who, after application of 40 Mg · ha^{-1} of rock wools, did not find any increase in the grain yield of both spring barley and wheat.

Gilewska [2] points out that the possible reason of different achieved results in affecting rock wools on yield parameters of grown plants can lie in interdependence between the phenol compounds and formaldehyde contents in rock wools and yield formation.

Table 3

The effect of Nobasyp and Agrodrap on quantitative yield parameters of spring barley

Treatment		Grain			Straw		
number	designation	[g · pot^{-1}]	[%]		[g · pot^{-1}]	[%]	
1	0	12.45 a	100.0	—	14.81 a	100.0	—
2	NS_1	15.50 a	124.5	—	16.68 a	112.6	—
3	AD_1	13.53 a	108.7	—	16.45 a	111.1	—
4	NPK	46.06 c	370.0	100.0	51.42 c	347.2	100.0
5	$NPK+NS_1$	54.01 c	433.8	117.3	59.72 c	403.2	116.1
6	$NPK+NS_{1/2}$	46.64 c	374.6	101.3	49.60 bc	334.9	96.5
7	$NPK+AD_1$	39.91 b	320.6	86.7	48.12 bc	324.9	93.6
8	$NPK+AD_{1/2}$	42.44 bc	340.9	92.1	44.81 b	302.6	87.1
$LSD_{0.05}$		5.474			6.419		
$LSD_{0.01}$		7.453			8.738		

LSD – limit of significant difference at the level $\alpha = 0.05$ and $\alpha = 0.01$.

Application of NPK fertilizers (var. 4) has statistically significantly increased the yields of grain, straw and the contents of nitrogenous substances and decreased the starch content what is equal to textbook knowledge about the effect of nitrogenous fertilizers on qualitative and quantitative parameters of the spring barley (Tables 3 and 4).

The grain yield increase amounted to 370 % that means it was 10 times higher than has been generally known while applying the fertilizers. Similar was the straw yield increase. From the above-mentioned results is clear that the effectiveness of Agrodrap and Nobasyp was positive, but in comparison with NPK fertilizers it was significantly lower – the crucial role in grain and straw yield formation played direct (NPK) fertilizers. This result corresponds with the earlier data of Kováčik [5] who has emphasized unreplaceable role of fertilizers in keeping up the permanent soil fertility as well as the necessity to apply both indirect (soil amended material) and direct fertilizers in plant nutrition.

Combined application of Nobasyp and NPK fertilizers (var. 5 and 6) resulted in the highest grain (in the case of both Nobasyp doses – 10 Mg · ha^{-1} and 20 Mg · ha^{-1}) and straw (just while applying the dose of 20 Mg · ha^{-1}) yields. At the same time non-significant decrease in N substance contents and the increase in starch content (var. 5) have been achieved what is considered to be a positive finding from a quantitative viewpoint. On the other hand, combined application of Agrodrap and NPK fertilizers was not well-founded, because the yields achieved by interactive effect of NPK fertilizers and Agrodrap (var. 7 and 8) were lower than the ones achieved by solely use of NPK fertilizers (var. 4, Table 3). Agrodrap, unlike Nobasyp, at the used dose of 20 Mg · ha^{-1} combined with NPK fertilizers significantly eliminated the negative effect of

NPK fertilizers on the increase of N substance content in the grain (var. 7) and insignificantly on the decrease of starch content (Table 4).

Table 4

The effect of Nobasyp and Agrodrap on qualitative yield parameters of spring barley

Treatment		Crude protein			Starch		
number	designation	[g · pot^{-1}]	[%]		[g · pot^{-1}]	[%]	
1	0	8.76 a	100.0	—	67.40 a	100.0	—
2	NS$_1$	8.63 a	98.5	—	67.14 a	99.6	—
3	AD$_1$	8.80 a	100.5	—	66.34 a	98.4	—
4	NPK	16.56 c	189.0	100.0	57.84 b	85.8	100.0
5	NPK+NS$_1$	16.11 bc	183.9	97.3	58.54 b	86.9	101.2
6	NPK+NS$_{1/2}$	16.26 c	185.6	98.2	57.70 b	85.6	99.8
7	NPK+AD$_1$	15.37 b	187.9	99.4	59.13 b	87.7	102.2
8	NPK+AD$_{1/2}$	16.46 c	175.5	92.8	57.32 b	85.0	99.1
LSD$_{0.05}$		0.817			3.959		
LSD$_{0.01}$		1.133			5.495		

LSD – limit of significant difference at the level $\alpha = 0.05$ and $\alpha = 0.01$.

Achieved positive influence of combined application of NPK fertilizers and both kinds of rock wool on spring barley qualitative parameters (in case of applied Nobasyp also on quantitative parameters) has exceptional meaning in malt barley growing.

Agrochemical parameters have been also determined in the tested materials. Both rock wools inhibited the soil acidity; they moderately increased pH of the soil, calcium content as well as magnesium content (significantly – Table 5, var. 2 and 3). Their application has not caused the increase of salts in the soil.

Table 5

Some agrochemical and pedological parameters of the soil after the experiment

Treatment		pH$_{KCl}$	Ca	Mg	^{1}CEC	^{2}Vw	EC
number	designation		[g · kg^{-1}]		[mmol · kg^{-1}]	[g · cm^{-3}]	[mS · cm^{-1}]
1	0	5.76 c	1.80 a	0.25 a	174.95 a	1.22 d	0.03 a
2	NS$_1$	5.90 c	2.00 bcd	0.47 e	237.35 d	1.15 b	0.03 a
3	AD$_1$	5.92 c	1.90 abc	0.38 b	204.99 c	1.19 bcd	0.02 a
4	NPK	5.17 a	1.85 ab	0.26 a	180.78 a	1.21 cd	0.25 d
5	NPK + NS$_1$	5.61 bc	2.15 d	0.48 e	205.27 c	1.09 a	0.23 c
6	NPK + NS$_{1/2}$	5.35 ab	2.05 cd	0.44 d	199.36 bc	1.18 bcd	0.25 d
7	NPK + AD$_1$	5.30 ab	2.10 d	0.42 c	196.99 bc	1.16 bc	0.21 b
8	NPK + AD$_{1/2}$	5.22 a	2.05 cd	0.39 b	186.26 ab	1.19 bcd	0.25 d
LSD$_{0.05}$		0.334	0.175	0.1693	15.905	0.0563	0.0155
LSD$_{0.01}$		0.463	0.243	0.235	22.074	0.0754	0.0215

^{1}CEC – cation exchange capacity, ^{2}Vw – volume weight of dry soil.

Based on the knowledge of the parameters of rock wools used in hydroponic plant growth, there has been expected that their application will increase the cation exchange capacity (CEC) and decrease volume weight of dry soil (bulk density). The assumptions have been confirmed (Table 5).

Loose Nobasyp in comparison with scrapped Agrodrap increased the CEC more significantly as a result of its even application into the soil. The effect of both materials was statistically highly significant. Both kinds of rock wool decreased the bulk density, loosening (aerating) effect of Nobasyp on the soil has been more significant than the Agrodrap effect.

Effect of NPK fertilizers on agrochemical soil parameters corresponded to general knowledge presented by different authors [8]. Their application has statistically significantly decreased the pH value and increased salts content (Table 5, var. 5). Their combined application with Nobasyp and Agrodrap inhibited the negative influence of NPK fertilizers.

Both waste rock wools have had a positive effect on the total carbon content and the organic matter quality, thereby have increased the humic acid share in the soil, whereas Agrodrap has had more significant effect on these parameters than Nobasyp (Table 6).

Table 6

Some pedological parameters of the soil after the experiment

Treatment		C_{OX}	C_{HK} from C_{OX}	C_{FK} from C_{OX}	HA/FA
number	designation	[g · kg^{-1}]	[%]		
1	0	11.66 a	12.01	18.87	0.636
2	NS_1	11.90 ab	11.81	18.49	0.639
3	AD_1	12.74 cd	11.77	17.27	0.682
4	NPK	11.67 a	12.63	16.57	0.762
5	NPK + NS_1	12.30 bc	12.03	16.54	0.727
6	NPK + $NS_{1/2}$	12.62 cd	11.75	17.62	0.669
7	NPK + AD_1	12.95 d	12.71	15.89	0.800
8	NPK + $AD_{1/2}$	12.59 cd	13.13	16.99	0.773
$LSD_{0.05}$		0.589			
$LSD_{0.01}$		0.817			

The combined application with NPK fertilizers has increased the positive effect of the wools on the increase of the organic matter quality of the soil.

Conclusions

The application of both kinds of rock wool had a positive but not statistically significant effect on the grain and straw yield of spring barley. The Nobasyp effect on the yield was more positive than the one of Agrodrap.

Combined application of Nobasyp (20 Mg · ha^{-1}) and NPK fertilizers resulted in the highest grain and straw yields. On the other hand, combined application of Agrodrap and NPK fertilizers (under the use of both doses – 10 and 20 Mg · ha^{-1}) was not well-founded, because the yields achieved by interactive effect of NPK fertilizers and Agrodrap were lower than the ones achieved by solely use of NPK fertilizers. The rock wool + NPK fertilizers application inhibited the negative effect of nitrogen on increase of crude protein content and the decrease of starch in the barley grain.

Both kinds of rock wool significantly increased the cation exchange capacity, while the effectiveness of Nobasyp was higher than Agrodrap. Loosening (aerating) effect of Nobasyp on the soil is also more significant than the Agrodrap effect. Both kinds of rock wool moderately increased pH of the soil and have had a positive effect on the total carbon content and the organic matter quality.

The positive effect of Nobasyp on quantitative and qualitative parameters of spring barley and some soil parameters enables Nobasyp to be accepted not as a waste product but as a soil remediate substance, or an indirect fertilizer.

Yield of grain and straw has been affected in a statistically significant way by NPK fertilizers what at the same time decreased both the quality of barley grain and the starch content and increased the content of nitrogenous substances.

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ODDZIAŁYWANIE ODPADÓW WEŁNY MINERALNEJ NA PLONOWANIE JĘCZMIENIA JAREGO (*Hordeum vulgare* L.) I NIEKTÓRE WŁAŚCIWOŚCI GLEBY

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Abstrakt: W doświadczeniu wazonowym, prowadzonym w hali wegetacyjnej Słowackiego Uniwersytetu Rolniczego w Nitrze (48°18′ N, 18°05′ E), założonym na glebie płowej (25 kg gleby w wazonie) badano oddziaływanie dwóch odpadów wełny mineralnej (Nobasyp i Agrodrap) na parametry plonu jęczmienia jarego i niektóre agrochemiczne parametry gleby.

Uzyskane wyniki wykazały, że stosowanie obydwóch rodzajów wełny mineralnej miało pozytywny, ale statystycznie nieistotny wpływ na plon ziarna i słomy jęczmienia jarego. Wpływ Nobasypu na plon był bardziej widoczny niż ten wywołany przez Agrodrap. Łączne zastosowanie Nobasypu (20 Mg · ha^{-1}) oraz nawozów NPK skutkowało największymi plonami ziarna i słomy. Z drugiej strony, łączne stosowanie Agrodrapu oraz nawozów NPK (w obydwu zastosowanych dawkach – 10 i 20 Mg · ha^{-1}) nie było w pełni uzasadnione, ponieważ plony uzyskane w wyniku wzajemnego działania nawozów NPK i Agrodrapu były mniejsze niż te uzyskane po użyciu wyłącznie nawozów NPK. Stosowanie wełny mineralnej i nawozów NPK łagodziło ujemny wpływ azotu, który powodował wzrost zawartości białka surowego i obniżanie zawartość skrobi w ziarnie jęczmienia.

Obydwa rodzaje wełny mineralnej znacząco zwiększały kationową pojemność wymienną, czyniły glebę bardziej pulchną, umiarkowanie podnosiły wartość pH gleby oraz miały pozytywny wpływ na ogólną zawartość węgla i jakość materii organicznej.

Pozytywne oddziaływanie Nobasypu na ilościowe i jakościowe parametry jęczmienia jarego oraz niektóre parametry gleby umożliwia jego uznanie za substancję ulepszającą glebę lub nawóz pośredni, a nie jako produkt odpadowy.

Słowa kluczowe: wełny mineralne(bazalt), poprawa właściwości gleby, substancje ulepszające glebę (nawozy pośrednie), jęczmień jary

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ASSESSMENT OF THE IMPACT OF WASTE FROM POULTRY HATCHERY AND POSTCELLULOSE LIME ON SELECTED INDEXES OF LIGHT SOIL FERTILITY

OCENA ODDZIAŁYWANIA ODPADU Z WYLĘGARNI DROBIU ORAZ WAPNA POCELULOZOWEGO NA WYBRANE WSKAŹNIKI ŻYZNOŚCI GLEBY LEKKIEJ

Abstract: The effect of conditioned waste from poultry hatchery on selected parameters of light soil was examined. Basing on the obtained results it was found that after applying the conditioned waste, the contents of organic matter increased, which was manifested in the increase in both mean content of organic carbon and total nitrogen in soil. Furthermore, a mean significant increase in the contents of available forms of phosphorus and magnesium was also found. As the effect of applying both the organic waste from poultry hatchery and the postcellulose lime, a significant increase in exchangeable acidity and a decrease in contents of available forms of potassium were observed with reference to the control. The use of postcellulose lime reduced the contents of available forms of magnesium in soil.

Keywords: waste from poultry hatchery, postcellulose lime, organic matter, light soil, soil fertility

In many branches of industry including food industry, considerable amounts of industrial waste are produced, the nature uses of which are limited by strict legal rules and regulations. Many of the waste types are rich in organic substance containing macro- and microelements of high fertilizing value. Both a considerable fraction of light soils in the area of our country and a negative balance of organic matter in arable soils justify the interest in these types of wastes. One of them is the waste from poultry hatcheries and, owing to considerable amount of calcium (approx. 40 % CaO), it can improve soil fertility inducing changes in values of acidity indexes.

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The aim of this study was to assess the usefulness of soil conditioner containing the waste from poultry hatchery and postcellulose lime on the value of selected indexes of light soil fertility.

Material and methods

A strict one-factor field experiment was carried out in 2005–2007 in Ciele, near Bydgoszcz, to assess the effect of conditioned waste from poultry hatchery and postcellulose lime on selected parameters of light soil. The experiment was established in Luvisols of 6th class (clay fractions content – 4 %) characterised by very acid reaction, low abundance in available forms of phosphorus and potassium, and mean abundance in available forms of magnesium. The tested factor of the experiment was a soil conditioner applied in two doses: 2 Mg · ha^{-1} and 4 Mg · ha^{-1}. The experiment was carried out in a system of randomized blocks with three replications. An area of the experimental plot was 40 m^2. In the farm where the experiment was carried out, no root crops were cultivated, so spring triticale was the test plant in the first year of the study and oat in the second and third years. Soil conditioner dosages were established in such a way that no more than 170 kg N · ha^{-1} was introduced into soil together with the organic material.

The soil conditioner from poultry hatchery included: waste eggs rejected from the incubating process, cracked, not-fertilised, and dead eggs, and dead and invalid chicks. This material, mixed with sawdust, was hygienised with calcium oxide and stored in prism for six months. The chemical composition of soil conditioner and postcellulose lime are presented in Tables 1 and 2.

Table 1

The proporties of soil conditioner from poultry hatchery

Parameter	Unit	Value
pH in H_2O	pH	12.0
Dry matter (d.m.)	[%]	51.0
Organic matter	[g · kg^{-1}]	603.0
Total nitrogen (N)		72.6
Ammonium nitrogen (NH_4-N)		1.98
Total phosphorus (P)		1.60
Potassium (K)		2.10
Calcium (Ca)		290.0
Magnesium (Mg)		3.50

The conditioned waste from the poultry hatchery contained 40 % CaO (Table 1). Therefore the scheme of experiment apart from control object, included also the objects on which postcellulose lime was used (Table 2) in the amount equivalent to the dose of the component contained in the applied conditioner. Both tested conditioner from poultry hatchery and calcium fertilizer were applied in autumn 2004.

Table 2

The chemical composition of postcellulose lime

Parameter	Unit	Content
CaO	[%]	40
Chloride (Cl)		2.85
Sulphur (S)		1.1

Five fertilizing objects were taken into consideration:

- – K – the control (mineral fertilisation only),
- – S_1 – soil conditioner in a dose of 2 Mg · ha^{-1},
- – S_2 – soil conditioner in a dose of 4 Mg · ha^{-1},
- – Ca_1 – postcellulose lime in a dose of 1 Mg · ha^{-1},
- – Ca_2 – postcellulose lime in a dose of 2 Mg · ha^{-1}.

In every year of the field experiment, after the plant harvest time, soil samples were taken from the soil layer (0–25 cm) to determine their indexes as follows: exchangeable acidity, the contents of avalilable forms of potassium and phosphorus determined by the Egner-Riehm (DL) method, magnesium by AAS method. Additionally, there were determined: total nitrogen by Kjeldahl's method, and the content of organic carbon in soil with Tiurin's method.

The results obtained were statistically performed by using variance analysis and Tukey's test at the level of significance $\alpha = 0.05$.

Results and discussion

It was found that the applied soil conditioner and the postcellulose lime induced changes in soil pH values with reference to the control (Table 3). In all the analysed soil samples the values of pH were higher than the pH value determined for the control (pH_{KCl} 4.4). The highest value of pH (4.7) with reference to the control was found in the soil where the conditioner from poultry hatchery was applied in a dose of 4 Mg · ha^{-1}. The same conditioner used in a dose of 2 Mg · ha^{-1} resulted in the increase in pH-values by 0.2 unit on average with reference to the control. Under the influence of postcellulose lime the pH value was shaped independently on the dose applied, on average at the level of 4.5. It is worth noticing that the used waste materials as components of the tested conditioner induced changes of soil reaction in comparison with the control, from the very acidic into acidic one.

The effect of waste organic materials on soil reaction (pH) is not explicit. It depends on chemical composition mostly and it is connected with their origins [1, 2]. Dechnik and Wiater [1] report about acidifying organic waste on the basis of molasses and straw extraction. In turn, Gondek and Filipek-Mazur [3] demonstrate advantageous effect of tanning sediments on the increase in soil pH value. The extracts (from molasses, rye and potato) used in the research of Łabętowicz et al [4] showed less clear influence on soil reaction. However, no change in soil reaction (pH) was stated under the effect of applied compost consisted of straw, sawdust and lignite [5].

Table 3

Impact of conditioner from poultry hatchery and postcellulose lime on selected soil properties

Years of study	Fertilisation objects/Parameter					Mean	$LSD_{0.05}$
	Control	S_1	S_2	Ca_1	Ca_2		
	pH in KCl						
2005	4.4	4.7	4.9	4.6	4.5	4.7	
2006	4.5	4.5	4.6	4.5	4.6	4.5	
2007	4.4	4.5	4.7	4.4	4.5	4.5	
Mean	4.4	4.6	4.7	4.5	4.5	4.5	
C-organic [$g\ C \cdot kg^{-1}$]							
2005	5.87	8.32	7.25	6.12	6.16	6.74	0.833
2006	6.65	7.96	7.64	6.48	6.69	7.09	0.860
2007	6.36	7.67	7.54	6.44	6.16	6.84	0.834
Mean	6.29	7.98	7.48	6.35	6.34	6.89	0.248
Nitrogen content [$g\ N \cdot kg^{-1}$]							
2005	0.61	0.73	0.74	0.53	0.60	0.64	0.174
2006	0.68	0.72	0.75	0.55	0.60	0.66	0.046
2007	0.64	0.70	0.74	0.53	0.60	0.64	0.101
Mean	0.64	0.72	0.74	0.54	0.60	0.65	0.053
P – available [$mg\ P \cdot kg^{-1}$]							
2005	33.77	40.27	38.23	27.63	28.63	33.71	2.646
2006	27.87	38.90	36.60	26.83	29.50	30.34	3.446
2007	28.20	38.53	35.43	25.87	30.30	29.95	3.025
Mean	29.95	36.57	36.76	26.78	29.48	31.90	0.901
K – available [$mg\ K \cdot kg^{-1}$]							
2005	37.10	38.10	35.03	34.07	33.80	35.62	3.119
2006	46.27	31.27	32.37	33.47	31.60	34.99	4.480
2007	38.57	33.80	31.87	32.93	32.07	33.85	3.509
Mean	40.65	34.39	33.09	33.49	32.49	34.82	1.045
Mg – available [$mg\ Mg \cdot kg^{-1}$]							
2005	23.53	23.40	24.50	21.37	21.73	23.77	1.792
2006	23.57	24.53	25.53	21.27	22.33	23.45	1.706
2007	23.57	24.13	24.43	20.83	21.73	22.94	1.434
Mean	23.56	24.02	24.82	21.16	21.93	23.10	0.427

The study revealed that the highest content of organic carbon was found in the soil where soil conditioner was applied in doses 2 $Mg \cdot ha^{-1}$ and 4 $Mg \cdot ha^{-1}$ (7.98 $g \cdot kg^{-1}$ and 7.48 $g \cdot kg^{-1}$, respectively). Considering the control, the growth was on average 26.9 % and 18.9 %. Worth noticing is the fact that the content of organic carbon in soil on the plot where lower dose of the conditioner was used lowered as the years passed. Also in the research by Wiater and Dębicki [6], soil organic carbon was systematically reduced under the effect of sewage sludge as the research years went by. Mean carbon content on plots fertilized with defecation lime was slightly higher than in soil of the control. In the research of Skowrońska and Wiater [7], Kopeć et al [8], as well as Gondek and Filipek-Mazur [3] the application of organic waste also induced the increase in soil organic carbon by more than 50 % with reference to the control.

The investigations carried out revealed a clear increase in average content of total nitrogen in soil from the plots where conditioner from poultry hatchery was used with reference to the control. Depending on the conditioner dose the increase ranged from 12.5 % in the plot with a dose of 2 Mg · ha^{-1} up to 15.6 % in a plot with a dose of 4 Mg · ha^{-1}. Fertilisation with postcellulose lime in a dose of 1 Mg · ha^{-1} significantly lowered average total nitrogen content in soil, by 15.6 % with reference to the control. A higher dose of postcellulose lime did not differentiate significantly this element soil fertility, maintaining its value at the level similar to that observed on the unfertilized object.

Since the soil conditioner from poultry hatchery used in the research contained considerable amounts of organic matter and nitrogen, its modifying effect on these parameters is very clear. Worth noticing is the fact that the use of the conditioner in a single or double dose had no effect on diversifying the discussed parameters, which can be explained as its small ability to decay under the condition of the experiment.

The content of available forms of nitrogen in soil was the highest on the objects where soil conditioner from poultry hatchery was used. Comparing with the soil content before starting the field experiment, the increase was on average by 22.1 % in soil applied with a dose of 2 Mg · ha^{-1}, and 22.7 % in soil with dose of 4 Mg · ha^{-1} of the conditioner. The study by Bohacz and Korniłowicz-Kowalska [9] revealed that the application of creatine-bark composts and creatine-bark-straw composts also enriched soil with available forms of phosphorus. However, according to Dechnik and Skowrońska [10], the use of molasses extract and straw contributes to lowering the contents of available forms of this element in soil. In soil of the object where postcellulose lime was used in a dose of 1 Mg · ha^{-1}, an average content of available forms of phosphorus was the lowest and reached 26.78 mg · kg^{-1}. This reduction was significant and by 10.6 % lower with reference to soil in the control. The reduction in available forms of phosphorus was probably induced by acid reaction of soil and the cultivated plants which took up larger amounts of phosphorus as a result of higher nitrogen consumption, using soil reserves. It should be emphasized that the content of available forms of phosphorus was the highest in the first year of the experiment and lowered in the next years on the objects where soil conditioner was used with a lower dose of postcellulose lime. The decline in available forms of this element may be explained by acid soil reaction which probably retards some part of phosphorus available for plants.

Basing on the carried out experiment, it was found that average content of available forms of potassium in soil on all fertilized objects was significantly lower with reference to the control. The lowest mean content of available forms of this element was found in soil fertilized with 2 Mg · ha^{-1} of postcellulose lime. This reduction was by 20.07 % in comparison with soil from the control object. The conditioner with dose of 4 Mg · ha^{-1} reduced the content of the available for plants forms of potassium by

18.6 %. These observations confirm the results of the research made by Wiater and Dębicki [6] who used ceratine-bark-ureic granulates. These authors achieved reduction in available forms of potassium in soil even by 20 % in the first year of the experiment and by 10 % in the second one. Whereas an essential increase in potassium available for plants under the effect of applying molasses extract was observed by Dechnik and Skowrońska [10]. According to Gondek and Filipek-Mazur [3], tanning sludge increased soil available potassium forms by 40 % on average. Therefore it is important what waste we use, which is inseparably connected with its chemical composition and its impact on soil properties. The observed reduction in soil available potassium in presented experiment was probably connected with higher absorption of this element by cultivated plants. Since the soil conditioner contained small amounts of potassium, the plants absorbed it from soil resources.

Mean content of available forms of magnesium was the highest in soil of the objects where the conditioner based on the waste from poultry hatchery was used and amounted to 24.82 mg $\cdot$ kg^{-1} and 24.02 mg $\cdot$ kg^{-1}. In comparison with the control, the increase reached 5.3 % when a dose of the conditioner per hectare equaled 4 Mg $\cdot$ ha^{-1} and 1.9 % in case of 2 Mg $\cdot$ ha^{-1} application. Under the effect of fertilizing with postcellulose lime in a dose of 2 Mg $\cdot$ ha^{-1}, the available forms of this element were reduced by approx. 6.9 %, whereas after applying the dose of 1 Mg $\cdot$ ha^{-1} – by 10.2 %. Such considerable differences between the conditioner and postcellulose lime were caused by a nearly 0.4 % content of magnesium in the conditioner which additionally enriched the fertilised soil with that element. In Błazik and Wiater's study [11], sawdust with addition of defecation lime increased available forms of magnesium in soil, but according to Skowrońska and Wiater [7], distillery waste induced a decrease in soil available magnesium by approx. 10 %. Since light soils are formed on loose or clayey sands, their contents of available magnesium forms are connected to the most degree with soil acidity and lower with the reduction in pH value [12]. It contributes to forming of magnesium deficiencies in considerable areas of arable lands [13] and the use of organic wastes may counteract Mg deficit.

Conclusions

1. After using the conditioner containing waste from poultry hatchery, a change in the class of reaction of fertilised soil (as regards pH values) was observed.
2. Under the effect of the used soil conditioner, an increase in average contents of: organic carbon, total nitrogen, available forms of both phosphorus and magnesium was observed, while a decrease in the potassium content was noticed after applying the conditioner and postcellulose lime, with reference to the control.
3. The available forms of phosphorus and potassium content in soil where the conditioner was applied reduced in the years of the experiment.
4. According to the effects of research, the conditioner produced on the basis of poultry hatchery waste caused significant positive changes in chemical properties of very acid and poor light soil. It points out the possibility of practical usage of this product in returning the soil of low agricultural usage its productive ability.

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OCENA ODDZIAŁYWANIA ODPADU Z WYLĘGARNI DROBIU ORAZ WAPNA POCELULOZOWEGO NA WYBRANE WSKAŹNIKI ŻYZNOŚCI GLEBY LEKKIEJ

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Abstrakt: Badano wpływ kondycjonowanego odpadu z wylęgarni drobiu oraz wapna pocelulozowego na wybrane parametry gleby lekkiej. Na podstawie uzyskanych wyników badań stwierdzono, że po zastosowaniu kondycjonera glebowego wzrosła zawartość materii organicznej, co przejawiało się wzrostem średniej zawartości węgla organicznego oraz azotu ogółem w glebie. Ponadto stwierdzono w tych warunkach znaczny wzrost średniej zawartości przyswajalnych form fosforu i magnezu w glebie. W wyniku zastosowania zarówno odpadu organicznego z wylęgarni drobiu, jak i wapna pocelulozowego, stwierdzono znaczny wzrost wartości kwasowości wymiennej oraz zmniejszenie zawartości przyswajalnych form potasu w porównaniu z obserwowanymi w obiekcie kontrolnym. Zastosowanie wapna pocelulozowego powodowało zmniejszenie zawartości przyswajalnych form magnezu w glebie.

Słowa kluczowe: odpad z wylęgarni drobiu, wapno pocelulozowe, materia organiczna, gleba lekka, żyzność gleby

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EFFECT OF PYRETHROIDS ON STRESS-INDUCED BIOSYNTHESIS OF SELECTED HAEMOPROTEINS IN *Saccharomyces cerevisiae* YEAST CELLS

WPŁYW PYRETROIDÓW NA INDUKOWANĄ WARUNKAMI STRESU BIOSYNTEZĘ WYBRANYCH BIAŁEK HEMOWYCH W KOMÓRKACH DROŻDŻY *Saccharomyces cerevisiae*

Abstract: The cellular response to stress is a basic protective mechanism enabling cells to adapt to changing environmental conditions. Logarithmic cultures of *Saccharomyces cerevisiae* yeast are a good model for research on this topic, because the stress response is accompanied by induction of biosynthesis of haemoproteins such as catalase and cytrochromes. In wild-type yeast cells growing on YPG medium containing 2 % glucose, catalase activity in the logarithmic culture is low. Cytochrome spectra under these conditions are flat, indicating low respiratory complex activity. Stress conditions induce expression of the gene CTT1, which codes for catalase T, and the enzyme is synthesized *de novo*. Within a short time catalase T activity increases sharply, attaining a value characteristic of the stationary phase of growth. Similarly, induced biosynthesis of cytochromes takes place under stress conditions. This study investigated the effect of pyrethroids on stress-induced biosynthesis of these haemoproteins in *S. cerevisiae* cells. The experiments were conducted on a standard wild-type strain of yeast. The yeast cultures were grown in liquid YPG medium. The effect of pyrethroids (deltamethrin, esfenvalerate and cypermethrin) on induced biosynthesis of haemoproteins was studied under conditions of alcohol stress or osmotic stress (induced by sodium chloride or sodium nitrate). Catalase activity was determined and low-temperature cytochrome spectra of the yeast cells were performed. Application of pyrethroids and stress conditions at the same time was found to inhibit synthesis of haemoproteins, ie catalase T and cytochromes.

Keywords: pyrethroids, stress, catalase, cytrochromes, yeast

Environmental stress is induced by a variety of factors, including changes in temperature, UV, ionizing radiation, changes in osmotic pressure, dehydration, metal ions, changes in environmental acidity, enzyme inhibitors, amino acid analogues,

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pesticides or lack of nutrients. Under stress conditions, a number of genes are expressed in cells, leading to induction of synthesis of a specific group of proteins and to temporary inhibition of the division cycle [1]. These proteins take part in various metabolic processes, including redox reactions, antioxidant protection, carbohydrate metabolism, DNA repair, and intercellular signal transmission. They are also involved in maintaining the pool of native cellular proteins and in cell wall modification [2]. The purpose of the cellular response to stress is to protect cell components from the dangerous effects of stress factors. The typical cellular response to changing environmental conditions is synthesis of a specific group of proteins called heat shock proteins (HSP). The action of various stress factors leads to an increase in the level of reactive forms of oxygen in cells as well as changes in the level of antioxidant enzyme activity [3]. The fact that various kinds of stress induce similar physiological effects has made it possible to attribute to ROS the role of second messengers, because during stress the concentration of free radicals in the cell increases. It has also been postulated that the effective concentration of reactive forms of oxygen as transmitters is very small, so that they can perform their function even when oxygen access is limited. Changes in the concentration of reactive forms of oxygen can be a factor triggering a stress response in a cell [4].

Nevertheless, attributing to ROS – highly reactive molecules characterized by low selectivity – the role of a second messenger raises many doubts. Opponents of this hypothesis argue that proteins characteristic for the stress response can be synthesized in anaerobic conditions after a stress factor has acted on them [5].

Pyrethroids are synthetic insecticides. They are esters of primary or secondary alcohols containing at least one double bond and chrysanthemic acid [2,2-dimethyl-3--(2-methylpropenyl)-cyclopropanecarboxylic acid), or halogen analogues of this acid [6]. Pyrethroids are neurotoxins that have a powerful effect on the nervous system of insects. Authors of numerous publications claim that the effects of pyrethroids on parts of an organism other than the nervous system may be associated with free radical generation [7]. In studies on the non-specific effects of pyrethroids on various organisms, oxidative stress markers are often determined, including lipid peroxidation level, activity of catalase, superoxide dismutase or glutathione peroxidase, and antioxidant concentration [8].

The aim of this study was to investigate the effect of selected pyrethroids on the stress response. Two types of haemoproteids were chosen as markers of stress response intensity: catalase and cytochromes, which are synthesized in cells of the yeast *S. cerevisiae* under stress conditions.

Material and methods

Yeast strains: SP-4α leu1 arg4, wild-type strain [5].

Media: The yeast were grown on liquid YPG medium – 1 % yeast extract, 1 % peptone, 2 % glucose.

Plotting of cytochrome spectra: Cytochrome spectra were determined in whole yeast cells frozen in liquid nitrogen. The yeast cells were centrifuged and dried on filter paper. A few $Na_2S_2O_4$ crystals were added to the yeast. Cytochrome spectra were measured in a cuvette constructed by T. Biliński, in a wavelength range of 500–650 nm with a 1-mm layer of yeast cells frozen in liquid nitrogen. Absorption value was measured relative to a baseline connecting points representing absorption minima. The maximum absorption value was read at a wavelength of 551 nm for cytochrome c, 558 nm for cytochrome b and 605 nm for cytochrome a + a^3 [9].

Preparation of yeast cell extracts for enzyme determination: The collected yeast cells were washed with phosphate buffer (0.05 M, pH 6.8), centrifuged again, and then suspended in 1 cm^3 of this buffer, cooled in ice for 10 min and disrupted with glass beads (diameter 0.4–0.5 mm) in a Bosch homogenizer for 2 min. The glass beads, undisrupted cells and cell wall fragments were centrifuged for 5 minutes. The supernatant was transferred to clean test tubes, kept in ice and used for enzyme determination.

Protein determination: Protein in the yeast cell extracts was determined using the Lowry method [10].

Determination of catalase activity: Total catalase activity was determined spectrophotometrically using the method described by Beers and Sizer [11].

Incubation of yeast cells with pyrethroid was carried out in the logarithmic phase of growth for 2 h in standard aeration conditions. To assess the effect of the insecticide on yeast cells under stress conditions, stress conditions were applied simultaneously with various concentrations of the pesticide.

Stress conditions: Alcohol stress conditions were obtained by adding ethyl alcohol to the culture at a concentration of 6 % [12]. Osmotic stress conditions were obtained by adding 0.3 M sodium chloride or 0.5 M $NaNO_3$ to the logarithmic phase culture [13, 14].

The following pyrethroids were applied in the study:

– deltamethrin-(S)-alpha-cyano-3-phenoxybenzyl(1R)-cis-3-(2,2-dibromovinyl)-2,2--dimethylcyclopropane-carboxylate;

– cypermethrin-(R,S)-alpha-cyano-3-phenoxybenzyl(1RS)-cis,trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-carboxylate; and

– esfenvalerate-(S)-alpha-cyano-3-phenoxybenzyl (S)-2-(-4-chlorophenyl)-3-methylbutyrate.

Results

Catalase T activity in a wild-type yeast cell depends on growth conditions. In yeast cells in the logarithmic phase of growth catalase activity is very low, while in the stationary phase it is about 25 times higher (Table 1). It is in accordance with the results obtained by other authors.

After pyrethroids were added to the logarithmic phase of culture there was no increase in catalase activity, which would indicate induced biosynthesis of this enzyme (Table 1). When the stationary phase of yeast culture was incubated with pyrethroids for 2 h a decrease in catalase activity was noted.

Table 1

Catalase activity in SP4 yeast cells after incubation with various concentrations of pyrethroid, expressed as % of the logarithmic or stationary control

Culture conditions	Logarithmic phase of culture			Stationary phase of culture		
Control	Catalase activity					
	1.8 U · mg^{-1} protein · cm^{-3}			45.6 U · mg^{-1} protein · cm^{-3}		
	100 %			100 %		
Pyrethroid concentration [µg · cm^{-3}]	esfen-valerate	delta-methrin	cyper-methrin	esfen-valerate	delta-methrin	cyper-methrin
20	98	95	98	87	96	93
40	100	87	96	82	85	80
60	98	89	96	76	71	65
80	92	91	96	60	42	58

Activity of cytochromes of the respiratory chain has been studied on the basis on cytochrome spectra analysis. Yeast are facultative anaerobes. If glucose is available in the medium, they obtain energy from fermentation, that is why the absorbance values for individual cytochromes in the logarithmic phase are low (Table 2).

Table 2

Effect of pyrethroids on cytochromes of the respiratory chain in SP4 yeast cells in the logarithmic and stationary phase of cultures

Conditions applied			Relative cytochrome absorbance		
			b	c	a + a^3
Logarithmic phase of culture	control		0.09	0.11	0
	esfenvalerate	40 µg · cm^{-3}	0.09	0.11	0
	deltamethrin		0.085	0.1	0
	cypermethrin		0.09	0.11	0
Stationary phase of culture	control		0.31	0.35	0.25
	esfenvalerate	40 µg · cm^{-3}	0.31	0.34	0.25
	deltamethrin		0.3	0.34	0.25
	cypermethrin		0.31	0.33	0.24

Glucose depletion causes a change in the way yeast respire, from fermentation to oxidative phosphorylation, and on the cytochrome spectrum in the stationary phase one may observe absorbance maxima for cytochromes. Incubation of yeast cells with pyrethroids does not cause significant changes in cytochrome absorbance (Table 2).

In wild-type yeast cells growing on YPG medium containing 2 % glucose, cytochrome spectra are flat in the logarithmic phase, which indicates low activity of respiratory complexes. When yeast cells from the logarithmic phase of culture are subjected to stress conditions, they alter their metabolism and begin a synthesis of many proteins, including cytochromes. Stress conditions were found to induce cytochrome biosynthesis within 2 h so that the number of cytochromes in the cells increased to high values characteristic of the stationary phase.

This study found that cytochrome biosynthesis is induced most strongly by NaCl osmotic stress, followed by alcohol stress. Pyrethroid alone added to the logarithmic phase of culture does not cause an increase in absorbance of the cytochrome spectrum. In the stationary phase of culture, 2 h incubation with pyrethroid does not significantly affect the absorbance value for individual cytochromes (Table 3). Simultaneous application of stress conditions and addition of pyrethroid to the yeast culture inhibits cytochrome biosyntesis.

Table 3

Effect of simultaneous application of stress and pyrethroid on catalase and cytochromes of the respiratory chain in SP4 yeast cells in the logarithmic phase of culture

Conditions applied			Catalase activity U · mg^{-1} protein · cm^{-3}	Relative cytochrome absorbance		
				b	c	$a + a^3$
Control			1.8	0.09	0.1	0
Osmotic stress (NaCl)			28.4	0.43	0.65	0.03
+	esfenvalerate	40 μg · cm^{-3}	1.71	0.04	0.05	0
+	deltamethrin		1.83	0.05	0.08	0
+	cypermethrin		1.8	0.06	0.06	0
Alcohol stress			22.2	0.37	0.44	0.03
+	esfenvalerate	40 μg · cm^{-3}	1.65	0.05	0.06	0
+	deltamethrin		1.78	0.08	0.08	0
+	cypermethrin		1.75	0.08	0.07	0
Osmotic stress ($NaNO_3$)			24.5	0.18	0.22	0.02
+	esfenvalerate	40 μg · cm^{-3}	1.63	0.01	0.01	0
+	deltamethrin		1.73	0.04	0.06	0
+	cypermethrin		1.8	0.03	0.05	0

The analysed yeast cells were capable to a stress response, which was manifested by increased catalase activity and higher cytochrome absorbance under conditions of osmotic stress, induced by adding 0.3 M NaCl or 0.5 M sodium nitrate, and 6 % alcohol stress (Table 3).

When osmotic stress was applied simultaneously with selected pyrethroids at a concentration of 40 μg · cm^{-3} to assess inhibition of induction of catalase T synthesis by the pyrethroids, no increase in catalase activity was noted. This observation was confirmed when other types of stress were applied together with pyrethroid. This suggests that pyrethroids inhibit induced biosynthesis of catalase T in *S. cerevisiae* yeast cells.

Discussion

In laboratory practice one way of determining the intensity and range of the cellular stress response is determination of the activity level of selected proteins induced by stress factors. *Saccharomyces cerevisiae* yeast are often used in research on the stress response. They have two types of catalase – cytoplasmatic catalase T and peroxysomal catalase A. Catalase T activity is a frequently used stress response marker [13–16].

In induced biosynthesis of catalase in yeast there is a frequently observed stress response induced by incubation of a logarithmic phase culture with certain pesticides, such as ammonium glyphosinate [15].

It was found, that the activity of catalase in logarythmic phase of yeast culture did not change after treatment with pyrethroids, while in the stationary phase it is decrease. In a previously published study there was also found that long-term yeast cell culture on a medium with various concentrations of deltamethrin decreased catalase activity [16]. Activity of this enzyme is a frequently used as oxidative stress marker in studies on the effects of pesticides on various organisms. Research on the effects of fenvalerate and its metabolite p-chlorophenylisovalerianic acid on the erythrocytes of rats has found that both fenvalerate and its metabolite can cause oxidative stress in erythrocytes, manifested as a significant decrease in activity of antioxidant enzymes, including catalase [17]. A single dose of only 0.001 % LD_{50} of cypermethrin or fenvalerate caused changes in catalase activity in the erythrocytes of rats [7]. In these studies catalase activity was found to increase maximally on the third day after application of cypermethrin or cypermethrin together with fenvalerate, while maximum catalase activity after application of fenvalerate was noted after 7 days. In the fish *Channa punctatus* (Bloch) exposure to deltamethrin caused catalase activity to decrease in the liver, kidneys and gills. Cypermethrin affects catalase activity in the liver and kidneys of the fish *Oreochromis niloticus* and *Cyprinus carpio* [18]. After exposure to a low concentration of cypermethrin – 1/20 LD_{50} – an increase in catalase activity was noted in the liver of both fish species, while in the kidneys catalase activity in *C. carpio* increased as much as 960 %, but in *O. niloticus* there was a 276 % decrease in comparison with the control.

Cytochromes are another haemoproteid that undergoes in induced biosynthesis in yeast. Induction of cytochromes of the respiratory chain caused by entering the stationary stage of growth or by reoxygenation stress has been well described [19]. The maximum absorption on the cytochrome spectrum is directly proportional to the concentration of respiratory chain cytochromes; there is even a method that makes it possible to quantitatively determine cytochrome concentration on the basis of absorbance values [20]. Yeast seem to be the most convenient eukaryotic organism for studying the role of the mitochondrial system in pesticide toxicity. Yeast can easily survive inhibition of almost all mitochondrial function, eg by anoxia, and tolerate respiratory inhibitors and genetic changes leading to respiratory deficiency [21].

Błaszczyński et al [21] found that the mitochondria are the site in yeast where paraquat is reduced to a toxic free-radical form. This hypothesis was supported by analysis of cytochrome spectra of yeast cells. After incubation by paraquat the absorbance maxima for cytochromes b and c disappeared. According to Cochemé and Murphy [22], complex I is the site in the respiratory chain of yeast where superoxide anion radical is generated during incubation with paraquat. Other pesticides also cause damage to yeast cell mitochondria. Cadmium and thiuram applied together show synergistic toxicity and induce mitochondrial petite mutants in yeast [23].

Pesticides can also cause mitochondrial damage in the cells of higher eukaryotes. Paraquat induces cell death in dopaminergic neurons through a mechanism in which p53 and the mitochondrial apoptotic pathway are linked. Paraquat has been found to decrease activity of mitochondrial complex I and potential of mitochondrial transmembrane and to induce the release of cytochrome c from the mitochondria [24]. Chlorpyrifos-induced toxicity was characterized by the loss of mitochondrial potential, the appearance of nuclear condensation and fragmentation in placental cells [25]. Atrazine is a widely used triazine herbicide. A series of experiments with sperm and isolated mitochondria suggest that atrazine inhibits mitochondrial function, and mitochondrial F(1)F(0)-ATP synthase is a molecular target of atrazine [26].

A bioassay is a system for monitoring toxicity of chemicals in the environment via the biological responses of experimental organisms. In this study the stress response of yeast cells was used to test the toxicity of pyrethroids. The stress factors applied in the logarithmic phase of culture induce expression of the gene CTT1, which encodes catalase T [27]. Under these conditions catalase T is synthesized *de novo* and within a short time achieves a high level of activity.

Many compounds can inhibit the cellular stress response. Cycloheximide is known to prevent synthesis of stress proteins by inhibiting protein synthesis in cytoplasm. Some pesticides, such as paraquat, can destroy the internal membrane of mitochondria and cause cytochrome absorbance to decrease within a short incubation time.

In this study inhibition by pyrethroids of induced biosynthesis of catalase and cytochromes was described. At this stage of research it is difficult to determine the stage at which biosynthesis of these proteins is inhibited. Both proteins are haemoproteids, so it may be postulated that pyrethroids affect the haeme biosynthesis pathway. Haeme and iron metabolism play a key role in maintaining integrated cellular metabolism. Haeme is necessary for activating the haeme-responsive transcriptional activator HAP1, involved in the expression of numerous genes [28]. Biosynthesis of haeme begins in the mitochondrion with synthesis of delta-aminolevulinic acid, and subsequent stages take place in the cytoplasm. The main product of the haeme biosynthesis pathway in yeast cells grown under aerobic conditions is haeme [9]. Yeast do not accumulate porphobilinogen and porphyrin [29]. Accumulation of haeme in any cellular compartment could be very dangerous, because this compound is a potential source of oxygen free radicals [28]. A previously published study found that pyrethroids can damage yeast cell membranes [30]. They may also damage mitochondrial membranes and prevent transport of coproporphyrynogen. After yeast cells were incubated with pyrethroids, a number of respiratory-incompetent rho^{-} mutants were found to increase [31]. One possible explanation for the inhibition of induced biosynthesis of the haemoproteids studied may be the free-radical effect of pyrethroids. On the haeme biosynthesis pathway there are several enzymes that are sensitive to the presence of free radicals, such as ferrochelatase, which catalyzes insertion of iron into the protoporphyrin ring [9].

Conclusions

1. The effect of pyrethroid on catalase activity depends on phase of yeast culture growth.
2. Pyrethroid alone added to the yeast culture does not cause significant changes in cytochrome absorbance.
3. Stress conditions (NaCl, alcohol, $NaNO_3$) induce biosynthesis of catalase and cytochrome proteins in logarithmic yeast cells.
4. Application of pyrethroid and stress conditions at the same time was found to inhibit synthesis of haemoproteins, ie catalase T and cytochromes in logarithmic yeast cells.

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WPŁYW PYRETROIDÓW NA INDUKOWANĄ WARUNKAMI STRESU BIOSYNTEZĘ WYBRANYCH BIAŁEK HEMOWYCH W KOMÓRKACH DROŻDŻY *Saccharomyces cerevisiae*

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Abstrakt: Odpowiedź komórek na stres jest podstawowym mechanizmem ochronnym, pozwalającym dostosować się do zmieniających się warunków środowiska. Logarytmiczne hodowle drożdży *Saccharomyces cerevisiae* są dobrym modelem do tego typu badań, ponieważ reakcji na stres towarzyszy m.in. indukcja biosyntezy białek hemowych, takich jak katalaza i cytochromy. W komórkach drożdży szczepu dzikiego rosnących na pożywce YPG zawierającej 2 % glukozy w logarytmicznej fazie wzrostu hodowli poziom aktywności katalazy enzymu jest niski. Widma cytochromowe w tych warunkach są płaskie, co informuje o małej aktywności kompleksów oddechowych. Warunki stresu wywołują indukcję ekspresji genu CTT1 kodującego katalazę T i enzym ten jest syntetyzowany *de novo.* W ciągu krótkiego czasu poziom aktywności katalazy T gwałtownie wzrasta, osiągając wartość charakterystyczną dla stacjonarnej fazy wzrostu komórek drożdży. Podobnie pod wpływem stresu zachodzi indukowana biosynteza białek cytochromowych. Badano wpływ pyretroidów na przebieg indukowanej przez warunki stresu biosyntezę tych białek hemowych w komórkach drożdży *S. cerevisiae*. Doświadczenia przeprowadzono na standardowym szczepie dzikim drożdży. Hodowle drożdży prowadzono w pożywce płynnej YPG. Wpływ pyretroidów (esfenwalerat, deltametryna, cypermetryna) na indukowaną biosyntezę białek hemowych badano w warunkach stresu alkoholowego lub osmotycznego (wywoływanego przez chlorek sodu lub azotan(V) sodu). Oznaczano aktywność katalazy i wykonano niskotemperaturowe widma cytochromowe komórek drożdży. Stwierdzono, że równoczesne stosowanie pyretroidów i warunków stresu powoduje zahamowanie indukowanej syntezy białek hemowych, to jest katalazy T i cytochromów.

Słowa kluczowe: pyretroidy, stres, katalaza, cytochromy, drożdże

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EFFECT OF FOLIAR TREATMENT WITH SELENIUM AND COOKING ON THE NUTRITIONAL QUALITY OF POTATOES

WPŁYW NAWOŻENIA DOLISTNEGO SELENEM I GOTOWANIA NA JAKOŚĆ ŻYWIENIOWĄ ZIEMNIAKÓW

Abstract: In accurate small-plot experiments with potatoes we explored the effect of foliar applications of Se in the form of sodium selenate(IV) on tuber yields, the Se concentration in the tops and tubers of raw and boiled potatoes and in French fries, in the 'Karin' and 'Ditta' varieties. The experiments were established in Žabčice near Brno in 3 variants: (1) control; (2) 200 g Se $\cdot$ ha^{-1}; (3) 400 g Se $\cdot$ ha^{-1}. The mean total yields of tubers per ha were the highest in the control variant (19.99 Mg $\cdot$ ha^{-1}) but applications of selenium reduced the yields statistically insignificantly (to 16.79 and 18.39 Mg $\cdot$ ha^{-1}, respectively). The 'Ditta' variety produced higher yields. The average content of Se increased with the applied dose as compared with the control and equals: in the tops 0.255; 0.739 and 0.767 mg $\cdot$ kg^{-1} d.m., in raw tubers 0.214; 0.564 and 0.917 mg $\cdot$ kg^{-1} d.m., in boiled tubers 0.200; 0.523 and 0.915 mg $\cdot$ kg^{-1} d.m. and in French fries 0.223; 0.425 and 0.574 mg $\cdot$ kg^{-1} d.m. The content of Se decreased more markedly in French fries by heat processing. On the basis of the achieved results foliar nutrition appears to be a suitable and economically reasonable measure towards a targeted increase in the content of Se in potato tubers from the viewpoint of the positive effects of Se on the human organism.

Keywords: potatoes, selenium, foliar nutrition, yields, tops, tubers, French fries

Of late the importance of antioxidants in foodstuffs has been continuously growing since they improve the quality of the food and in this way protect human health. This group of substances also includes selenium. The source of selenium for the human organism is vegetables, fruit and agricultural produce [1] the predominant part being in the form of selenomethionine which is biologically more accessible for humans [2].

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In the human organism selenium is a component of biologically active proteins. Approximately 30 proteins with an enzyme activity have been confirmed of which the most important is glutation peroxidase which helps to protect lipoprotein membranes against the effect of hydroperoxides and other toxic compounds of oxygen [3]. Selenium is also a component of thioredoxin reductase which plays an important role in regeneration of the antioxidant system and is also a component of proteins partaking in the production of the thyroid hormone [4]. In these selenoproteins Se appears in the form of selenocysteine amino acid [5]. Selenium plays a part in the prevention of cardiovascular diseases and cancer. Se deficiency gives rise to a number of health problems related to the immune system, virus infections, male reproduction organs, thyroid gland, asthma and various inflammations [6].

To a large extent the content of Se in plants is based on its concentration in the soil; most soils contain between 0.01 to 0.2 mg Se · kg^{-1} [7] in the various oxidation degrees – selenide (Se^{2-}), selenate(IV)* (SeO_3^{2-}) and selenate(VI) (SeO_4^{2-}) [8]. The water-soluble fraction of Se in the soil is taken up by plants and the plants prefer selenates(VI) to selenates(IV). Apart from that the plant can take up Se in the form of amino acids such as selenomethionine [4, 9]. Soil pH affects Se uptake and as it decreases the uptake of selenate(IV) and selenate(VI) is considerably restricted [10].

Since potatoes (*Solanum tuberosum* L.) are consumed on a regular basis, they are seen as a suitable crop for exploring Se supplementation thus increasing its content in the population. The Recommended Dietary Allowance (RDA) of Se for adult males and females is 0.055 mg · day^{-1} [11]. According to the German and Austrian Nutrition Society and the Swiss Nutrition Association a daily dose of 0.03–0.07 mg Se · day^{-1} is sufficient for an adult female and male [12]. However in most EU countries the daily intake of Se is below the RDA level [13] and according to Dietary Reference Intakes (DRI) [11] the acceptable upper limit of Se intake for adults is 0.4 mg · day^{-1}.

Tubers of selected potato varieties grown in the Czech Republik were analysed and their content of selenium was seen as insufficient [14]. The Se concentration in potato tubers can be elevated by foliar applications of Se, in this way contributing to the solution of the problem of Se deficiency in human nutrition as the studies of many researches confirmed [2, 3, 15–19].

The objective of the present experiment was to explore the effect of foliar Se applications on yields of potato tubers and on the content of Se in the tops and in variously treated tubers – raw, boiled and in French fries.

Material and methods

Foliar applications of selenium were applied in a small-plot trial established on experimental plots of the School Farm of the Mendel University of Agriculture and Forestry Žabčice in Brno (altitude 179 m a.s.l.). Table 1 shows the agrochemical characteristics of the soil prior to the establishment of the experiment.

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* Formerly known as selenite.

The soil was leached in Mehlich's III solution (CH_3COOH, NH_4NO_3, NH_4F, HNO_3 and EDTA). Phosphorus was determined by colorimetry and K, Ca and Mg using the AAS method. The available Se portion was extracted with a 2 $\text{mol} \cdot \text{dm}^{-3}$ HNO_3 solution and then AAS-determined.

Table 1

Results of analyses of a medium heavy soil (acc. to Mehlich III method)

Nutrient	Content [$\text{mg} \cdot \text{kg}^{-1}$]	Supply
Phosphorus	87.5	good
Potassium	204.8	good
Magnesium	383.6	very high
Calcium	3 399.2	high
Selenium	0.11	—
pH/$CaCl_2$	6.07	mildly acid

Two potato varieties, the early 'Karin' and semi-early 'Ditta', were used in the experiment and two levels of foliar selenium application in the form of sodium selenate(IV) were tested. The experiment was established in four replications. The pattern of the experiment was as follows:

1. unfertilised control,
2. 200 g $\text{Se} \cdot \text{ha}^{-1}$ on the 48th day of vegetation,
3. 400 g $\text{Se} \cdot \text{ha}^{-1}$ split
   - 1st dose 200 $\text{g} \cdot \text{ha}^{-1}$ applied on the 48th day of vegetation,
   - 2nd dose 200 $\text{g} \cdot \text{ha}^{-1}$ applied on the 55th day of vegetation.

The potatoes were planted on 3 April 2007. Selenium was applied at the beginning of bud setting, ie at the beginning of tuber formation. During vegetation the plot was kept free of weeds and treated with pesticides against the Colorado potato beetle (*Leptinotarsa decemlineata*) and late blight (*Phytophtora infestans*).

The dry spring weather (April) influenced the time of harvest. The 'Karin' variety was harvested on the 99th day of vegetation on 2 July 2007 and 'Ditta' on the 106th day of vegetation on 9 July 2007. Planning the harvest dates was based on the highest demand of these varieties on the Czech market. At harvest the tops and tubers were sampled and the hectare tuber yields were determined. The tops and tubers were rinsed in water; the tubers were then peeled and prepared for the respective method of processing – dried, raw, boiled and French fries. All parts of the potatoes were dried at 60 ^{o}C, then homogenised and prepared for analysis.

The samples were mineralised by decomposition of the samples in a mixture of HNO_3 and H_2O_2 in the MILESTONE MLS 1200 MEGA microwave. After transforming into the defined volume the sample was analysed on the UNICAM 939 "SOLAR" atomic absorption spectrophotometer using the method of hydride production by means of the "vapour system" UNICAM VP 90. The results were statistically processed using the STATISTICA 8 programme and method of multifactorial ANOVA followed by Tukeye's test at a 95 % level of significance.

Results and discussion

Table 2 shows the results of yields. It was discovered statistically significant differences between the varieties; the total yields of the 'Ditta' variety were higher than 'Karin' in all the three variants.

Table 2

The effect of selenium application on tuber yields

Variant	Karin		Ditta	
	[Mg · ha^{-1}]	[%]	[Mg · ha^{-1}]	[%]
1	16.03^{a}	100.0	23.95^{b}	100.0
2	11.17^{c}	69.7	21.20^{b}	88.5
3	15.22^{ac}	94.9	19.89^{ab}	83.0

* The same letters indicate insignificant differences among variants and varieties ($p < 0.05$).

No statistically significant difference was detected among the variants of the 'Ditta' variety, although the yields decreased with the increasing dose of Se. The highest yields, ie 23.95 Mg · ha^{-1}, were achieved in the control variant not fertilised with Se (Table 2).

The yields of variant 2 of the 'Karin' variety decreased statistically significantly compared with variant 1. With the highest dose of selenium in variant 3 the differences compared with the other two variants were insignificant. The results are in compliance with those of Jůzl et al [15] who also achieved the highest yields with the Se-unfertilised control variant of the potato variety 'Ditta' (25.9 Mg · ha^{-1}) as compared with 'Karin' (22.3 Mg · ha^{-1}). However, Jůzl et al [15] in their experiments applied Se in doses of 12, 24, 48 and 72 kg Se · ha^{-1} into the soil in the form of Na_2SeO_3. With increasing Se doses the tuber yields decreased and between the varieties the yields differed. On the other hand Turakainen et al [19] achieved statistically significantly lower tuber yields in the control variant not fertilised with Se; however they applied only 0.075 and 0.3 mg Se · kg^{-1} of soil in the form of Na_2SeO_4. These low doses of Se into the soil and another form of Se (Na_2SeO_4) probably caused the increasing of yield of potatoes.

The Se content in raw tubers of the 'Karin' variety (Table 3) increased with the applied dose of 0.2; 0.474 and 1.025 mg · kg^{-1} d.m.; the difference was statistically significant only between variant 3 and variants 1 and 2. The tendency in the selenium content of the 'Ditta' variety was similar (0.227; 0.654 and 0.809 mg · kg^{-1} d.m.). The difference was statistically significant only between variants 1 and 3. No differences were detected between the varieties. Poggi et al [2] achieved similar results; with foliar applications of 50 and 150 g Se · ha^{-1} they saw a linear increase in its content in the potato tubers. Likewise Jůzl et al [16] reported that even after a foliar application of only 100 g · ha^{-1} the Se concentration in tubers increased as much as 2.81 times when compared with controls not fertilised with selenium.

Table 3

The effect of applications of selenium on its content in raw tubers

Variant	Karin		Ditta	
	[mg · kg^{-1} d.m.]	[%]	[mg · kg^{-1} d.m.]	[%]
1	0.200^{a}	100.0	0.227^{ab}	100.0
2	0.474^{abc}	237.0	0.654^{bcd}	288.1
3	1.025^{d}	512.5	0.809^{cd}	356.4

The Se content in boiled tubers of both varieties (Table 4) was seen to increase (0.2; 0.494 and 0.841 mg Se · kg^{-1} d.m. in 'Karin' and 0.2; 0.552 and 0.989 mg Se · kg^{-1} d.m. in 'Ditta'). In both varieties the differences among the variants were statistically in significant. The differences between the varieties in the case of the individual variants were statistically not significant. The effect of heat treatment of the tubers by boiling on the content of selenium was insignificant. Average concentrations of Se in boiled tubers of the respective variants were comparable with the Se concentration in raw tubers (Tables 3 and 4).

Table 4

The effect of applications of selenium on its content in boiled tubers

Variant	Karin		Ditta	
	[mg · kg^{-1} d.m.]	[%]	[mg · kg^{-1} d.m.]	[%]
1	0.200^{a}	100.0	0.200^{a}	100.0
2	0.494^{b}	247.0	0.552^{b}	276.0
3	0.841^{c}	420.5	0.989^{c}	494.5

The Se content in French fries (Table 5) of the variety 'Karin' also increased with increasing Se doses (0.249; 0.421 and 0.814 mg Se · kg^{-1} d.m.); between variant 1 and 2 there was no statistically significant difference. The Se content in tubers of variant 3 was significantly higher than in the other variants. The Se content in French fries of the variety 'Ditta' was similar (0.2; 0.428 and 0.333 mg Se · kg^{-1} d.m.) and a statistically significant difference was detected only between variants 1 and 2.

Table 5

The effect of applications of selenium on its content in French fries

Variant	Se content in French fries			
	Karin		Ditta	
	[mg · kg^{-1} d.m.]	[%]	[mg · kg^{-1} d.m.]	[%]
1	0.249^{ab}	100.0	0.200^{b}	100.0
2	0.421^{a}	169.1	0.428^{a}	214.0
3	0.814^{c}	326.9	0.333^{ab}	166.5

Frying reduced the content of Se compared with raw tubers. This reduction was significant in variant 3 of the variety 'Ditta'; among varieties this difference was insignificant. Jůzl et al [15] reached the same conclusions saying that the Se content in boiled potatoes decreased by 15 % and in French fries by 22 % in an experiment where doses of 12, 24, 48, 72 kg Se · ha^{-1} were applied. The same authors [18] monitored similar results in an experiment with soil (12 and 24 kg Se · ha^{-1}) and foliar (200 and 400 g Se · ha^{-1}) applications; after heat treatment the Se content in potato tubers decreased. Frying reduced the Se content more than boiling.

The Se content in tops (Table 6) of the 'Karin' variety also increased with the applied dose (0.237; 0.695 and 1.013 mg Se · kg^{-1} d.m.); the difference between variant 1 and 3 was statistically significant.

Table 6

The effect of applications of selenium on its content in tops (ppm in dry matter)

Variant	Karin		Ditta	
	[mg · kg^{-1} d.m.]	[%]	[mg · kg^{-1} d.m.]	[%]
1	0.237^{a}	100	0.272^{a}	100
2	0.695^{ab}	293.2	0.782^{ab}	287.5
3	1.013^{b}	427.4	0.518^{ab}	190.4

No difference was discovered among the variants of the 'Ditta' variety. Between the varieties no significant differences were discovered either. The Se content in the tops was comparable with the Se content in tubers. On the other hand Jůzl et al [18] discovered that after foliar application of 400 g Se · ha^{-1} the content of Se in the aboveground parts of potato was 3 times higher than in the tubers. The same authors [17] also pointed out that after soil application of selenium in doses ranging between 12 and 72 kg · ha^{-1} the tubers contained half the amount of Se than the potato tops.

Conclusions

The results imply that the application of Se did not increase yields and that with an increasing dose of foliar selenium its content in the potato tubers and in tops increased. Heat treatment of tubers by boiling did not change the content of selenium, but in French fries it reduced the content of Se. These differences in contents of Se in boiled tubers and French fries were probably caused by different processing temperatures (boiling 100 ^{o}C and frying 190 ^{o}C). Foliar applications of 200 and 400 g Se · ha^{-1} are seen as an effective way to increase the nutritional quality of potatoes.

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WPŁYW NAWOŻENIA DOLISTNEGO SELENEM I GOTOWANIA NA JAKOŚĆ ŻYWIENIOWĄ ZIEMNIAKÓW

Abstrakt: W ścisłym doświadczeniu mikropoletkowym badano wpływ nawożenia dolistnego selenu w formie selenianu(IV) sodu na plony bulw ziemniaków odmian ‘Karin’ i ‘Ditta’, zawartość Se w łętach i bulwach ziemniaków surowych i gotowanych oraz we frytkach. Doświadczenia założono w Žabčicach koło Brna w 3 wariantach: (1) kontrola; (2) 200 g Se · ha^{-1}; (3) 400 g Se · ha^{-1}. Największe średnie plony bulw uzyskano w obiekcie kontrolnym (19,99 Mg · ha^{-1}), a stosowanie selenu zmniejszało statystycznie nieznacząco plony (odpowiednio o 16,79 i 18,39 Mg · ha^{-1}). Odmiana ‘Ditta’ wytwarzała większe plony. Średnia zawartość Se zwiększała się wraz z zastosowaną dawką Se w porównaniu z obiektem kontrolnym i wynosiła w łętach 0,255; 0,739 i 0,767 mg · kg^{-1} s.m., w bulwach surowych 0,214; 0,564 i 0,917 mg · kg^{-1} s.m., w gotowanych bulwach 0,200; 0,523 i 0,915 mg · kg^{-1} s.m. i we frytkach 0,223; 0,425 i 0,574 mg · kg^{-1} s.m. Zawartość Se zmniejszała się wyraźniej we frytkach w wyniku obróbki termicznej. Na podstawie uzyskanych wyników można stwierdzić, że nawożenie dolistne wydaje się być odpowiednim i ekonomicznie uzasadnionym sposobem w kierunku osiągnięcia większej zawartości Se w bulwach ziemniaków z punktu widzenia pozytywnego wpływu Se na organizm ludzki.

Słowa kluczowe: ziemniaki, selen, nawożenie dolistne, plony, łęty, bulwy, frytki

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LINURON, DDT AND ORGANOCHLORINE PESTICIDE RESIDUES IN PLANTS FROM NORTH-EASTERN POLAND

LINURON, DDT I POZOSTAŁOŚCI PESTYCYDÓW CHLOROORGANICZNYCH W MATERIALE ROŚLINNYM PÓŁNOCNO-WSCHODNIEJ POLSKI

Abstract: The aim of this study was to investigate the DDT, organochlorine and linuron pesticide residues in agricultural products and herbs from North-Eastern Poland in 2000–2007. Total analyzed 3399 samples: field vegetables, greenhouse vegetables, fruit, field crops and herbs.

The determination of 20 active substances: DDT (*p,p'*-DDT, *o,p'*-DDT, *p,p'*-DDE, *p,p'*-DDD), aldrin, dicofol, dieldrin, endosulfan(α, β, sulfate), endrin, HCH (α-HCH, β-HCH, γ-HCH and δ-HCH), heptachlor, heptachlor epoxide(endo, exo), metoxychlor and linuron was carried by gas chromatography with selective detectors: EC and NP. Pesticide residues of linuron were detected only in carrot samples; DDT and metabolites of degradation in carrots, parsley, wheat, and flower of mallow, endosulfan in black curry and mushrooms; dicofol only in flower of mallow; lindan in parsley and wheat. Aldrin, heptachlor and metoxychlor were not found in any sample. Evaluation of detected contamination was carried out on the basis of appropriate regulation of Minister of Health on maximum residue limits (MRLs) in foodstuffs, on the basis of the register of biological substances in Poland and on the basis of EU directives.

Violations of MRLs were found in the case of endosulfan, degradation products of DDT, dieldrin, lindan, dicofol and linuron in small percent. After estimating long and short term risk of exposure to these compounds, it has been stated that food from North-Eastern Poland is safe considering the presence of organochlorine pesticides, and detection of pesticide residues was incidental.

Keywords: food contamination, organochlorine pesticides, DDT, linuron, monitoring, gas chromatography

Contamination of the environment and food by pesticide residues is an important topical issue in many areas of the world. Food has never been free of harmful components, but nowadays, the problem is serious. Pesticides were extensively used as insecticides, fungicides, herbicides in agriculture, for pest control in forestry and vector control in hygiene against diseases like malaria and typhus. Today residues of *p,p'*-DDT

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and other chlorinated pesticides are ubiquitously distributed and despite the ban in most industrialized countries in the early 1970s, significant concentrations are determined worldwide in water, sediment, agricultural commodities and soil samples of formerly affected environments [1–5].

Organochlorine (OCPs) pesticides were banned in Poland in 1975. OCPs pesticides act by binding to the acetylcholinesterase enzyme, disrupting nerve function, resulting in paralysis and death, yet their persistence in the environment is not well understood. The general population is mainly exposed to OCPs pesticides through the ingestion of contaminated foods (such as cereals, vegetables and fruits) which are directly treated with OCPs pesticides or are grown in contaminated fields. The long-term persistence of these pesticides, therefore, still poses problems for the environment, for farming in general and for organic farming in particular. The OCPs in fish, meat, rice, vegetables and mothers' milk [4] were found at high concentrations. The residues of pesticide could affect the final consumers especially when these commodities are freshly consumed. The total dietary intake of pesticide residues that remain on agricultural commodities are known as carcinogens and/or toxins, and therefore it is desirable to reduce these residues. The levels of pesticide residues are controlled by the maximum residue limits (MRLs) which are established by each country and sometimes cause conflicts because residue levels acceptable in one country could be unacceptable in another. Many countries have given high priority to monitor and control pesticide residues on treated crops [6–8]. Methods were developed and implemented as part of regulatory programs to detect products that exceed MRLs. In Poland, the MRLs of pesticide residues for specific commodities are established in Decree of Polish Minister of Health.

The aim of this study was to determine levels of organochlorine and linuron contaminants in vegetable, fruits, agricultural crops and herbs from north-eastern region of Poland called "Green Lungs of Poland" owing to its relatively clean environment. The analysis of organochlorine pesticide, DDT and its metabolites and linuron was performed by gas chromatography with specific EC and NP detectors.

Materials and methods

Fruit samples used in this study (Table 1) included: apples, currant, cherry, pear, plum, raspberry, strawberry, blueberry, berry and chokeberry, while vegetable samples included: road beet, cabbage, cauliflower, carrot, cucumber, onion, parsley, green been, broccoli, celery, tomato, leek; indoor cultivation: mushroom, paprika, tomato, lettuce, cucumber, cabbage. Among field crops were: potatoes, rape, wheat, barley, oat, rye, sugar-beets. The analysis was also conducted for herbs: folia: lemon balms, salvia, nettle, peppermint, apple and black currant, the flower: elder, chamomile, mallow; herb of St-John's-wort and horsetail. The main part of samples (2289 samples) were from government monitoring program in North-Eastern Poland conducted in 2000–2007 including Warmia-Mazury, Podlasie and Lublin region; remaining samples were from individual farm and gardeners from North-Eastern Poland (1110 samples) (Table 1). The sample size was at least 1 kg for small and medium size of fresh product. The minimum weight for large sample size was 2 kg (for example broccoli and cabbage), where the unit was generally more than 250 g (Codex Alimentarius, 2000). The portion of raw agricultural commodity prepared as the analytical sample for determination of pesticide residues was carried out by matrix solid phase dispersion. A representative

Table 1

Number and kind of analyzed crops, samples with residues, above or under maximum residues level

Product range		No. of samples		No. of samples with residues	No. of samples < MRLs	No. of samples > MRLs
Commodity		M	O			
Agricultural crops 257 (M) 44 (O)	potato	117	31	nd	0	0
	rape	10	0	nd	0	0
	oat	12	0	nd	0	0
	winter-spring wheat	64	13	**2**	0	2
	barley	12	0	nd	0	0
	rye	7	0	nd	0	0
	sugar-beet	35	0	nd	0	0
Fruit 955 (M) 520 (O)	apple	338	134	nd	0	0
	currant	118	10	**17**	5	12
	cherry	176	121	nd	40	0
	pear	13	0	nd	0	0
	plum	21	0	nd	0	0
	rasberry	15	38	nd	0	0
	strawberry	260	151	nd	0	0
	blueberry	0	12	nd	0	0
	berry	0	12	**1**	0	1
	chokeberry	14	42	nd	0	0
Field vegetable 623 (M) 420 (O)	red beet	33	0	nd	0	0
	cabbage	129	0	nd	0	0
	carrot	110	51	**14**	11	3
	cauliflower	51	98	0	0	0
	cucumber	135	0	0	0	0
	onion	45	45	0	0	0
	parsley	7	0	**2**	0	2
	green been	35	0	0	0	0
	broccoli	25	115	0	0	0
	celery	3	0	0	0	0
	tomato	45	100	0	0	0
	leek	5	11	0	0	0

Table 1 contd.

Product range		No. of samples		No. of samples with residues	No. of samples < MRLs	No. of samples > MRLs
Commodity		M	O			
Greenhouse vegetable 454 (M) 56 (O)	mushroom	70	0	**1**	1	0
	paprika	17	4	0	1	0
	tomato	199	35	0	2	0
	lettuce	46	2	0	0	0
	cucumber	120	15	0	0	0
	cabbage	2	0	0	0	0
Herbs 70 (O)	folia of peppermint	0	18	0	0	0
	folia of apple	0	4	0	0	0
	folia of black currant	0	8	**2**	0	2
	flower of elder	0	8	0	0	0
	flower of chamomile	0	4	0	0	0
	flower of mallow	0	8	**2**	1	1
	herb of st-john's-wort	0	4	0	0	0
	herb of horsetail	0	4	0	0	0
	folia of salvia	0	4	0	0	0
	folia of nettle	0	4	0	0	0
	folia of lemon balm	0	4	0	0	0

M – samples from national monitoring in North-Eastern Poland, O – samples from individual farm and gardeners in North-Eastern Poland, nd – not detected, MRLs – maximum residues level.

portion of the analytical sample 2 was blended using a food processor and mixed thoroughly with the solid-phase materials (Florisil PR (Floridon Co., USA), Silicagel 60 (70–230 mesh, Merck) and neutral alumina (70–230 mesh, activity IB supplied by Merck). All adsorbents were activated by heating overnight at 150 ^{o}C before use, allowed to cool and stored in well-closed flask. Freshly activated adsorbents were used for this study. Anhydrous sodium sulfate p.a. used for drying was heated for 4 hrs. at 500 ^{o}C. The clean-up step was performed by column chromatography. The homogenous mixture was transferred into a glass chromatography column containing 5 g of anhydrous sodium sulfate, 2 g of deactivated alumina or activated silica gel. The analytes were eluted with solvents of increasing polarity in 15 cm^3 fractions: *n*-hexane-ethyl ether (9:1), *n*-hexane-ethyl ether (8:2), *n*-hexane-ethyl acetate (7:3). Elution was performed by gravity flow. The three fractions were combined and concentrated to about 1 cm^3 using vacuum evaporator with temperature programmed bath (40 ^{o}C). The final volume of the eluates was adjusted to 2 cm^3 by addition of *n*-hexane-acetone mixture (9:1).

Pesticide-grade ethyl acetate, acetone, *n*-hexane and anhydrous sodium sulfate were obtained from Merck (Darmstadt, Germany). Pesticide standards of DDT (*p,p'*-DDT;

p,p'-DDT; *p,p'*-DDE; *p,p'*-DDD), aldrin, dicofol, dieldrin, endosulfan(α, β, sulfate), endrin, HCH (α-HCH, β-HCH, γ-HCH and δ-HCH), heptachlor, heptachlor epoxide(endo, exo), metoxychlor, linuron were purchased from Dr. Ehrenstorfer (Ausberg, Germany). Individual stock standard solution of pesticide was prepared by dissolving 50–60 mg of each compound in 50 cm^3 of acetone and stored in amber bottles at 4 ^oC. A mixed standard solution was prepared from the stock solutions with a concentration of 10 $mg \cdot dm^{-3}$. A series of calibration standards were prepared by diluting 10 $mg \cdot dm^{-3}$ of the mixed standard solution to produce a final concentration of 0.01; 0.1; 0.2 and 0.5 $mg \cdot dm^{-3}$ in acetone.

Gas chromatographic determination

The Agilent HP 6890 N gas chromatograph equipped with an ECD and with HP-5 fused capillary column (30-m length, 0.32-mm internal diameter, and 0.25-mm film thickness) was used. The injection port temperature was 250 ^oC and the detector temperature was 300 ^oC. The column temperature was programmed as follows: the initial temperature of 100 ^oC was increased at a rate of 10 $^oC \cdot min^{-1}$ up to 250 ^oC and held for 25 min, from 250 to 300 ^oC at a rate of 50 $^oC \cdot min^{-1}$ was used and held for 5 min at the final temperature. Helium carrier gas at a flow rate of 1.2 $cm^3 \cdot min^{-1}$ was used. Two microlitres of the extract were injected and the retention time of peak was compared with that retention time of the calibration standards (in matrices) to determine the residue quantitatively.

Results and discussion

GC-ECD provided good responses even at very low concentrations because of its selective and sensitive detectors.

Among 3399 analyzed samples (2000–20007 years) pesticide residues of organochlorine pesticides were detected in 20 samples of fruit (total 1475 samples of fruit), in 2 field crops (total 301 samples) and in 4 samples of herbs (total 70 samples). The pesticide residues of linuron and organochlorine compounds were detected in 18 samples of field vegetables and greenhouse vegetables (total 1553 samples). Among the determination of organochlorine pesticides (Table 2) in raw fruit were detected products of DDT decomposition and endosulfan, in vegetables – lindane (γ-HCH), products of DDT decomposition and herbicide – linuron. In samples of field crops were found products of DDT decomposition and lindane. In investigated herbs were detected dicofol, products of DDT decomposition and endosulfan.

Levels of determined pollutants in individual years are presented in Table 3.

Linuron is a selective herbicide which is most readily absorbed through the root system. It is labeled for use in soybean, cotton, potato, corn, bean, pea, winter wheat, asparagus, carrot, and fruit crops. It accumulates and metabolizes differently in different plants. Susceptible plants transport linuron through the foliage, while tolerant plants can metabolize linuron into inactive products. Linuron inhibits photosynthesis in susceptible plants causing them to lose color, wilt, and die. In crops, linuron has low residual action

Table 2

The mean levels of organochlorine and herbicide linuron in vegetables, fruit, agricultural crops and herbs in North-Eastern Poland 2000–2007

Active substance	LOD [mg · kg^{-1}]	Fruit (n = 1477)		Vegetable (n = 1560)		Agricultural crops (n = 275)		Herbs (n = 70)	
		Mean value [mg · kg^{-1}]	No. of positive samples	Mean value [mg · kg^{-1}]	No. of positive samples	Mean value [mg · kg^{-1}]	No. of positive samples	Mean value [mg · kg^{-1}]	No. of positive samples
Aldrin	0.005	nd	0	nd	0	nd	0	nd	0
p,p’-DDT	0.005	9.50	1	0.02	3	15.5	1	27.57	1
o,p’-DDT	0.005	1.05	1	nd	0	1.70	1	1.40	1
p,p’-DDE	0.005	0.44	1	0.025	1	0.37	1	0.27	1
p,p’-DDD	0.005	0.32	1	nd	0	0.75	1	0.81	1
Dicofol	0.005	nd	0	nd	0	nd	0	0.17	1
Dieldrin	0.005	nd	0	0.12	1	nd	0	nd	0
Endosulfan sum	0.005	0.14	17	0.3	1	nd	0	0.94	2
α-HCH	0.005	nd	0	nd	0	nd	0	nd	0
β-HCH	0.005	nd	0	nd	0	nd	0	nd	0
γ-HCH (lindane)	0.005	nd	0	0.06	1	0.45	1		
δ-HCH	0.005	nd	0	nd	0	nd	0	nd	0
Heptachlor sum	0.005	nd	0	nd	0	nd	0	nd	0
Metoxychlor	0.005	nd	0	nd	0	nd	0	nd	0
Linuron	0.005	nd	0	0.12	12	nd	0	nd	0

LOD – limit of detection.

Table 3

Detection of pesticide residues (concentration over maximum residue levels in agricultural crops, fruit, vegetables and herbs – marked in bold)

Year	Commodity	No. of samples with residues	Pesticide	Concentration [mg · kg^{-1}]	MRL [mg · kg^{-1}]
2000	carrot	1	*p,p'*-DDT	0.007	0.01
	carrot	3	linuron	0.18; **0.22**; 0.05	0.2
	black berry	2	endosulfan	0.08 ;0.01	0.05
2001	carrot	5	linuron	0.05; 0.05; 0.07; 0.07; 0.08	0.2
	parsley	1	γ-HCH	**0.06**	0.01
	wheat	1	γ-HCH	**0.45**	0.01
	black berry	7	endosulfan	**0.07**; **0.08**; **0.16**; **0.21**; **0.35**; **0.45**	0.05
2002	carrot	1	linuron	0.17	0.2
	black berry	3	endosulfan	**0.1**; **0.13**; **0.13**	0.05
	folia of black currant	2	endosulfan	**0.25**; **1.62**	0.05
	flower of mallow	1	dicofol	0.17	20
2003	carrot	1	linuron	0.04	0.2
	black-currant	3	endosulfan	**0.2**	0.05
	flower of mallow	1	*p,p'*-DDD *p,p'*-DDE *o,p'*-DDT *p,p'*-DDT	**0.81** **0.27** **1.40** **27.57**	0.01 0.01 0.01 0.01
2004	mushroom	1	endosulfan	0.03	0.05
	black-currant	3	endosulfan	0.02; 0.04; **0.3**	0.05
2005	parsley	1	*p,p'*-DDT	**0.07**	0.01
	wheat	1	*p,p'*-DDD *p,p'*-DDE *o,p'*-DDT *p,p'*-DDT	**0.75** **0.37** **1.70** **15.50**	0.01
2006	carrot	1	*p,p'*-DDT dieldrine *p,p'*-DDE	0.005 **0.12** **0.025**	0.01 0.01 0.01
	black berry	1	endosulfan	0.06	0.05
	blue berry	1	*p,p'*-DDD *p,p'*-DDE *o,p'*-DDT *p,p'*-DDT	**0.32** **0.44** **1.05** **9.50**	0.01 0.01 0.01 0.01
2007	carrot	2	linuron	0.02; **0.4**	0.2

and persistence. Linuron has been detected in 12 samples of carrots (0.02–0.4 mg · kg^{-1}). Two samples of carrots had linuron concentration below Maximum Residues Level (for carrots is 0.2 mg · kg^{-1}). The highest detected concentration was two times higher than Maximum Residues Level and average concentration in 12 samples of carrots were 0.12 mg · kg^{-1}. Endosulfan is a chlorinated hydrocarbon insecticide of the cyclodiene subgroup which acts as a contact poison in a wide variety of insects and mites. It is used primarily on food crops like tea, fruits, vegetables and on cereals. The breakdown product, endosulfan sulfate, has been observed in several field studies involving plants [9, 10]. The sulfate is more persistent than the parent compound, accounting for 90 % of the residue in 11 weeks. On most fruits and vegetables, 50 % of the parent residue is lost within three to seven days. Endosulfan residues have been found in 20 samples: blackcurrant berry, black berry, leaves of black currant berry and mushrooms at low concentrations. They have been detected in 0.01–0.45 mg · kg^{-1}. The average concentration in fruit was 0.14 mg · kg^{-1}, vegetables 0.03 mg · kg^{-1} and herbs 0.95 mg · kg^{-1}.

DDT has been banned in developed countries because of long-term persistence and accumulation but pesticides are still detected. The resignation from using DDT is an expression of the rule of the foresight, because it is not yet known how it is accumulated in fat tissues and how it influences health of people. DDT is classified as "moderately toxic" by the US National Toxicological Program and "moderately hazardous" by WHO. DDT is a persistent organic pollutant with a half life of 2–15 years, and is immobile in most soils. Breakdown products in the soil environment are DDE (1,1-dichloro-2,2-bis(*p*-dichlorodiphenyl)ethylene) and DDD (1,1-dichloro-2,2-bis(*p*--chlorophenyl)ethane) which are also highly persistent and have similar chemical and physical properties. These products together are known as total DDT. In this study DDT and its metabolite was detected in berry: *p,p'*-DDD – 0.32 mg · kg^{-1}, *p,p'*-DDE – 0.44 mg · kg^{-1}, *o,p'*-DDT – 1.05 mg · kg^{-1}, *p,p'*-DDT – 9.50 mg · kg^{-1}. Concentration of all metabolites were 11.31 mg · kg^{-1}. In one sample of wheat were found *p,p'*-DDD – 0.75 mg · kg^{-1}, *p,p'*-DDE – 0.37 mg · kg^{-1}, *o,p'*-DDT – 1.70 mg · kg^{-1}, *p,p'*-DDT – 15.50 mg · kg^{-1} isomers and in flower of mallow: *p,p'*-DDD – 0.81 mg · kg^{-1}, *p,p'*-DDE – 0.27 mg · kg^{-1}, *o,p'*-DDT – 1.40 mg · kg^{-1}, *p,p'*-DDT – 27.57 mg · kg^{-1}. The concentration of p,p'-DDT isomer in all analyzed cases was the highest. *p,p'*-DDT was detected in the parsley – 0.07 mg · kg^{-1} and carrot – 0.025 mg · kg^{-1}. Dieldrine was detected only in carrots and dicofol only in flower of mallow (0.17 mg · kg^{-1}). γ-HCH was detected in parsley 0.06 mg · kg^{-1} and wheat 0.45 mg · kg^{-1}. Aldrin, heptachlor and metoxychlor were not found in any sample.

Conclusions

The results show that linuron pesticide was only present in carrots. Detected organochlorine suggests that these pesticides were not in common use in fruit and vegetable samples found in this region. In order to minimize health risk as well as for enforcement activities, monitoring of pesticide residues is increasingly important and essential [6, 12].

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LINURON, DDT I POZOSTAŁOŚCI PESTYCYDÓW CHLOROORGANICZNYCH W MATERIALE ROŚLINNYM PÓŁNOCNO-WSCHODNIEJ POLSKI

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Abstrakt: Celem badań była ocena poziomu skażeń płodów rolnych oraz surowców zielarskich pochodzących z północno-wschodniej Polski pod kątem obecności linuronu, DDT i związków chloroorganicznych.

W latach 2000–2007 w Laboratorium BPŚOR w Białymstoku przebadano ponad 3000 próbek. Badane asortymenty to: warzywa spod osłon, płody rolne, owoce, surowce zielarskie. Oznaczenia 20 substancji aktywnych: czterech izomerów DDT (*p,p'*-DDT, *p,p'*-DDT, *p,p'*-DDE, *p,p'*-DDD), aldryny, dikofolu, dieldryny, endosulfanu(α, β, siarczanu), endryny, czterech izomerów HCH (α-HCH, β-HCH, γ-HCH i δ-HCH), heptachloru, heptachloru epoksydu(endo, exo), metoksychloru i linuronu wykonano metodą chromatografii gazowej z zastosowaniem specyficznego mikrodetektora EC. Pozostałości linuronu stwierdzono w uprawie marchwi, DDT i produkty rozkładu w marchwi, pietruszce, pszenicy, kwiecie ślazu, endosulfan w porzeczce czarnej i jagodzie, dikofol – w kwiecie ślazu, lindan w pietruszce i pszenicy, a dieldrynę w marchwi. Ocenę wykrywanych skażeń prowadzono zgodnie z rozporządzeniem Ministra Zdrowia i Opieki Społecznej w sprawie największych dopuszczalnych pozostałości w środkach spożywczych, rejestrem substancji biologicznych w Polsce oraz zgodnie z dyrektywą Unii Europejskiej.

Przekroczenia największych dopuszczalnych poziomów (NDP) stwierdzono w przypadku endosulfanu, produktów rozkładu DDT, dieldryny, dikofolu, lindanu i linuronu w niewielkim procencie. Po ocenie krótko- i długoterminowego ryzyka narażenia ludności na te związki stwierdzono, iż żywność pochodząca z Podlasia jest bezpieczna pod kątem obecności pestycydów chloroorganicznych, a stwierdzone obecności tych związków są incydentalne.

Słowa kluczowe: zanieczyszczenie żywności, DDT, linuron, pestycydy chloroorganiczne, monitoring, chromatografia gazowa

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CHEMICAL CONTAMINATION OF SOIL RESULTING FROM THE STORAGE AND DISASSEMBLY OF MOTOR VEHICLES

SKAŻENIA CHEMICZNE GLEBY W WYNIKU SKŁADOWANIA I DEMONTAŻU POJAZDÓW SILNIKOWYCH

Abstract: The objective of this study was to determine the degree of chemical contamination of light soil used as a disposal site for storing motor vehicles unfit for operation. Dismantled motor vehicles and parts of motor vehicles were stored in sector A, while worn-out, inoperable motor vehicles were stored in sector B. Heavy metal content was 5.3-fold higher in soil from sector A than in soil from sector B. The greatest differences were observed between the two sectors with respect to the content of copper, lead, chromium and zinc, while the slightest – with regard to the content of nickel, cadmium and mercury. Taking into account the total content of heavy metals, the investigated soils may be considered chemically degraded.

Keywords: soil contamination, heavy metals, motor vehicles

Motor vehicles that are no longer used or operated are stored in areas specifically designated for this purpose. Parts of disassembled vehicles are often stored at unroofed facilities or in the open area. This may lead to local soil contamination with both mineral and organic compounds. The paper presents the results of studies on the content of heavy metals and selected macronutrients in light soil used as a disposal site for collecting and storing dismantled motor vehicles and motor vehicle parts.

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Materials and methods

The study was conducted at a facility located in town N, where scrapped motor vehicles and their parts are sorted and pre-processed. The facility is divided into two sectors, A and B. Dismantled motor vehicles and parts of motor vehicles are stored in sector A, while worn-out, junked or discarded motor vehicles are stored in sector B. Soil samples taken at a depth of 0–25 cm. Four composite samples were prepared for each section. P and K contents were determined by Egner-Riehm method, pH in 1 mol HCl $\cdot$ dm^{-3}, contents of heavy metals by the AAS method after acid-base extraction, trace mercury with the AMA 254.

Results and discussion

The disposal site for collecting and storing dismantled motor vehicles and motor vehicle parts was located on light soil developed from loose sand (sector A) and slightly loamy sand (sector B). Soil particle size distribution [%] in sectors A and B was as follows:

Size fractions	Sector A	Sector B
Sand (2.0–0.05 mm)	92.91	83.84
Silt (0.05–0.002 mm)	6.38	14.74
Clay (< 0.002 mm)	0.71	1.39

An analysis of selected agrochemical properties of soil revealed considerable differences between the two sectors (Table 1).

Table 1

Content of organic carbon and available nutrients in soil, and soil reaction

Property	Unit	Sector A		Sector B	
		mean	range	mean	range
Organic C	[g $\cdot$ kg^{-1}]	4.3	2.0–7.2	10.1	6.3–16.6
Available P	[mg $\cdot$ kg^{-1}]	51.7	11.7–73.0	107.0	58.1–131.1
Available K	[mg $\cdot$ kg^{-1}]	40.2	15.8–72.2	82.4	56.8–108.7
Available Mg	[mg $\cdot$ kg^{-1}]	14.9	10.5–15.5	23.0	17.5–27.5
Reaction	pH	7.8	7.7–7.9	7.5	7.4–7.7

Soil samples collected in sector B contained 2.4-fold more organic carbon, over 2-fold more available phosphorus and potassium and 1.5-fold more available magnesium than soil samples taken in sector A. However, it should be noted that the content of the above nutrients in soil varied widely.

The main indicator of chemical contamination is the concentration of metals displaying a variable degree of toxicity in soil. The investigated soils differed considerably with respect to heavy metal content (Table 2).

Table 2

Heavy metal content of soil [mg · kg^{-1}]

Metal	Sector A			Sector B		
	minimum	maximum	mean	minimum	maximum	mean
Pb	7.2	319.8	163.5	9.2	31.4	20.3
Cd	0.18	1.04	0.61	0.27	0.56	0.41
Zn	39.8	1305.0	672.4	57.4	241.4	149.4
Cu	4.84	1868.0	936.4	9.25	61.8	35.5
Mn	122.0	378.6	250.3	119.4	224.7	172.0
Ni	5.10	46.6	25.8	5.70	16.31	11.0
Cr	9.53	251.7	130.6	7.87	35.5	21.7
Hg	0.028	0.066	0.047	0.033	0.051	0.042

Mean values show that soil in sector A contained 5.3-fold more heavy metals than soil in sector B. There were significant differences between the two sectors as regards the concentrations of particular metals. In soil samples collected in sector A the content of copper, lead, chromium and zinc increased 26.3-, 8.0-, 6.0- and 4.5-fold, respectively, compared with soil samples taken in sector B. The content of nickel, cadmium and mercury rose from 2.3 to 1.1 times. The minimum heavy metal contents determined in the investigated soil exceeded the permissible limits for light soils [1], which indicates that their maximum concentrations can be considered highly toxic.

The mean percentage share of particular heavy metals in their total content (100 %) is illustrated in Fig. 1.

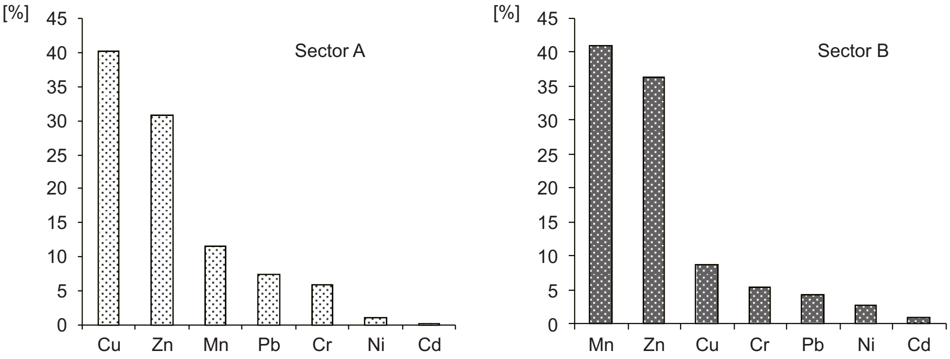

Fig. 1. Percentage share of heavy metals in their total content

In soil from sector A copper accounted for as much as 43 %, compared with 8.6 % in soil from sector B. Although the manganese content of soil was higher in sector A, the percentage of this element in the total content of heavy metals in soil was lower than in sector B. The percentage content and relative share of zinc were comparable in both analyzed soils.

The results of laboratory analyses confirmed that the investigated soils were contaminated with heavy metals to a different degree. Soil remediation aimed at its restoration to useable condition requires a method enabling the effective removal of one or more heavy metals whose concentrations exceed toxicity thresholds [2, 3].

The assessment of the chemical contamination of soil indicates the need to continue research in this subject. Such investigations contribute to environmental pollution reduction and may provide important data for thematic reports [4, 5].

Conclusions

1. The storage of worn-out or dismantled motor vehicles and motor vehicle parts results in the chemical degradation of soil.
2. The concentration of heavy metals (primarily copper and zinc) is substantially higher at sites used for storing parts of dismantled and inoperable motor vehicles than at sites used for storing junked, worn-out motor vehicles.

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SKAŻENIA CHEMICZNE GLEBY W WYNIKU SKŁADOWANIA I DEMONTAŻU POJAZDÓW SILNIKOWYCH

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Abstrakt: Badania miały na celu określenie skażeń chemicznych gleby lekkiej, na której składowano pojazdy silnikowe wycofane z eksploatacji. W sektorze A składowano zdemontowane pojazdy i ich części, a w sektorze B gromadzono pojazdy wycofane eksploatacji. W glebie sektora A sumaryczna zawartość metali ciężkich była 5,3 razy większa niż w glebie sektora B. Szczególnie duże różnice stwierdzono w zawartości miedzi, ołowiu, chromu i cynku, a najmniejsze niklu, kadmu i rtęci. Ogólna zawartość metali ciężkich sprawia, że gleby te należy zaliczyć do zdegradowanych chemicznie.

Słowa kluczowe: skażenie gleby, metale ciężkie, pojazdy silnikowe

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EFFECT OF CADMIUM AND ZINC ON METABOLISM OF SPINACH

WPŁYW KADMU I CYNKU NA METABOLIZM SZPINAKU

Abstract: Changes in metabolism of spinach (*Spinacia oleracea* L., 'Matador' cv.) plants exposed to stress caused by two heavy metals: cadmium and zinc were studied. The distribution of these elements in biomass (in root, leaf blade, petiole), chlorophyll content and activity of glutamate 5-kinase [E.C.2.7.2.11] the enzyme catalyzing the first step of proline biosynthesis were investigated in pot experiments. Results of the experiment revealed the toxic effects of cadmium and zinc at both tested levels (2.5, 25 mg Cd and 50, 500 mg Zn $\cdot$ kg^{-1}) for spinach. Under these conditions, decrease of glutamate kinase activity in spinach plants grown on contaminated treatments compared with untreated control was found. Allosteric regulation of glutamate kinase activity by free proline creates a possibility for an increase in glutamic acid content due to the synthesis of glutathione and subsequently phytochelatines in plant cells. For this reason the rates of Cd and Zn applied into soil decreased the glutamate kinase activity. The higher doses of both metals negatively affected yield of spinach dry biomass, but only Cd dose (25 mg Cd $\cdot$ kg^{-1} soil) was associated with the significant inhibition of aboveground biomass compared with control treatment (by 14 %). The highest contents of both elements were analyzed in blades of spinach leaves. Chlorophyll content was decreased only by higher cadmium dose.

Keywords: chlorophyll, glutamate kinase, toxic elements, stress, spinach

Cadmium is a widespread heavy metal released into the environment by energy production, metal-working industries, traffic, and phosphate fertilizers. Zinc toxicity is a problem in areas of natural Zn deposits, spoil heaps from mining and around zinc

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smelters. The increase of Zn and Cd accumulation in plant biomass can also be due to the application of sewage sludge to the soil [1, 2].

Heavy metals such as cadmium and zinc were found to induce oxidative stress in plants. Because of their atomic properties, heavy metals can induce uncontrolled redox reactions that will lead to the oxidation of membranes and proteins and the disruption of cellular homeostasis [3]. Plants can accumulate heavy metals in their tissues due to their great ability to adapt to variable properties of the environment. Cadmium and zinc can easily interact with iron, one of the most important elements for plant growth and metabolism. According to Siedlecka and Krupa [4] strong Fe-dependency to Cd mobility within the plant and adaptation of photosynthetic dark phase to Cd stress were found. Zinc is known to replace Fe from chelate complexes forming corresponding heavy metal chelates [5]. Cadmium inhibited the oxidative mitochondrial phosphorylation, reduced activity of plasma membrane ATPase and strongly affected the activity of several enzymes, such as glucose-6-phosphate dehydrogenase, isocitrate dehydrogenase, Rubisco and carbonic anhydrase. Cd ions can inhibit the activity of several antioxidative enzymes [6].

Metabolisms of glutathione, organic acids, peroxidases, stress proteins, proline and related amino acids, as well as mechanisms of compartmentalization, lignification, and root development are affected by the presence of the toxic elements and many other stress agents. Accumulation of free proline in plants has been often reported as a response to a wide range of environmental stresses including presence of heavy metals [7]. The glutamate kinase enzyme catalyzed the first step of proline biosynthesis, ie the conversion of glutamic acid to γ-glutamyl phosphate. Determination of glutamate kinase activity seems to be a suitable tool for monitoring the stress-induced changes of plant metabolism during the whole plant growth period [8].

This study was focused on the investigation of changes in the plant metabolism under a chronic stress caused by contaminants. The glutamate kinase activity [E.C.2.7.2.11] was investigated as a plant response to stress caused by Cd and Zn application into soil.

Material and methods

Adaptation of spinach (*Spinacia oleracea* L., ‘Matador’ cv.) plants to excessive cadmium and zinc levels in soil was investigated in pot experiment repeated for three years. For experiment, spinach seeds (20 seeds obtained from SEMO Ltd. Smržice, Czech Republic) were sown into plastic pots containing soil mixture as specified below. The plants (10 plants per pot) were cultivated from April to June under natural light and temperature conditions at the experimental hall of the Czech University of Life Sciences in Prague, Czech Republic. The water regime was controlled and the soil moisture was kept at 60 % MWHC.

For culivation of spinach plants, 5 kg of chernozem soil ($pH_{KCl} = 7.2$, $C_{ox} = 1.83$ %, CEC = 258 mval · kg^{-1}) was thoroughly mixed with 0.5 g N, 0.16 g P, and 0.4 g K applied in the form of ammonium nitrate and potassium hydrogen phosphate for control treatment and with the same amount of nutrients plus toxic element (Cd in $CdCl_2$ or Zn in $Zn(CH_3COO)_2 \cdot 2H_2O$) for treated variants. Two rates of Cd (Cd1 = 2.5, Cd2 = 25 mg · kg^{-1}) and Zn (Zn1 = 50, Zn2 = 500 mg · kg^{-1}) were applied. Each treatment was performed in five replications. Spinach plants were planted up to the stage of full leaves development.

The dried aboveground spinach biomass was used for determination of total Cd and Zn contents. The plant material was decomposed by a dry ashing procedure. The ash was dissolved in 1.5 % HNO_3 [9].

Varian SpectrAA-400 (Australia) atomic absorption spectrometer with a GTA-96 graphite tube atomizer was applied for Cd determinations. A pyrolytically-coated tube with a L'vov platform was used for all Cd measurements. Flame atomization (air-acetylene flame) was applied (Varian SpectrAA-300 atomic absorption spectrometer, Australia) for Zn determinations. The quality of the plant and soil analyses was verified using the RM 12-02-03 Lucerne.

Glutamate kinase was isolated from acetone-dried spinach fresh aboveground biomass and was extracted from 1 g of acetone powder by 10 cm^3 phosphate buffer (50 mM, pH 7.6) for 30 min and centrifuged (2 °C, 22000 g) for additional 20 min. Solid ammonium sulphate was added (2.12 g to every 10 cm^3 filtered supernatant) and the mixture was centrifuged (2 °C, 22000 g) for 30 min. The pellets were suspended in 50 mM phosphate buffer (pH 7.6). After incubation the activity of the enzyme was determined spectrophotometrically by modification of the hydroxamate method [10].

Chlorophyll was extracted from spinach fresh aboveground biomass by acetone. Contents of chlorophylls *a* and *b* were determined spectrophotometrically at wavelength λ = 644 and 663 nm.

Results and discussion

Plant responses to the excessive cadmium and zinc contents in soil were assessed on the basis of decreased spinach dry matter after application of higher Cd and Zn rates (Fig. 1) and increased concentrations of these elements in the aboveground biomass

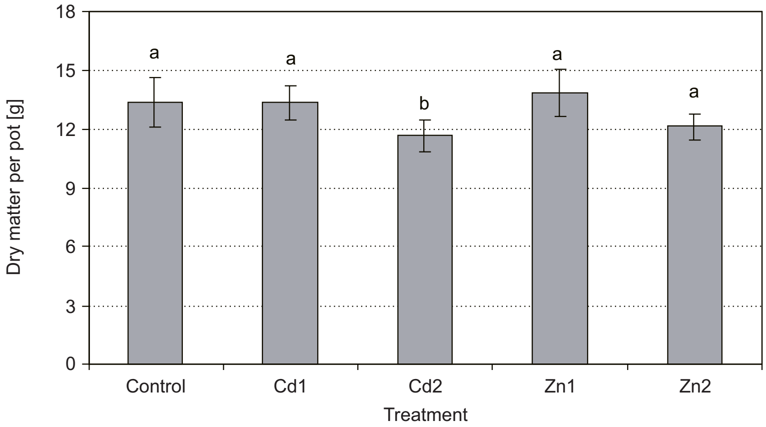

Fig. 1. Yield of spinach dry matter [g per pot]; upper letters refer to the effects of Cd and Zn addition – the same letters mean not significant difference at the level P < 0.05

(Table 1). Both lower Cd and Zn rates did not affected yield of spinach dry matter. The higher Cd dose (25 mg Cd · kg^{-1} soil) was associated with the significant inhibition of aboveground biomass. The biomass yields of Cd2 treatment was reduced to ca 14 % in contrast to control treatment. The yield inhibition by Zn2 level was much less pronounced (9 %).

Table 1

Cd and Zn contents in spinach aboveground biomass [mg · kg^{-1}]

Treatment	Cd content	Zn content
	[mg · kg^{-1}]	
Control	0.44 ± 0.01	53.9 ± 3.4
Cd1	1.99 ± 0.12	
Cd2	7.30 ± 0.21	
Zn1		95.0 ± 5.8
Zn2		158.5 ± 7.2

Application of both rates of cadmium and zinc led to the increase of both elements contents in plant biomass in the pot experiment in contrast to control treatment (Table 1). Compared with the untreated control, the cadmium content in leaves was enhanced up to 4.5–fold (Cd1) and 16.6-fold (Cd2), while the zinc content in aboveground biomass was increased 1.8-fold (Zn1) and 2.9-fold (Zn2). Our data correspond with those by Tlustoš et al [11] who reported that excessive amounts of toxic elements in contaminated soil inhibited plant growth and development due to their phytotoxicity. Kabata-Pendias and Pendias [12] reviewed plant response to increased levels of Cd in soil and showed a great difference in the ability of various plant species to absorb this metal. The highest Cd concentration was determined in leafy and root vegetables.

The results of distribution of cadmium and zinc in spinach biomass showed the concentrations of both elements as following leaf blade > root > petiole (Table 2). Tlustoš et al [13] have presented similar results for spinach. According their results 63.8 % of Cd content was found in spinach aboveground biomass and 36.2 % in roots.

Table 2

The distribution of cadmium and zinc in spinach biomass

Treatment	Cd	Zn
	[mg · kg^{-1}]	
Root		
Control	0.27 ± 0.01	34.9 ± 2.6
Cd1	1.41 ± 0.06	
Cd2	5.47 ± 0.05	
Zn1		65.7 ± 3.7
Zn2		131.5 ± 3.5

Table 2 contd.

Treatment	Cd	Zn
	[mg · kg^{-1}]	
Leaf blade		
Control	0.75 ± 0.01	90.7 ± 1.3
Cd1	2.90 ± 0.02	
Cd2	7.59 ± 0.07	
Zn1		121.3 ± 3.7
Zn2		276.7 ± 3.1
Petiole		
Control	0.43 ± 0.01	30.9 ± 5.4
Cd1	1.50 ± 0.02	
Cd2	4.82 ± 0.08	
Zn1		63.7 ± 4.0
Zn2		95.3 ± 6.7

Determination of chlorophyll content confirmed its decrease only on Cd2 and Zn2 treatments (Table 3). Our previous results (Cd rate was 90 mg · kg^{-1}) showed damage caused by cadmium led to the bleaching of chlorophylls [8]. In accordance with these data, cadmium and zinc treatments considerably inhibited growth and progressively reduced chlorophyll contents of duckweed plants [14]. Moreover, the cadmium-treated plants were found to be completely photobleached in those experiments.

Table 3

Chlorophyll contents in spinach leaves [mg · g^{-1}]

Treatment	Chlorophyll *a*/Chlorophyll *b*	Chlorophyll *a*	Chlorophyll *b*
		[mg · g^{-1}]	
Control	2.759	0.080 ± 0.004	0.029 ± 0.003
Cd1	2.793	0.081 ± 0.007	0.029 ± 0.005
Cd2	3.182	0.070 ± 0.010	0.022 ± 0.006
Zn1	2.964	0.083 ± 0.006	0.028 ± 0.005
Zn2	3.076	0.080 ± 0.009	0.026 ± 0.002

Plant stress evoked by cadmium and zinc treatments was associated with a reduced activity of glutamate kinase (Fig. 2), the enzyme catalyzing the first step of proline biosynthesis from L-glutamate. Activity of this enzyme is regulated in plants via a feedback mechanism by content of proline [15]. Results of the glutamate kinase activity showed a strong effect of both elements. Effect of zinc as an essential element in the glutamate kinase activity was significantly lower compared with Cd toxic element. Allosteric regulation of glutamate kinase activity by free proline enables to increase the

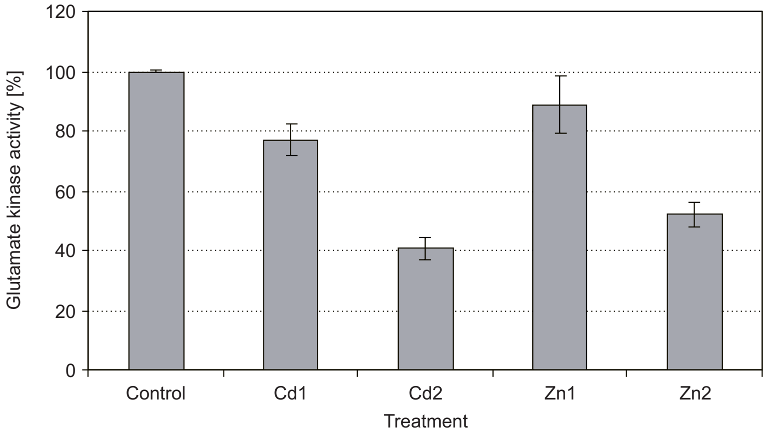

Fig. 2. Changes of glutamate kinase activities of spinach; activity of glutamate kinase of untreated control = = 100 %

content of glutamate that is required for the formation of a peptide bond between the g-carboxyl group of glutamate and the α-amino group of cysteine and used in synthesis of phytochelatins via glutathione in plant cells [8, 16].

Conclusions

1. Application of cadmium and zinc led to the increase of both elements contents in plant biomass in the pot experiment in contrast to control treatment.

2. The higher doses of both metals negatively affected yield of spinach dry biomass, but only Cd dose (25 mg Cd · kg^{-1} soil) was associated with the significant inhibition of above-ground biomass compared with control treatment (by 14 %). The yield inhibition by Zn2 level was much less pronounced (9 %).

3. The results of distribution of cadmium and zinc in spinach biomass showed the concentrations of both elements as following leaf blade > root > petiole.

4. Determination of chlorophyll content confirmed its decrease only on Cd2 and Zn2 treatments.

5. Allosteric regulation of glutamate kinase activity by free proline creates a possibility for an increase in glutamic acid content due to the synthesis of glutathione subsequently phytochelatines in plant cells. For this reason the rates of Cd and Zn applied into soil decreased the glutamate kinase activity.

Acknowledgements

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WPŁYW KADMU I CYNKU NA METABOLIZM SZPINAKU

Abstrakt: Badano zmiany metabolizmu roślin szpinaku (*Spinacia oleracea* L., odmiany 'Matador') eksponowanych na stres powodowany przez dwa metale ciężkie: kadm i cynk. W doświadczeniu wazonowym badano rozmieszczenie tych pierwiastków w biomasie (w korzeniach, blaszkach i ogonkach liściowych), zawartość chlorofilu oraz aktywność kinazy glutaminianowej [E.C.2.7.2.11], enzymu katalizującego pierwszy stopień biosyntezy proliny. Wyniki doświadczenia wykazały toksyczny wpływ kadmu i cynku na szpinak w obydwu zastosowanych dawkach (2,5 i 25 mg Cd oraz 50 i 500 mg Zn · kg^{-1}). W tych warunkach stwierdzono spadek aktywności kinazy glutaminianowej w roślinach szpinaku rosnących w obiektach zanieczyszczonych metalami w porównaniu z obiektem kontrolnych, bez ich dodatku. Regulacja allosteryczna aktywności kinazy glutaminianowej przez wolną prolinę indukuje możliwość zwiększenia zawartości kwasu glutamiowego w wyniku syntezy glutationu, a następnie fitochelatyn w komórkach roślin. Z tego względu dawka Cd i Zn zastosowana do gleby zmniejszyła aktywność kinazy glutaminianowej. Większa dawka obydwu metali ujemnie oddziaływały na plon suchej masy szpinaku, ale tylko dawka 25 mg Cd · kg^{-1} gleby wywołała znaczne zahamowanie (o 14 %) wzrostu nadziemnej biomasy w porównaniu z obiektem kontrolnym. Największe zawartości obydwu metali stwierdzono w blaszkach liściowych szpinaku. Zawartość chlorofilu zmniejszyła się tylko po zastosowaniu większej dawki kadmu.

Słowa kluczowe: chlorofil, kinaza glutaminianowa, pierwiastki toksyczne, stres, szpinak

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URBAN ENVIRONMENT INFLUENCE ON SELECTED PROPERTIES OF FOREST SOILS OF LUBLIN

WPŁYW ŚRODOWISKA MIEJSKIEGO NA WYBRANE WŁAŚCIWOŚCI GLEB LEŚNYCH MIASTA LUBLINA

Abstract: The assessment of urban environment influence on forest soils located in the city of Lublin was made by the determination of Ulrich soil flexibility index as well as Siuta index – the indicator referred to anthropogenic acidification of ecosystems. Selected heavy metals – lead, nickel, copper, zinc and cadmium – contents were also determined, both total content and in form of available for plants. The indicator of anthropogenic acidification of ecosystems Siuta index suggests small influence of Lublin agglomeration on forest soils located within its boundaries. Ulrich soil flexibility index determined for soils located in Lublin and for reference soils did not show significant differences. This implies similar resistance to anthropopressure. Total nickel, copper, zinc, cadmium and lead content in Lublin forest soils one may consider as natural, when compared with limit numbers of these metals in surface soil horizons (0–20 cm), according to the Decree of Polish the Minister of Environment on soil and earth quality standards. Elevated zinc content was noted in the lessive (grey-brown podsolic) soil reference profile. The share of available forms of Ni, Cu, Zn, Pb and Cd in their total contents was similar in Lublin forest soils and in reference ones. The city agglomeration influence on heavy metal contents in forest soils located within the boundaries of Lublin is slight in relation to reference profiles.

Keywords: anthropopressure, Siuta index, Ulrich indicator, heavy metals

The influence of the urban environment causes significant changes of the natural environment of forests present within this area. Besides plant degradation, also soil is especially threatened by the diverse anthropogenic pressure. This includes both mechanical and chemical influence. The multitude and variety of emission sources within the city significantly impedes definition of their participation in degradation of forest soils present in urban areas. The evaluation of pressured soils and their comparison with forest soils devoid of direct influence of anthropogenic factors may, with recognition of the open soil system and its close association with other elements of the natural and anthropogenic environment, become resistance indicator of forest soils

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degradation [1–3]. The share of forest terrains within the total area of Lublin is significant, exceeding 11 %. This area has various functions; it is a buffer zone between industrial plants located within Lublin and residential buildings, it has recreational functions with the inadequate tourism-sport infrastructure, it is also the home of protected flora specimens (Stary Gaj reservation) and is among Lublin's microclimate factors [4].

The examination target was the evaluation of the urban environment multilevel influence of the properties of forest soils through designation of the Ulrich soil flexibility indicator [5], an indicator associated with the anthropogenic acidification of ecosystems – level of the sorptive complex saturation by hydrogen cations [6] as well as selected components of heavy metals – lead, nickel, copper, zinc and cadmium in their total contents and also that available form for plants. Through designation of the aforementioned ecological soil indicators, assumed will be an attempt to characterize and evaluate the functioning and resistance to external factors of forest soils located within city limits.

Material and methods

The examinations encompassed six soil profiles located within two forest complexes of the Lublin city (Stary Gaj and Dąbrowa), which were applied to as bench mark profiles outside of the Lublin agglomeration. The examination material represents various soil types, which originate from Lublin location on the border of three relatively strongly diverse geographical mesoregions. The profiles collected in Stary Gaj are lessive (grey-brown podsolic) soils, created from loesses (reference profile located in the neighbourhood of Czesławice). The soil material collected in Dąbrowa is light, sandy, mainly brown-rusty and rusty soil (reference profile located in Prawiedniki).

The collected samples were analyzed for the basic soil properties: pH in 1 mol · dm^{-3} KCl solution and water, potentiometrically, organic carbon with the Tiurin method, exchangeable cations in NH_4Cl, granulometric composition with the areometrical method (Casagrande modified by Prószyński) [7, 8]. Forms of Cu, Cd, Pb, Ni and Zn available to plants were extracted by the Lindsay and Norvell method [9]. Mineralization of soil samples were conducted with *aqua regia*, method ISO 11466 [10]. The total content and forms of Cu, Cd, Pb, Ni and Zn available to plants were determined by the AAS 3300 Perkin Elmer.

Results and discussion

Lessive forest soils exhibited granulometric composition of silty formations (Table 1) and characteristics of clay relocating into the depth of the profile, which led to the creation of a distinct Bt horizon. The rusty-brown soils are dominated by sandy formations. The AEes horizon is created by light loamy sand, while the remaining, deeper horizons of BfeBv, C1 and C2 are created by weakly loamy sand and loose sand. In the investigated forest soils, the organic matter is accumulated mainly in the

Table 1

Selected parameters of investigated soils (ranges of values)

Location		1. Stary Las	2. Stary Las	3. Stary Las	4. Czesławice – reference profile	5. Dąbrowa	6. Dąbrowa	7. Dąbrowa	8. Prawiedniki – reference profile
pH H_2O		4.52–5.52	3.51–4.07	3.39–4.40	4.17–8.69	3.40–4.24	3.48–4.61	4.29–2.90	3.80–4.85
pH KCl		3.73–4.92	3.96–5.33	4.14–5.79	3.41–7.85	4.13–4.49	3.96–5.23	3.52–4.10	3.00–4.05
organic C [$g \cdot kg^{-1}$]		0.6–8.7	1.6–13.8	0.7–14.5	0.6–31.8	0.7–24.0	0.4–12.7	0.7–12.8	0.6–11.8
Base cations [$mmol(+) \cdot kg^{-1}$]		5.7–168.8	3.9–37.5	5.0–73.3	3.4–617.0	1.2–33.7	1.3–15.9	1.2–87.8	1.7–25.7
H [$mmol(+) \cdot kg^{-1}$]		26.4–189.0	34.5–176.3	22.5–195.0	3.8–423.8	19.5–255.0	13.5–198.8	17.7–171.7	16.5–326.3
Siuta index		25.84–88.92	54.22–93.73	46.41–92.64	0.60–99.11	88.34–95.97	91.11–95.33	66.16–95.67	90.81–94.95
UFI		0.07–0.71	0.04–0.40	0.05–0.48	0.01–0.99	0.02–0.10	0.02–0.03	0.03–0.32	0.02–0.06
Sand [%]		0.3–0.6	0.5–3.5	0.3–1.0	0.3–0.6	65.0–72.0	69.5–87.0	68.5–80.0	54.0–90.0
Silt [%]		54.7–59.6	50.2–65.5	39.3–69.2	52.7–68.7	14.1–20.1	5.9–17.5	14.9–19.5	4.0–29.0
Fine earth [%]		40–45	31–49	30–60	31–47	13–17	7–13	5–12	6–19
Colloids [%]		7–23	5–21	5–29	12–25	2–3	2–3	0–2	2–3
Total content [$mg \cdot kg^{-1}$]	Cd	0.00–0.52	0.00–0.48	0.00–0.44	0.00–0.42	0.00–0.44	0.15–0.44	0.14–0.35	0.16–0.35
	Pb	6.00–12.44	9.88–33.60	11.45–22.77	9.05–20.73	4.80–17.25	3.44–13.68	2.60–15.20	4.15–16.90
	Cu	2.20–8.56	5.15–10.72	4.92–12.33	3.28–8.71	2.14–5.48	1.79–5.02	0.52–2.90	1.94–6.74
	Zn	4.28–26.74	24.51–29.12	17.42–37.97	9.36–73.80	10.14–26.88	7.02–22.52	2.80–11.22	8.60–22.37
	Ni	2.91–11.14	7.48–15.95	6.84–22.08	3.99–11.62	2.11–4.58	2.18–3.19	0.25–1.46	2.73–4.07
Available form [$mg \cdot kg^{-1}$]	Cd	0.00–0.32	0.00–0.10	0.00–0.13	0.00–0.39	0.00–0.25	0.00–0.18	0.00–0.08	0.00–0.12
	Pb	0.57–3.26	0.93–8.78	0.88–9.36	0.36–12.62	0.29–12.03	0.16–8.51	0.21–10.35	0.15–12.04
	Cu	0.46–1.06	0.90–1.41	0.85–1.80	0.59–2.64	0.18–1.49	0.13–1.71	0.13–1.18	0.14–2.23
	Zn	0.09–10.69	0.14–10.85	0.13–14.05	0.14–23.69	0.53–11.22	0.41–16.94	0.28–7.95	0.40–22.25
	Ni	0.26–1.29	0.51–1.28	0.29–2.34	0.15–3.05	0.03–1.08	0.06–0.63	0.07–0.52	0.03–1.07

ectohumus. The highest value of organic carbon is noted in surface horizons and usually decreases with depth.

The investigated soils showed a low variability of reaction (pH), which oscillated within a small range between strongly acid and acid. This mainly concerns surface horizons. The acidification gradually diminishes into the lessive soil profiles, reaching the alkaline reaction in Cca horizons, while insignificantly oscillating in sandy soils. The pH of soils from forests located in Lublin does not differ from reference profiles. The lack of strong acidification of these soils, which is suggested by many authors as an anthropogenization indicator, points to the small influence of the city of Lublin to this area [1].

The soils created of sand were characterized by poor sorptive complex, while lessive soils had higher sorptive capacity. The sorptive complex base saturation was also higher diversified (from a few to 100 %). The sorptive complex base saturation in rusty and rusty-brown soils was low, in contrast to the investigated lessive soils, where the horizon of the parent material has almost full base saturation. The determined indicator of anthropogenic acidification of ecosystems – Siuta index [6], as the level of saturation of sorptive complex by hydrogen cations in forest soils located in Lublin in relation to reference profiles showed small influence of the city agglomeration on forest soils located within. A somewhat stronger saturation than that of profiles located outside of Lublin should be associated with the higher level of recreational utilization of forest terrains within city borders by the residents of Lublin [2].

The second ecological-soil indicator of soil degradation under influence of anthropogenic factors is the Ulrich flexibility index (UFI) [5], defined as the relation of the sum of exchangeable calcium and magnesium cations to the sorptive capacity. The comparison of UFI in forest soils located within Lublin to reference soils did not demonstrate significant differences. Therefore it is not possible to confirm a higher level of resistance to anthropogenic factors of forest soils other than the influence of the Lublin agglomeration [1].

The preliminary evaluation of Lublin forest soil contaminated by Ni, Cu, Pb, Cd and Zn was carried out based on limit content of heavy metals in the soil surface layer (0–20 cm) according to the Decree of the Minister of Environment on quality standards of soil and ground. The forest soils contained total of nickel, copper, zinc, cadmium and lead amounts that could be defined as natural [11], despite present neighbouring road and rail communication routes. Elevated zinc values have been noted in the reference profile of lessive soils. Based on the diverse location of sampling sites (outside of and in Lublin), it is possible to declare that the influence of city agglomeration on the content of heavy metals in forest soils is slight. The profile distribution of the heavy metal content was characterized by high variability. Accumulation in surface and Bt horizons was observed in lessive soils. In sandy soils, the highest content of total heavy metal was observed in the litter and accumulation horizon. Whereas the Ni, Cu, Zn, Pb and Cd content profile distribution in available form (extracted by DTPA) showed an accumulation of heavy metals in surface horizons. The available form share in total pool of these metals was diverse and usually higher in surface horizons of both lessive and rusty-brown soils as compared with lower layers. The comparison of the available form share in total content of Ni, Cu, Zn, Pb and Cd of investigated Lublin forest soils with reference profiles did not provide significant differences. This may be proof of insignificant influence of the urban environment on the appearance of heavy metals in forms more mobile and available to living organisms. Among examined elements, the most available metal, especially in surface horizons, was cadmium, subsequently zinc, lead, copper and nickel.

The accumulation of Ni, Cu, Zn, Pb and Cd occurs in surface horizons and may indicate significant role of humus in this process, although, it is not confirmed by statistical analysis (Table 2).

Table 2

Values of significant correlation coefficients (at $p < 0.05$) between selected soil properties

Parameter	C org.	Siuta index	Ulrich index	Sand fraction	Colloidal fraction	Silt fraction	Fine earth fraction	Total Pb	Total Cu	Total Zn
Siuta index	0.44									
Ulrich index	–0.42	–1.0								
Sand fraction		0.50	–0.53							
Colloidal fraction		–0.70	0.70	–0.75						
Silt fraction		–0.50	0.49	–0.97	0.61					
Fine earth fraction		–0.50	0.53	–0.94	0.86	0.83				
Total Cd		0.40	–0.38	0.39	–0.44		–0.41			
Total Pb	0.54			–0.48		0.51	0.39			
Total Cu		–0.70	0.64	–0.72	0.89	0.60	0.80	0.47		
Total Zn		–0.40	0.43	–0.45	0.53	0.46	0.40	0.37	0.60	
Total Ni		–0.50	0.51	–0.67	0.87	0.52	0.79	0.44	0.96	0.54

Profile distribution of heavy metals indicates also an influence of urban environment on analyzed soils. This is also confirmed by results obtained in reference soils. The correlation coefficients calculated for selected properties of soils (Table 2) indicate strong relationship of the heavy metal content with soil texture; it is worth recognising that the amount of Cd negatively correlates with the amount of fine earth. Also notable is the relationship of heavy metals with calculated Siuta and Ulrich indices. An exception was Pb, which significantly correlates with organic carbon content.

Conclusions

1. The city agglomeration influence on forest soils evaluated by the Siuta index is weak; the resistance of investigated soils to anthropopressure (Ulrich index) is similar to bench mark profiles.

2. The content of heavy metals in the investigated soils varies in ranges of values considered as natural. The available forms of heavy metals constitute significant share

of the total their amounts, but at the same time their content in bench reference profiles is similar.

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WPŁYW ŚRODOWISKA MIEJSKIEGO NA WYBRANE WŁAŚCIWOŚCI GLEB LEŚNYCH MIASTA LUBLINA

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Abstrakt: Dokonano oceny wpływu środowiska miejskiego na właściwości gleb leśnych zlokalizowanych w obrębie miasta Lublina, poprzez oznaczenie wskaźnika elastyczności gleb Ulricha, wskaźnika Siuty odnoszącego się do antropogennego zakwaszenia ekosystemów (stopień wysycenia kompleksu sorpcyjnego kationami wodorowymi), a także poprzez oznaczenie ogólnej zawartości wybranych metali ciężkich – ołowiu, niklu, miedzi, cynku i kadmu oraz ich form dostępnych dla roślin. Wskaźnik antropogennego zakwaszenia ekosystemów (wskaźnik Siuty) wskazuje na nieznaczne oddziaływanie aglomeracji lubelskiej na gleby leśne zlokalizowane w jej obrębie, zaś wskaźnik elastyczności gleb Ulricha w glebach leśnych zlokalizowanych w obrębie Lublina do gleb odniesienia nie wykazywało znaczących różnic. Nie można stwierdzić większej siły odporności na czynniki antropogenne gleb leśnych poza oddziaływaniem aglomeracji lubelskiej. Ogólna zawartość niklu, miedzi, cynku, kadmu i ołowiu w badanych glebach leśnych Lublina można ocenić jako naturalne, porównując je z granicznymi zawartościami metali ciężkich w powierzchniowej warstwie gleb (0–20 cm) zgodnie z Rozporządzeniem Ministra Środowiska w sprawie standardów jakości gleby i ziemi. Zwiększone zawartości cynku odnotowano natomiast w profilu odniesienia gleb płowych. Porównanie udziału formy dostępnej w ogólnej zawartości Ni, Cu, Zn, Pb i Cd w badanych glebach leśnych Lublina z profilami odniesienia także nie wykazało znaczących różnic. Wpływ aglomeracji miejskiej na zawartość oznaczonych metali ciężkich w glebach leśnych zlokalizowanych w obrębie miasta Lublina w stosunku do profili odniesienia jest niewielki.

Słowa kluczowe: antropopresja, wskaźnik Siuty, wskaźnik Ulricha, metale ciężkie

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INVESTIGATION OF HEAVY METAL SORPTION BY POTATO TUBERS

BADANIE SORPCJI METALI CIĘŻKICH PRZEZ BULWY ZIEMNIACZANE

Abstract: In the paper a potential application of potato tubers for the removal of heavy metals from aqueous solutions was investigated. Sorption of Cu^{2+}, Fe^{3+} and Pb^{2+} was studied for different salts in order to determine the influence of counter anions on the cation uptake by the investigated adsorbents. Potatoes in the form of raw and dried potato cubes and the peels were soaked in 5 % aqueous solution of the following salts: copper(II) acetate, chloride and sulfate, iron(III) chloride and nitrate(V) and lead(II) acetate and nitrate(V). After sample wet mineralization with HNO_3 and $HClO_4$, the total metal content was determined by the inductively coupled plasma atomic emission spectroscopy (ICP-AES). The inner structure of the applied sorbents was determined by using the scanning electron microscopy (SEM).

Sorption was found to be dependent on both the cation and the anion present in the solution. Acetate ions significantly enhanced the metal uptake from the solution. In all studied samples the uptake of Pb^{2+} was most effective in comparison with that for the other cations. Among the sorbents, the potato peels have taken higher, than it was obtained for the others, amount of metals, and the raw potato cubes were more effective compared with the dried ones. The metal uptake was positively correlated to the moisture and protein content as well as the amount of Ca^{2+} and K^+ ions in the initial samples. It was also related to the inner structure of used potato materials. The obtained results should be useful in the recovery of the heavy metals from the environmental samples.

Keywords: biosorption from solutions, heavy metals, potato sorbents

Contamination of surface and groundwaters by harmful substances, in particular the metal ions originated from the industrial effluents, is a commonly known problem. Metallic ores, their by-products and other minerals are widely utilized in different kinds of industries, so there are many industrial sources of such environmental pollution. Moreover, metal ions could penetrate the biological systems from the salt solutions used

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in agriculture, food processing or just in everyday life applications. Heavy metal cations are known to be toxic to plants, animals and human beings. Thus, they must be removed from wastewaters coming from mining, metallurgy, petroleum refining, chemical or tanning industry, etc. Furthermore, for the economic sake, metal ions should be recovered and used again in the industrial processes. Recently, many methods, like: ion exchange, reverse osmosis, membrane filtration, solvent extraction, coagulation and adsorption by various sorbents, are widely applied for metal ion removing in wastewater purification [1]. These methods are rather complicated, time consuming and expensive due to high costs of the operations and materials. It is therefore necessary to find alternative low-cost technology of separation of metallic pollutants from sewage waters. Many examples of usage of the agricultural by-products and biomass for the industrial effluent treatment are available in the literature. A range of materials have been already examined for this purpose. These include cotton bolls [2], *Sphagnum* peat [3], sugar beet pulp [4], lentil, rice and wheat shells [1], maize cobs [5], wood sawdust [6, 7], carbon derived from agricultural wastes [8, 9], just to mention a few. However, the new inexpensive, environmental friendly and locally available adsorbents of contaminations are still needed.

Potato production is one of the most important agriculture sector in Poland. Potato industry by-products, such as potato peels and the low-quality potato crops, are commonly utilized as animal foodstuff or the substrate in industrial processing [10]. However, the waste quantities cause problems with their disposal and utilization. According to literature, some trials were taken in the aim of using the potato waste products for microbial reduction of chlorate(VII) [11], as an antioxidant reagents [12, 13] or in the biofuels production [14, 15]. It was already reported, that potato starch due to anionic character could easily absorb heavy metal cations [16]. Thus, potatoes tubers containing starch should exhibited the same properties. To our knowledge, they have not been used until now for heavy metal removal from aqueous solutions.

Metal sorption by biomaterials, in particular by waste products, is of an empirical nature because of their complicated chemical composition and the structure influencing the process. Various parameters, such as a dosage and the adsorbent properties, contact time, an initial ion concentration, pH and temperature of the solution, could be important for optimization of the sorption process [1–9]. There are only a few reports about the influence of accompanying anions on the heavy metal ion uptake by biosorbents like starch [16] and algae [17].

The presented study was undertaken in order to evaluate the ability of raw potatoes and their by-products, such as potato peels and dried potato cubes, to absorb the metal cations from aqueous solution as well as to determine the counter anion effect on the sorption efficiency.

Material and methods

Commercially available potatoes (*Solanum tuberosum* L., ‘Irga’ cv.), their peels and dried potato cubes were used in the investigations. The potato tubers were carefully washed with tap water following by deionized water for removing surface impurities, then peeled and cut into cubes (of the side size about 5 mm). The peels were also cut into small pieces (approximately 5 mm × 5 mm × 1 mm). Samples of 50 g of both the raw potato cubes and the peels and 10 g of dried potato cubes were mixed with 100 cm^3 of 5 % aqueous solutions of copper(II) salts: $Cu(CH_3COO)_2$, $CuCl_2$, $CuSO_4$, iron(III) salts: $FeCl_3$, $Fe(NO_3)_3$, and lead(II) salts: $Pb(CH_3COO)_2$, $Pb(NO_3)_2$ (POCh, Poland), and shaken at 37 ^oC for 24 h with an agitation speed 200 rpm. Then, the samples were filtered off, threefold washed with deionized water and next with 99.8 % ethanol (POCh, Poland) and dried in a dryer for 12 h at 105 ^oC. Afterward, samples were powdered in a grinder, mineralized with HNO_3 and $HClO_4$ (2:1, v/v) and analysed. The samples were prepared and analysed in duplicate.

The sample total metal content was determined by using the inductively coupled plasma atomic emission spectroscopy (ICP-AES) and the JY 238 Ultrace Jobin Yvon Emission apparatus. Background content of the investigated heavy metals and the contents of calcium, phosphorus, potassium, magnesium, and sodium in the initial materials were also determined by the same method.

The total nitrogen contents in the initial samples were determined by the Kjeldahl method by using the Büchi 324 distilling apparatus. The results were converted into the protein contents [18]. Amount of starch in the samples, after their dissolving in calcium chloride solution, was measured by the polarimetric method [19]. The moisture content of the samples was calculated according to the percentage of the sample weight loss after drying at 105 ^oC for 12 h; the measurements were performed in duplicate.

Scanning electron microscopy images at different magnifications of the sample microstructure were taken with the scanning electron microscope SEM Jeol (JSM-5500LV).

Results and discussion

Metal ions were introduced to the solids as aqua complexes typical for aqueous solutions of the salts used for soaking of the potato materials. The cations could be accumulated due to interactions with inner water molecules of the sorbents as well as the oxygen or nitrogen atoms of their constituents, eg polysaccharides or proteins. Thus chemical composition and the moisture content of the material could very much influence the ion uptake.

The chemical characteristics of the sorbents before processing is presented in Table 1. The very low amounts of trace elements found in the samples, similar to those reported for potatoes by the other authors, could be classified as natural for this kind of material [20–22]. The same assumption concerns the starch and protein content of the samples typical for potato originated products [21, 23]. It should be noted, however, that the values obtained for the potato peels were a little bit higher than in the raw and dried potato cube case. Except for dried potato, the samples exhibited high moisture content.

Scanning electron microscopy was used for characterisation of the cells, the inner construction and surface morphology of the investigated adsorbents. The SEM micrographs shown in Fig. 1 revealed the structure differences of the samples.

Table 1

Chemical composition of potato sorbents used in the experiments (mean of two measurements ± standard deviation)

Product	Moisture	Starch	Protein	Ca	P	K	Mg	Na	Cu	Fe	Pb
	[%]	[% d.m.]*		$[\mathrm{mg \cdot g^{-1}\ d.m.}]$							
Raw potato cubes	76.04 ± 0.32	59.12 ± 0.76	9.31 ± 0.15	0.70 ± 0.01	2.52 ± 0.04	6.04 ± 0.05	1.23 ± 0.03	0.22 ± 0.02	0.02 ± 0.01	0.27 ± 0.04	0.02 ± 0.01
Dried potato cubes	5.90 ± 0.21	60.77 ± 0.03	9.46 ± 0.03	0.65 ± 0.01	2.25 ± 0.10	3.48 ± 0.19	0.99 ± 0.03	0.17 ± 0.01	0.03 ± 0.01	0.29 ± 0.05	0.02 ± 0.01
Potato peels	85.88 ± 0.23	40.32 ± 0.10	14.78 ± 0.01	2.15 ± 0.17	2.96 ± 0.06	17.80 ± 1.18	2.24 ± 0.05	0.29 ± 0.07	0.04 ± 0.01	0.34 ± 0.05	0.02 ± 0.01

* dry matter

Parenchyma tissue with globular starch granules outside of the cells and small intercellular spaces could be observed in the sample of raw potato cubes (Fig. 1a). Drying caused significant changes to the potato tissue. As could be seen in the Fig. 1b, dried potato cubes exhibited irregular, compact structure with destructed cell walls and poorly recognized starch grains as well as lower porosity than it was observed for raw potato [24]. In the potato peel case, the distinctive layers of phelloderm and phellogen containing the phellum (dead cells filled with air) and the others composed of cellulose, pectin, lignin and protein could be noticed (Fig. 1c). Such a variety of active components and extensive surface area of the sorbent could enhance the metal ion trapping into this material as it was actually confirmed in the study.

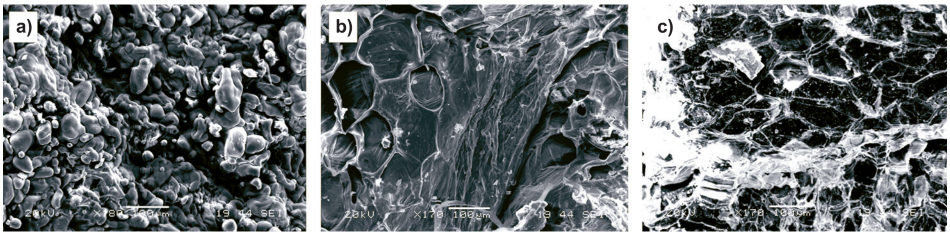

Fig. 1. SEM images of raw potato cube (a), dried cube (b) and potato peel (c)

The obtained results indicated sorption of Cu^{2+}, Fe^{3+} and Pb^{2+} ions by the all investigated potato sorbents (Fig. 2). The process efficiency was found to be dependent on both the cation and the anion present in the solution. In all studied samples the uptake of Pb^{2+} was most effective in comparison with that for Cu^{2+} and Fe^{3+} cations. The same was already noticed for potato starch [25].

Quantitative differences observed in the sorption efficiency could be caused by many factors. The results were strongly related to the moisture and protein content as well as the amount of Ca^{2+} and K^+ ions in the initial samples. It was somewhat surprising, that the starch content was found not to be the main factor influencing the process. The ion uptake was also dependent on the inner microstructure of used potato sorbents. The highest efficiency of the process was found for the potato peels. There might be several reasons for such results. The high moisture content seemed to be as much important as the layered structure and porosity of the peels causing favourable conditions for penetration the tissue by the hydrated metal ions. Moreover, the proteins, present in this material in significant amount, could also participate in binding of metal ions. An additional support might come from reasonable high concentration of the Ca^{2+}, K^+, Mg^{2+} and Na^+ cations which influenced the Cu^{2+}, Fe^{3+} and Pb^{2+} sorption by the cation-exchange mechanism [26].

Counter ions present in the solution noticeably influenced the content of particular cations in the sorbents. In all samples, acetate ions significantly increased the uptake, while sulfate ions were found to be least effective in the process. Similar results were obtained for starch soaked in copper salts [16]. The observed effect could be attributed to the anion hydration ability influencing amount of free water molecules available for

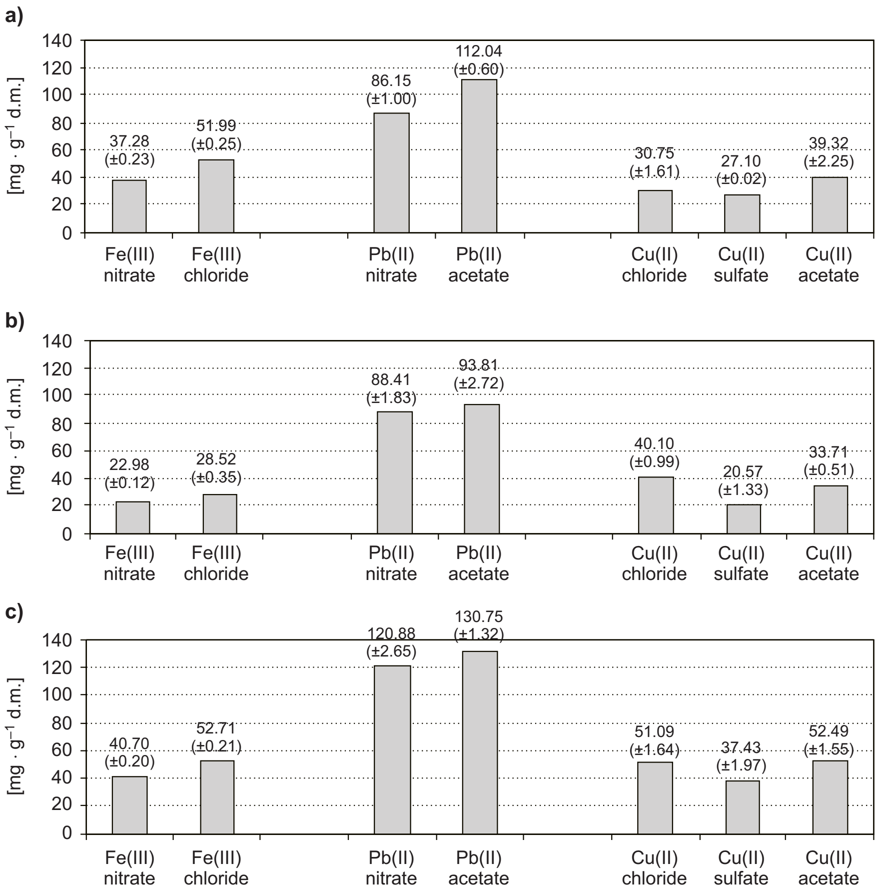

Fig. 2. Heavy metal content in raw potato cubes (a), dried potato cubes (b) and potato peels (c) (mean of two measurements ± standard deviation)

cation migration through the sorbent inner microstructure. Higher hydrated anions, like SO_4^{2-}, caused decreasing of amount of water molecules capable for cation hydration thus lowered amount of cations in the sorbent while the lower hydrated anions (like Cl^- or NO_3^-) acted in the opposite way. However, this regularity was not valid for acetate anions. In this case, probably, the anionic hydrolysis occurring in the solution slightly increased its pH value what could support cation binding to the OH^- and/or NH_2^- active groups of the sorbent components, like polysaccharide or proteins, and enhanced the sorption efficiency.

Conclusions

1. Potato tubers in the form of raw and dried potato cubes and potato peels, could absorb heavy metal ions from salt solutions.

2. Potato peels have taken more metals than other investigated sorbents, and the raw potato cubes were more effective compared to the dried ones.

3. The process was dependent on both the anion and the cation present in the solution. Uptake of Pb^{2+} was most effective in comparison with that for the other cations. Acetate ions significantly enhanced the metal uptake from the solution.

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BADANIE SORPCJI METALI CIĘŻKICH PRZEZ BULWY ZIEMNIACZANE

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Abstrakt: W pracy badano sorpcję metali ciężkich przez bulwy ziemniaczane oraz wpływ różnych anionów na wydajność tego procesu. Badano sorpcję Cu^{2+}, Fe^{3+} i Pb^{2+} z 5 % roztworów wodnych soli, takich jak octan, chlorek i siarczan(VI) miedzi(II), chlorek i azotan(V) żelaza(III) oraz octan i azotan(V) ołowiu(II). W roli sorbentów użyto surowe i suszone ziemniaki w postaci kostki oraz skórki ziemniaczane. Całkowitą zawartość metali w próbkach, po ich mineralizacji przy użyciu HNO_3 i $HClO_4$, oznaczono metodą ICP-AES. Wewnętrzną strukturę zastosowanych sorbentów określono przy użyciu skaningowej mikroskopii elektronowej (SEM).

Stwierdzono, że wydajność sorpcji zależy zarówno od rodzaju kationów, jak i od anionów obecnych w roztworze. Obecność jonów octanowych znacznie zwiększała pobieranie kationów z roztworu. Największą wydajność procesu, bez względu na zastosowany sorbent zaobserwowano dla jonów Pb^{2+}. Wśród sorbentów skórki ziemniaczane okazały się najbardziej efektywnym materiałem wiążącym badane kationy metali ciężkich, a najmniejszą wydajność uzyskano dla suszonej kostki ziemniaczanej. Zauważono pozytywną korelację pomiędzy ilością kationów metali związaną z danym sorbentem a jego wilgotnością, zawartością białka oraz ilością jonów Ca^{2+} i K^{+}. Ponadto, struktura wewnętrzna sorbentów była również czynnikiem wpływającym na efektywność procesu. Uzyskane wyniki wskazują, że bulwy ziemniaczane, a zwłaszcza skórki ziemniaczane mogą być przydatne jako biosorbenty do odzyskiwania metali ciężkich z próbek środowiskowych.

Słowa kluczowe: biosorpcja z roztworów; metale ciężkie; sorbenty ziemniaczane

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EFFECT OF BETOKSON SUPER AND FUSILADE PREPARATIONS AND INDOLEACETIC ACID (IAA) ON SOME PHYSIOLOGICAL PROCESSES OF THE RADISH (*Raphanus sativus* L.) AND THE YEAST (*Saccharomyces cerevisiae*)

ODDZIAŁYWANIE PREPARATÓW HERBICYDOWYCH: BETOKSON SUPER, FUSILADE ORAZ KWASU INDOLILOOCTOWEGO (IAA) NA WYBRANE PROCESY FIZJOLOGICZNE RZODKIEWKI (*Raphanus sativus* L.) ORAZ DROŻDŻY (*Saccharomyces cerevisiae*)

Abstract: Studies of non-specific effect of herbicides concern not only plants but also other organisms, mostly those which are directly exposed to the influence of herbicides in the natural environment. Reports on studies concerning a reaction on herbicides of phylogenetically distant organisms are more and more frequent. The author of this study examined the effect of commercial pesticide preparations Betokson super containing β-naphthaleneacetic acid (NOA), Fusilade containing fluazifop-*p*-butyl and indoleacetic acid (IAA) on selected parameters of the process of germination of the radish (*Raphanus sativus* L.) seeds and the growth of the yeast (*Saccharomyces cerevisiae*) cells. In the case of radish the effect of these preparations on the dynamics of seed germination and elongation of seedlings was analysed. In the case of yeast, the effect of the preparations on the dynamics of growth of cells in the wild strain in liquid medium was examined.

The experiments show that sprouts and seedlings are the most sensitive to the examined preparations; significant inhibition of elongation of seedling organs was observed in the presence of examined preparations in concentrations lower than 10 $\mu g \cdot cm^{-3}$. In this case it was observed that the strongest effects were induced by Betokson super preparation. The dynamics of sprouting of radish seeds and growth of yeast cells did not undergo significant changes under these conditions. Distinct inhibition of the process of sprouting and cell divisions in yeast was observed after adding the examined preparations in concentrations higher than 10 $\mu g \cdot cm^{-3}$. The parameters which characterise the growth of yeast cells may be used in studies of non-specific effect of herbicides.

Keywords: yeast, radish, NOA, IAA, fluazifop-*p*-butyl

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The studies concerning the toxicity of herbicides concentrate not only on plant organisms but also include other living organisms and particularly those organisms which are directly exposed to the herbicides in the natural environment. Reports on studies concerning the response to herbicides of phylogenetically distant organisms are more and more frequent, eg the studies concerning the toxicity of herbicides conducted by the Papaefthimiou et al [1] concerned two different test systems, one based on the isolated sciatic nerve of amphibian and the other on a microbial eukaryote. The studies conducted by the Venkov team [2] concerned the reaction of three tests based on yeast, transformed hematopoietic and mouse bone marrow cells. Comparing the response to auxin herbicides, the studies conducted by Fargasova [3] consisted of four biological subjects (*Daphnia magna*, *Tubifex tubifex*, *Scenedesmus quadricauda* and seeds of *Sinapis alba*).

In the study the yeast cells of *Saccharomyces cerevisiae* were chosen as well as the seeds and young seedlings of radish (*Raphanus sativus* L.) Both radish and yeast are model organisms commonly used for studies concerning the effect of xenobiotics, such as heavy metals or pesticides [4–8]. Tests based on measuring the sensitivity of the germination process and elongation of radicles are used in short-term ecotoxicological studies [9].

The purpose of the present study was to determine the effect of pesticide preparations (Betokson super, Fusilade) containing β-naphthaleneacetic acid (NOA) and fluazifop-*p*-butyl, respectively, as active substances and indoleacetic acid (IAA) on the selected parameters of the germination process on the seeds of radish (*Raphanus sativus* L.) and the growth of cells of yeast (*Saccharomyces cerevisiae*). In order to compare the results obtained for these two different organisms, the level of inhibition of the parameters in study was determined.

Material and methods

Seeds of radish (*Raphanus sativus* L.), 'Rowa' cv., from Przedsiębiorstwo Nasiennictwa Ogrodniczego i Szkółkarstwa Ożarów Mazowiecki were used in the experiment.

Radish seeds were sowed in 50-seed batches on Petri dish padded with circles of filter paper. The medium for germination was a water solution of herbicide preparations of Betokson super, Fusilade and indoleacetic acid (IAA) in the following concentrations: 2, 4, 6, 8, 10, 20, 30 $\mu g \cdot cm^{-3}$. Stock IAA in concentration of 100 $\mu g \cdot cm^{-3}$ was made by dissolving the appropriate amounts of IAA in DMSO. The dishes with seeds were incubated in an incubator at 25 ^{o}C in the dark.

After 24, 36 and 48 hours the number of germinated seeds was determined. The appearance of a radicle protruding to more than 2 mm was accepted as a criterion for germination [10]. After 48 hours from sowing the length of roots, hypocotyls and whole seedlings was measured with a ruler. Root, hypocotyl and whole seedling elongation inhibitory rates were calculated as differences between vegetables exposed to herbicides or IAA and the control sample cultured in distilled water. The inhibitory rates of the process of seed germination were determined in the same way.

The sensitivity of yeast cells of the SP4 wild strain of genotype Mat α leu1 arg4 to the examined herbicide preparations was determined by measuring the density of the culture [11]. The yeast cultures were grown on YPD liquid medium containing: 10 $g \cdot dm^{-3}$ of yeast extract, 10 $g \cdot dm^{-3}$ of peptones, 20 $g \cdot dm^{-3}$ of glucose and different concentrations of Betokson super and Fusilade preparations and indoleacetic acid (IAA). The cultures were incubated on a rotary shaker at 28 ^{o}C. The density of the cultures was determined after 24 and 48 hours from inoculation by counting the cells in the Malassezz hemocytometer chamber. The level of inhibition of the growth of yeast cells was calculated as a difference between the density of the culture conducted in the presence of herbicide preparations and the control sample cultured in medium which did not contain any supplements.

The herbicide preparations used in the experiments were bought in shops selling crop protection products. Indoleacetic acid (IAA) was bought in Sigma. The presented results are average values from at least 3 experiments conducted independently. After data were obtained from the experiments, average values and standard deviations (SD) were calculated.

Results

At the first stage of the experiment, there was determined the dynamics of germination of radish seeds in the presence of selected concentrations of herbicide preparations of Betokson super, Fusilade containing β-naphthaleneacetic acid (NOA) and fluazifop-*p*-butyl as active substances, respectively and indoleacetic acid (IAA) (Table 1). On the basis of obtained data the rates of inhibition of germination process were calculated as a difference between the percentage of germinated seeds in the control and the seeds in the samples with herbicides or IAA.

Table 1

Effect of herbicides preparations and YAA on the dynamics of germination of radish (*Raphanus sativus* L.) seeds

Concentrations of active substances [$\mu g \cdot cm^{-3}$]	Germinated seeds [%]					
	After 24 hours from sowing	Inhibition rate [%]	After 36 hours from sowing	Inhibition rate [%]	After 48 hours from sowing	Inhibition rate [%]
0 (control)	50.3	0	62.8	0	80.1	0
NOA						
2	54.0	0	63.4	0	87.0	0
4	63.4	0	68.2	0	76.3	4.7
6	62.0	0	68.5	0	78.4	2.6
8	62.0	0	62.0	1.3	82.1	0
10	56.3	0	68.1	0	80.2	0
20	62.1	0	63.0	0	78.5	2.6
30	51.8	0	64.2	0	73.7	8.8
Fluazifop-*p*-butyl						
2	60.5	0	67.3	0	76.1	0
4	66.6	0	69.3	0	80.4	0

Table 1 contd.

Concentrations of active substances [μg · cm^{-3}]	Germinated seeds [%]					
	After 24 hours from sowing	Inhibition rate [%]	After 36 hours from sowing	Inhibition rate [%]	After 48 hours from sowing	Inhibition rate [%]
6	67.3	0	68.0	0	85.2	0
8	72.0	0	74.1	0	86.0	0
10	55.2	0	64.2	0	85.2	0
20	42.5	15.5	63.1	0	80.8	0
30	30.1	40.1	45.1	28.2	56.7	29.2
IAA						
2	53.1	0	65.4	0	82.6	0
4	45.5	9.5	55.5	11.6	79.0	1.4
6	46.8	6.9	56.4	10.2	84.2	0
8	45.4	9.7	54.4	13.4	76.2	4.9
10	44.0	12.5	52.3	16.7	77.2	3.6
20	38.6	23.2	49.3	21.5	76.4	4.6
30	36.3	27.8	45.6	27.4	68.4	14.6

Inhibition of the germination process of radish seeds was the largest in the presence of exogenous indoleacetic acid (IAA). Fusilade preparation affected this parameter to a smaller degree. Only the largest concentrations of this substance (20 and 30 μg · cm^{-3}) led to an evident inhibition of the seed germination process. As far as Betokson super is concerned, it had almost no effect on the dynamics of germination of radish seeds. It should be stressed that negative effects of the examined preparations were more evident at the initial stage of seed germination (24 hours from sowing) than at later stages.

Next, the effect of the selected herbicide preparations and IAA on the length of radish seedlings and its organs (root, hypocotyl) was examined. Measurements were taken after four days from sowing the seeds. The obtained results are presented in Table 2. The inhibition rates of elongation of these organs and of whole seedlings were calculated as a difference between a mean length of radicle, hypocotyl and seedling from control seeds and a mean length of radicle, hypocotyl and seedling of seeds grown in the presence of herbicides or IAA.

The mean length of seedlings which developed in the presence of Fusilade, Betokson super and concentrations of IAA higher than 2 μg · cm^{-3} was smaller than the average length of seedlings in the control. The highest level of inhibition of seedling elongation was observed for Betokson super containing NOA as an active substance. In the case of exogenous indoleacetic acid used in small concentrations the mean length of seedlings was larger than in the control, which suggests that this compound stimulates seedling elongation.

While comparing the effect of the examined preparations on the process of elongation of the particular organs of seedlings it was observed that the examined preparations and exogenous indoleacetic acid inhibited the elongation of roots more than the elongation of hypocotyl.

The inhibition of root elongation was very big in the case of all examined concentrations of Betokson super. Similar effect was obtained only after the application of the highest concentration of Fusilade. When the concentration of Fusilade was low, the length of hypocotyl was medium, in spite of the fact that the mean length of roots and the whole seedling was reduced in these conditions. After application of low concentration of IAA (2 μg · cm^{-3}) the mean length of the seedling and the length of its organs was bigger.

Table 2

Effect of herbicide preparations and IAA on the length of radicles, hypocotyls and seedlings of radish (*Raphanus sativus* L.)

Concentrations of active substances [μg · cm^{-3}]	Mean length of seedlings and their organs ± standard deviation [mm]					
	Radicle	Inhibition rate [%]	Hypocotyl	Inhibition rate [%]	Seedling	Inhibition rate [%]
0 (control)	47.98 ± 2.5	0	13.9 ± 3.6	0	61.9 ± 5.3	0
NOA						
2	10.0 ± 3.1	79.1	6.8 ± 2.1	51.1	16.8 ± 2.4	72.8
4	6.9 ± 2.3	85.5	5.5 ± 2.8	59.9	12.5 ± 3.4	79.7
6	6.3 ± 1.4	86.8	5.4 ± 2.7	60.6	11.7 ± 2.1	80.9
8	4.2 ± 3.1	91.1	6.0 ± 1.8	56.4	10.2 ± 1.4	83.5
10	3.7 ± 2.4	92.1	5.1 ± 2.2	62.8	8.8 ± 2.4	85.5
20	3.1 ± 1.3	93.3	5.0 ± 1.6	63.8	8.1 ± 1.1	86.7
30	1.6 ± 1.3	96.5	4.1 ± 0.9	70.2	5.7 ± 0.6	90.5
Fluazifop-*p*-butyl						
2	37.4 ± 4.2	22.0	14.7 ± 4.2	0	52.1 ± 8.1	15.8
4	36.6 ± 5.2	23.6	15.5 ± 3.5	0	52.1 ± 4.1	15.8
6	38.8 ± 4.1	19.0	16.0 ± 6.4	0	54.8 ± 4.8	11.4
8	38.4 ± 3.8	20.8	18.1 ± 3.2	0	56.1 ± 2.4	9.2
10	41.3 ± 4.9	13.9	15.1 ± 4.7	0	56.4 ± 4.5	8.8
20	39.8 ± 2.1	16.9	13.5 ± 2.5	2.3	53.4 ± 3.9	13.6
30	4.5 ± 1.0	90.6	3.0 ± 0.8	78.1	7.5 ± 1.3	87.7
IAA						
2	54.3 ± 6.3	0	16.8 ± 5.3	0	71.2 ± 4.4	0
4	45.6 ± 2.1	4.8	13.6 ± 2.5	2.1	59.2 ± 5.6	4.3
6	40.0 ± 5.9	16.6	16.3 ± 3.7	0	56.4 ± 7.1	8.9
8	35.8 ± 6.3	25.2	12.9 ± 3.3	6.8	48.8 ± 4.2	21.1
10	36.9 ± 3.1	23.1	11.2 ± 1.5	18.9	48.1 ± 3.5	22.2
20	23.0 ± 2.7	51.9	9.3 ± 1.3	32.0	32.4 ± 4.2	47.6
30	21.4 ± 5.6	55.3	10.6 ± 2.4	23.7	32.0 ± 1.2	48.3

Next, there was examined the effect of herbicide preparations of Betokson super, Fusilade and IAA on the growth of yeast cells of the SP4 wild strain. Yeast cells were grown in standard conditions on liquid medium (YPD) supplemented with the concentrations of herbicide preparations and IAA presented in Table 3.

Young yeast cultures (24 hours) were the most sensitive to Fusilade preparation. After the cells were treated with concentrations of this preparations exceeding 10 μg · cm^{-3}, the density of the cultures was much smaller than in the control. The highest level of inhibition of cell growth was observed after the application of the biggest concentrations of the herbicide (100 and 200 μg · cm^{-3}). The other two preparations inhibited the growth of yeast cells when added in concentrations of 50 μg · cm^{-3} and higher. Older cultures (48 hours) were sensitive to Betokson super and Fusilade when these preparations were applied in concentrations exceeding 50 μg · cm^{-3}. Even applied in the highest concentration of 200 μg · cm^{-3} exogenous indoleacetic acid reduced the growth of yeast cells only to a small extent.

Table 3

Effect of Betokson super, Fusilade and IAA preparations on the dynamics of growth of yeast cells

Concentrations of active substances [μg · cm^{-3}]	Density of yeast cells culture of wild strain SP4			
	After 24 h from inoculation [cells · 10^6 · cm^{-3}]	Inhibition rate [%]	After 48 h from inoculation [cells · 10^8 · cm^{-3}]	Inhibition rate [%]
0 (control)	22.3	0	3.2	0
NOA				
10	26.7	0	5.4	0
20	23.4	0	5.5	0
50	3.9	82.5	3.0	6.25
100	0.4	98.0	0	100
200	0	100	0	100
Fluazifop-*p*-butyl				
10	19.1	14.3	3.8	0
20	17.6	21.1	4.6	0
50	10.1	54.7	3.0	6.2
100	4.0	82.1	1.6	50.0
200	2.7	87.8	0.9	71.9
IAA				
10	22.0	1.3	6.1	0
20	23.8	0	4.6	0
30	22.9	0	4.3	0
50	21.2	4.9	5.7	0
100	16.4	26.4	4.5	0
200	7.7	65.4	2.3	28.1

Discussion

Auxin is a plant hormone which regulates and influences many aspects of the growth and development of plants. Treatment of plants with exogenous IAA can result in a variety of physiological and morphological effects. One of the symptoms of treatment of Arabidopsis seedlings with natural or synthetic auxins is inhibition of root growth [12].

The obtained results show that exogenous IAA (4-(indol-3-yl)acetic acid) in concentration higher than 2 μg · cm^{-3} inhibits the elongation of radicle and hypocotyl. Only when IAA was applied in concentration of 2 μg · cm^{-3}, small effect stimulating the germination of radish seeds and elongation of seedlings was observed.

Yeast cells were more resistant to the effect of this phytohormone. Negative effects consisting inhibition of yeast cell division were visible after an application of about ten times higher doses than the doses effective for radish seedlings. These results agree with the data obtained by Prusty et al [13]. According to them, the application of higher concentrations of IAA inhibits the growth of yeast strains both in liquid and solid media. In this case the concentration inducing 50 % inhibition of growth measured as the OD growth in the culture was EC_{50} = 250 μM, which corresponds to about 43 $\mu g \cdot cm^{-3}$. In the present study the effective concentrations were higher both for logarithmic and for stationary cells. The differences are caused most probably by a different sensitivity of the wild strains used for the experiments, which differ from each other in the genetical backgrounds.

According to Prusty et al [13] IAA arrests yeast growth at all stages of the cell cycle and after removal of IAA the cells resume growth. At lower concentrations it induces filamentation and adhesion (invasive growth of yeast cells). These responses are mediated by family of transporters and the fungal transcription factor Yap1p, which is engaged in the response of yeast cells to oxidative stress. IAA also inhibits the growth of the *Ustilago maydis*, a fungal pathogen of corn.

NOA (β-naphthaleneacetic acid) is a synthetic derivative of auxin. In high concentrations (herbicide doses) synthetic auxins induce various defects of plant growth, such as stem curvature, leaf epinasty, inhibitions of root and shoot growth, decrease in leaf surface and opening of stomata. These defects lead to a decrease in transpiration, carbon assimilation, ageing and local necroses causing death in the end [14]. The results of our studies show that the process which is the most sensitive to NOA effect is elongation of radish seedlings (particularly the elongation of the radicle). The process of seed germination was inhibited only when the concentration of this substance was 10 times higher. The same concentration of NOA was also effective in the case of young, logarithmic yeast cells.

The results presented in this study show that natural auxin has a smaller inhibiting effect on root growth than its synthetic analogue NOA (Table 2).

According to the studies conducted by Walsh et al [15] a variety of synthetic auxins and natural auxins IAA are characterized by a different potency in the reduction in *Arabidopsis* root growth. Out of the compounds examined by the authors (novel picolinate auxin DAS534, 2,4 D and IAA) natural auxin also had the smallest inhibiting effect on root growth.

Fluazifop-*p*-butyl is an active ingredient of APS (aryloxyphenoxypropionates herbicides). They inhibit the lipid synthesis in plant by interfering with the activity of the enzyme acetyl-coenzyme A carboxylase (ACCase), acting on the meristematic level.

The results of the experiments presented in this study show that the parameter which was the most sensitive to the effect of this herbicide was the process of elongation of seedlings and radicles. The process of germination of seeds, just like the division of yeast cells were inhibited only when the doses of this herbicide were 5 times higher.

While comparing the response of organs of seedlings it should be stressed that the elongation of radicles was the most sensitive process to the effect of all the examined substances. It agrees with the observations of other researchers. While examining the effect of pollutants, such as cadmium, copper and chlorimuron-ethyl (used as herbicides) Wang and Zhou [16] found that the sensitivity of wheat to the toxicity of the three pollutants was in the following sequence: root elongation > shoot elongation >

> germination rate. Cheng and Zhou [17] and Song et al [18] identified in their studies concerning the toxicity of heavy metals (Cd, Cu, Pb, Zn) and a chemical (reactive X-3B red dye) to wheat that the inhibitory rate of root elongation was higher than the germination rate with the same concentration of pollutants. A similar relation was also identified in radish while comparing the sensitivity of organs of seedlings to sodium benzoate or Al-based coagulants.

Conclusions

The results showed that root elongation was a more sensitive indicator than seed germination and proliferation rate of yeast for evaluating the toxicity of herbicide preparations. But the parameters which characterise the growth of yeast cells may be used in studies of nonspecific effect of herbicides.

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Abstrakt: Badania dotyczące niespecyficznych oddziaływań pestycydów koncentrują się nie tylko na organizmach roślinnych, ale także obejmują inne organizmy, szczególnie te które są bezpośrednio narażone na ich działanie w środowisku naturalnym. Coraz częściej spotyka się relacje z badań dotyczących odpowiedzi na herbicydy odległych filogenetycznie organizmów. W prezentowanej pracy badano oddziaływanie preparatów pestycydowych Betokson super (zawierający kwas β-naftoksyoctowy, NOA), Fusilade (zawierający fluazifop-*p*-butylowy) i heteroauksyny (IAA) na wybrane parametry procesu kiełkowania nasion rzodkiewki (*Raphanus sativus* L.) oraz wzrostu komórek drożdży (*Saccharomyces cerevisiae*). Oznaczono wpływ badanych substancji na dynamikę kiełkowania nasion i wydłużanie organów siewek rzodkiewki oraz na dynamikę wzrostu komórek drożdży w pożywce płynnej. Najbardziej wrażliwe na badane substancje okazały się kiełki i młode siewki. Wysoki poziom inhibicji wydłużania organów siewek zanotowano dla mniejszych niż 10 $\mu g \cdot cm^{-3}$ stężeń substancji aktywnych badanych preparatów. Najsilniejsze efekty hamowania wydłużania siewek i korzeni zaobserwowano po zastosowaniu preparatu Betokson super. Natomiast dynamika kiełkowania nasion rzodkiewki i przyrost liczby komórek drożdży w tych warunkach nie ulegały znaczącym wahaniom. Wyraźne hamowanie procesu kiełkowania i podziałów komórkowych drożdży zaobserwowano dopiero po zastosowaniu większych niż 10 $\mu g \cdot cm^{-3}$ stężeń badanych substancji. Parametry charakteryzujące wzrost komórek drożdży mogą być wykorzystywane w badaniach nad niespecyficznymi oddziaływaniami herbicydów.

Słowa kluczowe: drożdże, rzodkiewka, NOA, IAA, fluazifop-*p*-butylowy

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EFFECT OF MAGNESIUM AND CADMIUM SUPPLEMENTATION ON YIELDS AND QUALITY OF POPPY (*Papaver somniferum* L.)

ODDZIAŁYWANIE DODATKU MAGNEZU I KADMU NA PLON I JAKOŚĆ MAKU (*Papaver somniferum* L.)

Abstract: In a vegetation pot experiment with poppy, ‘Opál’ variety, we explored the effect of soil supplementations of magnesium (0.78 g Mg · pot^{-1}) and as foliar dressing (3 % solution) in the form of $Mg(NO_3)_2$ at a natural (0.14 mg Cd · kg^{-1}) and increased level (1 mg Cd · kg^{-1}) of cadmium in the soil on the chemical composition of the plants, seed yields, content of morphine in the straw and cadmium content in the seeds.

The following six variants were used in the experiment: 1) N (control), 2) N + $Mg(NO_3)_2$ into the soil, 3) N + $Mg(NO_3)_2$ foliar dressing, 4) N (control) + Cd, 5) N + $Mg(NO_3)_2$ into the soil + Cd, 6) N + $Mg(NO_3)_2$ foliar dressing + Cd.

The level of magnesium in plants in the DC 41 stage (stem elongation growth) increased in the magnesium-fertilised variants. The level of Cd in plants grown in soil with a natural Cd content increased in all the Mg-fertilised variants to 0.29–0.45 mg Cd · kg^{-1} compared with 0.27 mg Cd · kg^{-1} in the control variant. Of the plants grown in soil with the increased Cd content the highest Cd level was monitored in the control variant (1.29 mg Cd · kg^{-1}), while in the other variants the Cd level decreased after the application of magnesium to 0.51 and 0.69 mg Cd · kg^{-1}, respectively. In the DC 41 stage the dry matter weight of one plant ranged irregularly from 2.16 to 3.82 g and the highest value was achieved in the variant with a supplementation of cadmium and nitrate form of magnesium.

Poppy seed yields were statistically insignificant in all variants and ranged between 2.28 and 2.74 g of seeds per plant.

The content of morphine in straw (empty capsule + 15 cm of stem) ranged only between 0.92 and 1.05 %, and the effect of magnesium or cadmium was insignificant.

At a natural level of Cd in the soil the differences in its content in seeds were not significant and ranged between 0.479 and 0.612 mg Cd · kg^{-1}. In variants where the soil was supplemented with Cd its content in the seeds increased significantly (1.413–2.176 mg Cd · kg^{-1}) compared with variants with a natural Cd content in the soil. When Mg was applied to soil with an increased Cd level we saw that the Cd level in seeds decreased significantly when compared both with the controls and to foliar application of magnesium.

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Applications of Mg increased its content in plants, stabilised yields and did not increase the Cd content in poppy seeds which increased only after goal-directed Cd supplementation in the soil.

Keywords: poppy, cadmium, magnesium, soil application, foliar application

In 2008 poppy was sown in the Czech Republic for purposes of the food (poppy seed) and pharmaceutical (alkaloids) industry in an area of almost 75,000 ha. This enormous increase in the area of sown poppy demonstrates, in the first place, the economic benefits which this crop, traditional in Central Europe, brings. That is why it is necessary to set up a compact technology of cultivation of this crop, the integral part of which is indisputably complete nutrition assuring a sufficient supply of nutrients acceptable for the plant. Apart from the indispensability of biogenic nutrients, to a great extent contributing to the formation of yields of poppy seed and poppy straw, we must also focus on the presence of substances which deteriorate the quality, particularly in terms of its subsequent consumption as food.

One of the many factors limiting the use of poppy as food is the content of heavy metals in the seeds, especially of cadmium. This alien element troubles the farmers as the results of controls of the Czech Agriculture and Food Inspection Authority carried out from 2002 to 2006 confirmed; the average content of Cd ranged between 0.56 and 0.70 $\mathrm{mg \cdot kg^{-1}}$ [1]. The use of poppy in the food industry is limited by the legislation of the Czech Republic, ie the Decree of the Ministry of Health [2], according to which the maximal admissible amount of Cd in 1 kg of oil plants intended for direct consumption and use is 0.50 $\mathrm{mg \cdot kg^{-1}}$ [3]. The risk of Cd entering the plant is enhanced especially in areas where the soil pH value is low, where the content of organic compounds is low and naturally where the content of Cd in the soil is high [4–7]; an amount as low as 6 mg of releasable Cd in 1 kg of soil is toxic for plants and for soil bacteria [7]. Also atmospheric deposits and mineral P fertilisers made from phosphates with a high content of cadmium play an important role in Cd contamination [8]. A number of literature sources describe the effect of macrobiogenic (P and Ca) and particularly microbiogenic elements (namely Zn) on the intake of Cd by the plant and its distribution and utilisation in tissues [9–16]. However in literature facts explaining the mutual effect of cadmium and magnesium and their impact on the plant organism and metabolism of poppy are rather sporadic [17]. And it is no other than magnesium which is an element having a positive effect not only on yields but particularly on the quality of the poppy seeds; it decreases Cd utilisation in seeds [17].

The present study is focused on the effect of soil and foliar applications of Mg in interaction with the natural content of Cd and with soil supplementation on the content of nutrients and Cd in poppy plants in the stage of elongation growth, on yields of poppy seeds, yields of straw (empty capsules + 15 cm of stem), the content of morphine in the straw and Cd content in seeds.

Material and methods

The vegetation pot experiment with poppy was established on 27 March 2007 in plastic pots which held 9.5 kg of medium-heavy soil characterised as Fluvisol. Table 1 gives the agrochemical characteristics of the soil before the establishment of the study.

Table 1

Agrochemical properties of soil (Melich III)

Soil acidity $pH/CaCl_2$	Content of available nutrients [$mg \cdot kg^{-1}$]			
	P	K	Ca	Mg
7.6	72	94	2074	111

Among the evaluated parameters of soil fertility was the soil reaction (1:5 soil: 0.01 $mol \cdot dm^{-3}$ $CaCl_2$ solution) and the contents of available nutrients (P, K, Ca and Mg) specified after soil extraction with the leaching agent Mehlich III (1:10 soil: leaching agent Mehlich III) [18]. The amount of phosphorus in the extract was determined colorimetrically (UV/VIS spectrophotometer ATI Unicam 8625). Potassium, calcium and magnesium were determined using the method of atomic absorption spectrophotometry (AAS) on the Carl Zeiss Jena AAS-30 apparatus. Apart from the content of available nutrients we determined the total content of Cd in the soil extract with *aqua regia* [19] and detected a low value of 0.14 $mg \cdot kg^{-1}$.

We explored the effect of both soil and foliar (3 % solution) applications of magnesium in the form of $Mg(NO_3)_2$ at a natural and increased level of cadmium (Table 2).

Table 2

Experiment design

Variant of fertilization		Dose of nutrients		Dose of cadmium
		Nitrogen	Magnesium	
		[$g \cdot pot^{-1}$]		[$mg \cdot kg^{-1}$]
1	N (control)	0.9	0.00	0
2	N + $Mg(NO_3)_2$ into the soil	0.9	0.78	0
3	N + $Mg(NO_3)_2$ foliar dressing	0.9	0.78	0
4	N (control) + Cd	0.9	0.00	1
5	N + $Mg(NO_3)_2$ into the soil + Cd	0.9	0.78	1
6	N + $Mg(NO_3)_2$ foliar dressing + Cd	0.9	0.78	1

Variants where magnesium was applied to the soil (variants 2 and 5) were fertilised on 28 March 2007 according to the above pattern, ie 0.78 g Mg per pot. Later on soil of variants 4 – 6 was supplemented with cadmium in a dose of 1 $mg \cdot kg^{-1}$ of soil in the form of cadmium acetate $(CH_3COO)_2Cd \cdot 2H_2O$. Nitrogen was balanced out in all the variants of fertilisation to the same level (0.9 g N per pot) with ammonium nitrate (34.5 % N). All the nutrients and cadmium were dissolved and applied to the soil in the form of watering. Shallow sowing of poppy, 'Opál' variety, was conducted after the soil had settled naturally (2 April 2007).

The plants began to emerge evenly 10 days after sowing. In the stage of 4–6 true leaves (9 May 2007) the plants were singled to 7 plants per pot. On 18 May 2007 foliar application of magnesium followed in variants 3 and 6 in the stage of 8–10 leaves. For the application we used a 3 % solution of $Mg(NO_3)_2$. On a regular basis during vegetation the plants were watered with demineralised water, sampled for chemical analyses, they were maintained weed-free and the preparations Karate 2,5 EC and Discus were used as pest control against beet aphid (*Aphis fabae*) and downy mildew of poppy (*Peronospora arborescens*).

The plants were harvested on 8 August 2007, ie 4 plants per pot. The results were assessed using the programme STATISTICA 7.1 CZ by the method of variance analysis (ANOVA) followed by Tukeye's test at a 95 % level of significance ($p \leq 0.05$).

Results and discussion

Analyses of the plant matter

The range of the nitrogen levels in the stage of elongation growth (DC 41) of all fertilisation variants was narrow, ie between 3.59 and 4.21 %; the type or form of the applied fertilisers was insignificant. Application of Mg fertilisers had a positive effect on the level of magnesium in the plant. Compared with the control (unfertilised) variants its content increased in variants where both soil and foliar fertilisation with this nutrient was applied (by 12.2–13.0 % and by 4.1–15.2 %, respectively). Magnesium fertilisation had no marked impact on the contents of the other macrobiogenic elements which ranged within the optimal values (Table 3).

Table 3

Chemical composition of the poppy plants in the stage of elongation growth (DC 41)

Variant of fertilization		N	P	K	Ca	Mg	S	Cd
		[% dry matter]						[mg · kg^{-1}]
1	N (control)	3.87	0.57	3.57	2.86	0.49	0.40	0.27
2	N + $Mg(NO_3)_2$ into the soil	4.00	0.53	3.95	3.02	0.55	0.40	0.29
3	N + $Mg(NO_3)_2$ foliar dressing	4.21	0.58	4.10	2.89	0.51	0.42	0.45
4	N (control) + Cd	3.59	0.60	3.82	2.51	0.46	0.40	1.29
5	N + $Mg(NO_3)_2$ into the soil + Cd	3.97	0.55	4.00	2.78	0.52	0.35	0.51
6	N + $Mg(NO_3)_2$ foliar dressing + Cd	3.90	0.49	3.29	2.65	0.53	0.36	0.69

After soil application of magnesium the amount of cadmium in plants grown in soil with natural cadmium content (variants 1–3) increased to 0.29 and after foliar application to 0.45 ppm Cd; compared with the control plants it increased 7.4 and 66.7 %, respectively. In plants grown in soil where the Cd level was purposely increased to 1 ppm (variants 4–6) the level of Cd was the highest in the control variant (variant 4) and the correlation between cadmium intake by the poppy plant and the level in the soil was close [20]. Much like magnesium the effect of the increased Cd doses did not markedly influence the content of macrobiogenic elements. The results of experiments of Zhang et al [21] imply that increased doses of Cd in the soil cause excessive accumulation of P and K in plant roots but it probably inhibits subsequent translocation into the aboveground parts of the plants. In our experiment cadmium concentration in the plant decreased relatively after soil application of the Mg fertiliser to 39.5 % of the control variant and after foliar application by almost one half (53.5 % of the value of the unfertilised variant). Literature also states that Mg intake on Cd-contaminated soil is higher [22]. This fact was not proved in our experiment and confirmed the results of Jiang et al [23] who discovered that the effect of the Cd level on the intake and translocation of Mg was low.

The dry matter weight of one plant fluctuated irregularly from 2.16 to 3.82 g and the highest values were achieved in variants after foliar application of the nitrate form of magnesium, ie both without and with cadmium supplementation (variant 3 and 6). The average intake of nutrients by poppy plants is dependent on the dry matter weight of one plant and its content. This is the main reason of the fluctuation in nutrient intake among the variants of fertilisation (Table 4). In terms of their amount, the order of the intake of nutrients was as follows: N > K > Ca > P > Mg > S. Cadmium intake by plants was on a low level and a more marked increase was seen after soil supplementation.

Table 4

Average nutrient and Cd uptake by the poppy plant in the stage of elongation growth (DC 41)

Variant no.	Weight of 1 plant	N	P	K	Ca	Mg	S	Cd
	[g dry matter d.m.]	[mg per plant]						[μg per plant]
1	2.35	90.9	13.3	83.8	67.2	11.5	9.4	0.63
2	2.24	89.6	11.8	88.5	67.6	12.3	8.9	0.64
3	2.50	105.3	14.5	102.5	72.3	12.7	10.5	1.00
4	2.16	77.5	12.9	101.2	66.5	9.9	8.6	2.70
5	2.18	86.5	11.9	87.2	60.6	11.3	7.6	1.10
6	3.82	148.9	18.7	125.6	101.2	20.2	13.7	2.60

Results of yields

Soil application of Cd did not have a marked effect on poppy seed yields. While poppy seed yields of the control (unfertilised) variant and in combination with soil application of $Mg(NO_3)_2$ (variants 4 and 5) slightly decreased compared with variants with a natural supply of Cd (by 2.0 % between variants 1 and 4 and by 6.2 % between variants 2 and 5), interaction between the increased Cd level in soil and foliar application of magnesium (var. 6) relatively increased its level in comparison with variant 3. ie by 6.1 %. This fact is at variance with results of the experiment which showed that soil application of cadmium reduced yields by as much as 25 % [20].

Table 5 shows that no statistically significant differences ($p \le 0.05$) in poppy seed yields were detected between variants with a natural (variants 1–3) Cd level in the soil and the yields ranged between 9.74 and 10.31 g of seeds per pot. On the basis of these results it is apparent that the effect of foliar nutrition with magnesium nitrate was higher than soil application. Foliar application of magnesium (variant 3) increased the production of poppy seed against the soil-supplemented variant (variant 2) by 5.7 %, as compared with the unfertilised variant (variant 1) by 5.9 %.

Table 5

Yields of poppy (seed and straw) and morphine content in straw

Variants of fertilization		Seed yield		Yields of straw $[g \cdot pot^{-1}]$	Content of morphine [%]
		$[g \cdot pot^{-1}]$	relative [%]		
1	N (control)	9.74 a	*100.0*	21.29 a	0.92
2	N + $Mg(NO_3)_2$ into the soil	9.75 a	*100.1*	24.23 ab	1.00
3	N + $Mg(NO_3)_2$ foliar dressing	10.31 a	*105.9*	27.53 b	0.93
4	N (control) + Cd	9.55 a	*100.0*	18.89 a	0.93
5	N + $Mg(NO_3)_2$ into the soil + Cd	9.15 a	*95.8*	19.22 a	1.05
6	N + $Mg(NO_3)_2$ foliar dressing + Cd	10.94 a	*114.6*	22.23 a	0.97

$p \leq 0.05$ – statistical significance at a 95 % level of significance; variants with the same letters (a) showed statistically insignificant differences.

The trends in yields of variants grown in soil with a purposely increased Cd level are similar. The increase in poppy seed yields after foliar application of magnesium was even more marked (Table 5) but not statistically significant ($p \leq 0.05$). On the contrary, soil application of magnesium nitrate reduced seed yields by 4.2 % as compared with the control variant.

Total per-pot yields of poppy straw (empty capsule + 15 cm of stem) fluctuated among the variants. In the experiment with a natural Cd level in the soil we detected a statistically significant ($p \leq 0.05$) increase in straw production as a result of foliar nutrition with magnesium (variant 3) as compared with the control variant. Where the soil was supplemented with Cd the straw yields of all variants decreased as against the variants with a natural Cd level. The effect of foliar application of magnesium was similar and increased yields by 17.7 % as against the control and by 15.7 % as against soil application, but not significantly ($p \leq 0.05$).

In all variants the morphine content in straw exceeded the average value given for the ‘Opál’ variety. In variants without Cd supplementation it ranged between 0.92 and 1.00 % and in variants where Cd was applied it ranged between 0.93 and 1.05 %. According to literary data there is a positive correlation between the Cd content and production of alkaloids in poppy (narcotine in seeds and morphine in the capsules) [24] coupled with the positive effect of Mg application on the morphine content [17, 25]; however in our experiment the doses of magnesium, form of its application and soil supplementation of cadmium did not have a significant effect on the morphine content.

The content of Cd in poppy seeds increased considerably after its soil application. Table 6 demonstrates that after soil supplementation with Cd its content in poppy seeds increased evenly in the control variant and in the variant with a foliar application of $Mg(NO_3)_2$, 3.5 times on average. In spite of the universally stated capacity of plants to accumulate most of the Cd in roots, followed by vegetative organs and least in generative organs, Chizzola [20, 26] presented similar conclusions as above saying that most of the Cd in the plant is utilised in seeds.

Table 6

Cd content in seeds

Variants of fertilization		Content of cadmium in seed		
		$[\mathrm{mg \cdot kg^{-1}}]$	relative^x [%]	relative^y [%]
1	N (control)	0.607 a	*100.0**	*100.0*
2	N + $Mg(NO_3)_2$ into the soil	0.479 a	*100.0***	*78.9*
3	N + $Mg(NO_3)_2$ foliar dressing	0.612 a	*100.0****	*100.8*
4	N (control) + Cd	2.094 a	*345.0**	*100.0*
5	N + $Mg(NO_3)_2$ into the soil + Cd	1.413 b	*295.0***	*67.5*
6	N + $Mg(NO_3)_2$ foliar dressing + Cd	2.176 a	*355.5****	*103.9*

^{x} – elevated Cd content in soil versus natural Cd content; ^{y} – $Mg(NO_3)_2$ application versus control; $p \le 0.05$ – statistical significance at a 95 % level of significance; variants with the same letters (a) showed statistically insignificant differences.

In variants with a natural level of Cd in the soil (variants 1–3) the content of cadmium in seeds did not differ significantly ($p < 0.05$). In the magnesium-unfertilised control variant (variant 1) its content was 0.607 $\mathrm{mg\ Cd \cdot kg^{-1}}$ of seeds. Hoffmann and Blasenbrei [27] discovered that the average Cd content in poppy seeds was 0.739 $\mathrm{mg\ Cd \cdot kg^{-1}}$ d.m. The Cd content dropped more markedly after supplementary soil fertilisation with $Mg(NO_3)_2$, but it was not statistically significant ($p < 0.05$). In relative terms the content of the heavy metal decreased by 21.1 % under the effect of soil application of Mg. Foliar application of magnesium did not much change the cadmium content in poppy seeds.

A similar trend was discovered in variants where cadmium was supplemented to the soil prior to sowing. The highest content of Cd in poppy seed (2.176 $\mathrm{mg \cdot kg^{-1}}$) was detected in the variant after foliar magnesium fertilisation (variant 6); its content was almost the same as in the control variant (Table 6). Soil application of $Mg(NO_3)_2$ significantly ($p \le 0.05$) reduced the content of cadmium in seeds, ie from 2.094 to 1.413 $\mathrm{mg \cdot kg^{-1}}$, ie by 32.5 %.

Conclusion

While the amount of Cd in plants grown in soil with a natural level of Cd increased after soil and foliar application of magnesium by 7.4 and 66.7 %, respectively, Mg fertilisation considerably reduced its content in the plant matter if the heavy metal was supplemented prior to sowing. Foliar nutrition with magnesium nitrate increased poppy seed yields much more than its soil application regardless of the soil level of Cd. Foliar application increased yields as compared with the magnesium-unfertilised variant both at a natural content of Cd and with intentional soil supplementation, ie by 5.9 % and 14.6 %, respectively. It was the very interaction between the increased content of soil

Cd and foliar Mg application that resulted in the highest poppy seed yields (10.94 g · pot^{-1}). Foliar Mg applications also had a positive effect on straw yields (empty capsule + 15 cm of stem). Soil application of Cd reduced straw yields regardless of Mg fertilisation. The magnesium dose, form of its application and cadmium supplementation of the soil had no significant effect on the morphine content.

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ODDZIAŁYWANIE DODATKU MAGNEZU I KADMU NA PLON I JAKOŚĆ MAKU (*Papaver somniferum* L.)

Abstrakt: W wazonowym doświadczeniu wegetacyjnym z makiem odmiany ‘Opal’ badano oddziaływanie magnezu stosowanego doglebowo (0,78 g Mg · wazon^{-1}) lub dolistnie (roztwór 3 %) w formie $Mg(NO_3)_2$ w warunkach naturalnej (0,14 mg Cd · kg^{-1}) oraz zwiększonej (1 mg Cd · kg^{-1}) zawartości kadmu w glebie na skład chemiczny roślin, plon nasion, zawartość morfiny w słomie oraz zawartość kadmu w nasionach.

Doświadczenie obejmowało sześć następujących wariantów: 1) N (kontrola), 2) N + $Mg(NO_3)_2$ doglebowo, 3) N + $Mg(NO_3)_2$ dolistnie, 4) N (kontrola) + Cd, 5) N + $Mg(NO_3)_2$ doglebowo + Cd, 6) N + + $Mg(NO_3)_2$ dolistnie + Cd.

Zawartość magnezu w roślinach w stadium DC 41 (wzrost wydłużeniowy łodygi) zwiększała się w wariantach nawożonych magnezem. Poziom Cd w roślinach rosnących na glebie z naturalną zawartością Cd wzrósł we wszystkich wariantach nawożonych magnezem do 0,29–0,45 mg Cd · kg^{-1} w porównaniu z 0,27 mg Cd · kg^{-1} w wariancie kontrolnym. Największą zawartość Cd w roślinach na glebie ze zwiększoną zawartością Cd stwierdzono w wariancie kontrolnym (1,29 mg Cd · kg^{-1}), podczas gdy w pozostałych wariantach poziom Cd zmniejszał się po zastosowaniu magnezu odpowiednio do 0,51 i 0,69 mg Cd · kg^{-1}. W stadium DC 41 plon suchej masy jednej rośliny wahał się nieregularnie od 2,16 do 3,82 g, a największą wartość osiągnął w wariancie z dodatkiem kadmu i azotanu(V) magnezu.

Plony nasion maku we wszystkich wariantach wahały się od 2,28 do 2,74 g nasion z rośliny i nie różniły się znacząco statystycznie.

Zawartość morfiny w słomie (puste główki + 15 cm łodygi) wahała się od 0,92 do 1,05 %, a wpływ magnezu lub kadmu był statystycznie nieistotny.

Przy naturalnej zawartości Cd w glebie, zawartości Cd w nasionach wahały się od 0,479 do 0,612 mg Cd · kg^{-1}, a różnice były statystycznie nieistotne. W wariantach, w których do gleby dodano Cd, jego zawartość w nasionach zwiększała się znacznie (1,413–2,176 mg Cd · kg^{-1}) w porównaniu z wariantami z naturalną zawartością Cd w glebie. Gdy Mg dodano do gleby ze zwiększoną zawartością Cd, stwierdzono, że poziom Cd w nasionach znacznie obniżył się w porównaniu zarówno z obiektami kontrolnymi, jak i z dolistnym stosowaniem magnezu.

Stosowanie Mg zwiększało jego zawartość w roślinach, ustabilizowało plony, a nie zwiększało zawartości Cd w nasionach maku, która wzrastała tylko po ściśle ukierunkowanym dodatku Cd do gleby.

Słowa kluczowe: mak, kadm, magnez, nawożenie doglebowe, dokarmianie dolistne

Varia

INVITATION FOR ECOPOLE '09 CONFERENCE

CHEMICAL SUBSTANCES IN ENVIRONMENT

We have the honour to invite you to take part in the 18th annual Central European Conference ECOpole '09, which will be held in **14–17 X 2009** (Thursday–Saturday) on Wilhelms Hill at Uroczysko in Piechowice, PL.

The Conference Programme includes oral presentations and posters and will be divided into four sections – SI, SII, SIII and SIV:

- **SI Chemical Pollution of Natural Environment and Its Monitoring;**
- **SII Environment Friendly Production and Use of Energy;**
- **SIII Forum of Young Scientists and Environmental Education in Chemistry;**
- **SIV Impact of Environment Pollution on Food and Human Health.**

On the first day the debates of sections SI and SII will take place. The second day will be started with an ecological excursion. Afterwards the plenary Session with lectures on **environmental education** as well as **grants within the EU Programmes** and presentation of **EU Centres of Excellence** will be held. Then the **Forum of Young Scientists** – the presentation (lectures and posters) of young scientists work will take place.

The main topic of the third day is the influence of environment quality on the **human health**.

During the Conference an exhibition of publications concerned with conference topics will be also organised.

The Conference language is English.

Contributions to the Conference will be published as:

- abstracts on the CD-ROM (0.5 page of A4 paper sheet format)
- extended abstracts (4–6 pages) in the semi-annual *Proceedings of ECOpole*;

full papers will be published in successive issues of the *Ecological Chemistry and Engineering/Chemia i Inżynieria Ekologiczna* (Ecol. Chem. Eng.).

Additional information one could find on the Conference website:

ecopole.uni.opole.pl

The deadline for sending the Abstracts is **15.07.2009** and for the Extended Abstracts: **1.10.2009**. The actualised list (and the Abstracts) of the Conference contributions accepted for presentation by the Scientific Board, one will find (starting from 15.07.2009) on the Conference website.

The papers must be prepared according to the Guide for Authors on Submission of Manuscripts to the Journal.

The Conference fee is 300 € (covering hotel, meals and transportation during the Conference). It could be reduced (to 170 €) for young people actively participating in the Forum of Young Scientists. But the colleague has to deliver earlier the Extended Abstract (4-6 pages) of his/her contribution (deadline is on 15.08.2009), and a recommendation of his/her Professor.

Fees transferred after 15.09.2009 are 10% higher.

At the Reception Desk each participant will obtain a CD-ROM with abstracts of the Conference contributions as well as Conference Programme (the Programme will be also published on the Conference website).

Further information is available from:
Dr hab. Maria Wacławek, prof. UO
Chairperson of the Organising Committee
of ECOpole '09 Conference
University of Opole
email: Maria.Waclawek@uni.opole.pl
and mrajfur@o2.pl
tel. +48 77 455 91 49 and +48 77 401 60 42
fax +48 77 401 60 51

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REGISTRATION FORM FOR THE ECOpole ’09 CONFERENCE

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Tel./fax . . . . email . . . .

Title of presentation . . . .

. . . .

KIND OF PRESENTATION

	YES	NO
Oral		
Poster		
Taking part in discussion		

ACCOMMODATION

14/15 X		15/16 X		16/17 X	
YES	NO	YES	NO	YES	NO

MEALS

Date	Breakfast	Lunch	Dinner
14 X	—	—	
15 X			
16 X			
17 X			—

✂ -

ZAPRASZAMY
DO UDZIAŁU W ŚRODKOWOEUROPEJSKIEJ KONFERENCJI
ECOpole ’09
w dniach 14–17 X 2009

SUBSTANCJE CHEMICZNE W ŚRODOWISKU PRZYRODNICZYM

Będzie to osiemnasta z rzędu konferencja poświęcona badaniom podstawowym oraz działaniom praktycznym dotycząca różnych aspektów ochrony środowiska przyrodniczego. Odbędzie się ona w ośrodku „Uroczysko” na Wzgórzu Wilhelma w Piechowicach. Doroczne konferencje ECOpole mają charakter międzynarodowy i za takie są uznane przez Ministerstwo Nauki i Szkolnictwa Wyższego. Obrady konferencji ECOpole ’09 będą zgrupowane w czterech Sekcjach SI–SIV:

Obrady konferencji ECOpole ’08 będą zgrupowane w czterech Sekcjach SI–SIV:

- **SI Chemiczne substancje w środowisku przyrodniczym oraz ich monitoring,**
- **SII Odnawialne źródła energii i jej oszczędne pozyskiwanie oraz użytkowanie,**
- **SIII Forum Młodych (FM) i Edukacja prośrodowiskowa w chemii**,
- **SIV Wpływ zanieczyszczeń środowiska oraz żywności na zdrowie ludzi**.

Materiały konferencyjne będą opublikowane w postaci:

- abstraktów (0,5 strony formatu A4) na CD-ROM-ie;
- rozszerzonych streszczeń o objętości 4-6 stron w półroczniku *Proceedings of ECOpole*;
- artykułów: w abstraktowanych czasopismach: *Ecological Chemistry and Engineering/Chemia i Inżynieria Ekologiczna* (*Ecol. Chem. Eng.*) oraz niektórych w półroczniku *Chemia – Dydaktyka – Ekologia – Metrologia*.

Termin nadsyłania angielskiego i polskiego streszczenia o objętości 0,5–1,0 strony (wersja cyfrowa + wydruk) planowanych wystąpień upływa w dniu 15 lipca 2009 r. Lista prac zakwalifikowanych przez Radę Naukową Konferencji do prezentacji będzie sukcesywnie publikowana od 15 lipca 2009 r. na stronie webowej konferencji

ecopole.uni.opole.pl

Aby praca (dotyczy to także streszczenia, które powinno mieć tytuł w języku polskim i angielskim, słowa kluczowe w obydwu językach) przedstawiona w czasie konferencji mogła być opublikowana, jej tekst winien być przygotowany zgodnie z wymaganiami stawianymi artykułom drukowanym w czasopiśmie *Ecological Chemistry and Engineering*, które jest dostępne w wielu bibliotekach naukowych w Polsce i zagranicą. Są one takie same dla prac drukowanych w półroczniku *Chemia – Dydaktyka – Ekologia – Metrologia*. Zalecenia te są również umieszczone na stronie webowej konferencji.

Koszt uczestnictwa w całej konferencji wynosi 1000 zł i pokrywa opłatę za udział, koszt noclegów i wyżywienia oraz rocznej prenumeraty Ecol. Chem. Eng. (razem blisko 2000 ss.) łącznie z materiałami Konferencji. Jest możliwość udziału tylko w jednym wybranym przez siebie dniu, wówczas opłata wyniesie 650 zł i będzie upoważniała do uzyskania wszystkich materiałów konferencyjnych, jednego noclegu i trzech posiłków (śniadanie, obiad, kolacja), natomiast osoby zainteresowane udziałem w dwóch dniach, tj. w pierwszym i drugim lub drugim i trzecim, winny wnieść opłatę w wysokości 800 zł. Opłata dla magistrantów i doktorantów oraz młodych doktorów biorących aktywny udział w Forum Młodych może być zmniejszona do 600 zł, przy zachowaniu takich samych świadczeń. Osoby te winny dodatkowo dostarczyć: rozszerzone streszczenia (4–6 stron) swoich wystąpień (**do 15.08.2009 r.**). Jest także wymagana opinia opiekuna naukowego. Sprawy te będą rozpatrywane indywidualnie przez Radę Naukową oraz Komitet Organizacyjny Konferencji. Członkowie Towarzystwa Chemii i Inżynierii Ekologicznej i Polskiego Towarzystwa Chemicznego (z opłaconymi na bieżąco składkami) mają prawo do obniżonej opłaty konferencyjnej o 25 zł. Opłaty wnoszone po dniu 15 września 2009 r. są większe o 10% od kwot podanych powyżej. Wszystkie wpłaty winne być dokonane na konto w Banku Śląskim:

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i mieć dopisek ECOpole ’09 oraz nazwisko uczestnika konferencji.

Po konferencji zostaną wydane 4–6-stronicowe rozszerzone streszczenia wystąpień w półroczniku *Proceedings of ECOpole*. Artykuły te winny być przesłane do **1 października 2009 r**. Wszystkie nadsyłane prace podlegają zwykłej procedurze recenzyjnej. Wszystkie streszczenia oraz program Konferencji zostaną wydane na CD-ROM-ie, który otrzyma każdy z uczestników podczas rejestracji. Program będzie także umieszczony na stronie webowej konferencji.

Za Komitet Organizacyjny
dr hab. inż. Maria Wacławek, prof. UO
Wszelkie uwagi i zapytania można kierować na adres:
Maria.Waclawek@uni.opole.pl
lub mrajfur@o2.pl
tel. 077 401 60 42
tel. 077 455 91 49
fax 077 401 60 51

Kalendarium

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ZGŁASZAM UCZESTNICTWO W KONFERENCJI ECOpole ’09
(Prosimy o wypełnienie zgłoszenia drukowanymi literami)

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RODZAJ PRZEWIDYWANEGO WYSTĄPIENIA:

	TAK	NIE
Referat		
Poster		
Głos w dyskusji		

TYTUŁ WYSTĄPIENIA . . . .

. . . .

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14/15 X		15/16 X		16/17 X	
TAK	NIE	TAK	NIE	TAK	NIE

ZAMAWIAM POSIŁKI

Data	Śniadanie	Obiad	Kolacja
14 X	—	—	
15 X			
16 X			
17 X			—

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15th ICHMET

15th International Conference on Heavy Metals in the Environment

September 19–23, 2010
Gdańsk, Poland

Organized by
Chemical Faculty, Gdańsk University of Technology (GUT)
together with
Committee on Analytical Chemistry of the Polish Academy Sciences (PAS)

15th ICHMET – is a continuation of a series of highly successful conferences that have been held in major cities of the world since 1975. These conferences typically draw 500–1000 participants from countries in many parts of the world. Well over 5000 scientists have taken part in this series of conferences including most leaders in the field. Apart from the city's natural beauty, Gdansk is logical choice for the 15th Conference to highlight the outstanding work that is being done on heavy metals in central Europe. The venue for the meeting will be the Gdansk University of Technology (GUT) which features many tourist attractions.

The Conference will include a number of invited lectures treating frontier topics prepared by specialist with international reputation, oral presentation and poster sessions. ICHMET welcomes contributions on all aspects of any heavy metal in the environment. All presentation will be connected with such topics as:

- Risk assessment and risk management pertaining to toxic metals in the environment
- Susceptibility and protection of children from toxic metals in their environment
- Measurement and exposure assessment
- Biomarkers of exposure and effects of heavy metals
- Gene-environment-metal interactions
- Trend tracking/analysis of heavy metal data – spatial and temporal
- Risk communication pertaining to heavy metals
- Life cycle analysis for metalliferous consumer products
- Soil quality criteria
- Remediation technologies
- Control strategies for heavy metal emissions and deposition
- Metal mixtures – mechanistic and epidemiological studies
- Nutrient-metal interactions
- Advancements in analytical tools (procedures and measurement devices)

- Toxicology of heavy metals, from cellular and genomic to ecosystem levels
- Heavy metals in foods
- Impact of global change on heavy metal cycle

For further information on the conference, please contact:

Professor Jacek Namieśnik (Conference Chairman)
Gdansk University of Technology,
Chemical Faculty, Department of Analytical Chemistry
G. Narutowicza 11/12, 80–233 Gdansk, (Poland),
e-mail: chemanal@pg.gda.pl
homepage: http://www.pg.gda.pl/chem/ichmet/

GUIDE FOR AUTHORS
ON SUBMISSION OF MANUSCRIPTS

A digital version of the Manuscript addressed –

Professor Witold Wacławek
Editorial Office of monthly *Ecological Chemistry and Engineering*
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Tel. +48 77 452 71 34, fax +48 77 455 91 49,
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The Editor assumes, that an author submitting a paper for publication has been authorised to do that. It is understood the paper submitted to be original and unpublished work, and is not being considered for publication by another journal. After printing, the copyright of the paper is transferred to *Towarzystwo Chemii i Inżynierii Ekologicznej* (*Society for Ecological Chemistry and Engineering*). In preparation of the manuscript please follow the general outline of papers published in the most recent issues of *Ecol. Chem. Eng*., a sample copy can be sent, if requested.

Papers submitted are supposed to be written in English language and should include a summary and keywords, if possible also in Polish language. If not then the Polish summary and keywords will be provided by the Editorial Office. All authors are requested to inform of their current addresses, phone and fax numbers and their email addresses.

It is urged to follow the units recommended by the *Systéme Internationale d'Unites* (SI). Graph axis labels and table captions must include the quantity units. The use of the following commonly applied expressions is recommended: mass – m/kg, time – t/s or t/min, current intensity – I/A; thermodynamic temperature – T/K, Celsius scale temperature – t/°C or θ/°C (if both time and Celsius scale units need to be used, the symbol θ/°C for temperature is to be taken) etc.

Symbols recommended by the International Union of Pure and Applied Chemistry (Pure and Appl. Chem., 1979, **51**, 1–41) are to be followed.

Graphics (drawings, plots) should also be supplied in the form of digital vector – type files, *e.g.* Corel-Draw, Grapher for Windows or at least in a bitmap format (TIF, PCK, BMP). In the case of any query please feel free to contact with the Editorial Office.

Footnotes, tables and graphs should be prepared as separate files.

References cited chronologically should follow the examples given below:

[l] Kowalski J. and Malinowski A.: Polish J. Chem. 1990, **40**(3), 2080–2085.

[2] Nowak S: Chemia nieorganiczna, WNT, Warszawa 1990.

[3] Bruns I., Sutter K., Neumann D. and Krauss G.-J.: *Glutathione accumulation – a specific response of mosses to heavy metal stress*, [in:] Sulfur Nutrition and Sulfur Assimilation in Higher Plants, P. Haupt (ed.), Bern, Switzerland 2000, 389–391.

Journal titles should preferably follow the Chem. Abst. Service recommended abbreviations.

Receipt of a paper submitted for publication will be acknowledged by email. If no acknowledgement has been received, please check it with the Editorial Office by email, fax, letter or phone.

ZALECENIA DOTYCZĄCE PRZYGOTOWANIA MANUSKRYPTÓW

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Redakcja Ecological Chemistry and Engineering
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ul. Oleska 48, 45–951 Opole
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Usilnie prosimy o stosowanie układu jednostek SI. Zwracamy uwagę, że osie wykresów oraz główki tabel powinny bezwzględnie zawierać jednostki stosownej wielkości. Polecamy symbolikę zalecaną przez PTChem (Symbole i terminologia wielkości i jednostek stosowanych w chemii fizycznej, Ossolineum, Wrocław 1989; Pure Appl. Chem. 1979, **51**, 1–41). Materiał graficzny (rysunki, wykresy), obok wersji na papierze, powinien również być dostarczony w postaci cyfrowych plików wektorowych, np. za pomocą programu: CorelDraw wersja 3.0–8.0, Grafer dla Windows lub przynajmniej bitowe (TIF, PCX, BMP). W przypadku trudności z wypełnieniem tego warunku Redakcja zapewnia odpłatne wykonanie materiału graficznego na podstawie dostarczonego szkicu, bliższe informacje można uzyskać telefonicznie 077 401 60 42.

Przypisy i tabele podobnie jak rysunki zapisujemy jako osobne pliki.

Literaturę prosimy zamieszczać wg poniższych przykładów:

[1] Kowalski J. and Malinowski A.: Polish J. Chem. 1990, **40**, 2080–2085.

[2] Nowak S.: Chemia nieorganiczna, WNT, Warszawa 1990.

[3] Bruns I., Sutter K., Neumann D. and Krauss G.-J.: *Glutathione accumulation – a specific response of mosses to heavy metal stress*, [in:] Sulfur Nutrition and Sulfur Assimilation in Higher Plants, P. Haupt (ed.), Bern, Switzerland 2000, 389–391.

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