Polish J. of Environ. Stud. Vol. 15, No. 6 (2006), 927-934

Original Research

The Effects of Copper on Soil Biochemical Properties and Its Interaction with Other Heavy Metals

J. Wyszkowska, J. Kucharski*, W. Lajszner

Department of Microbiology, University of Warmia and Mazury in Olsztyn, pl. Łódzki 3, 10-727 Olsztyn, Poland

Received: November 10, 2005
Accepted: June 23, 2006

Abstract

The effect of soil contamination with copper on soil biochemical properties and oat yields was assessed in a pot experiment. Copper was applied alone or in combination with other heavy metals. The study was conducted on samples of brown soil consisting of heavy loamy sand and brown soil developed from light silty clay. On days 28 and 56 of the experiment the following were determined: activity of dehydrogenases, urease, acid phosphatase and alkaline phosphatase in soil as well as oat yields.

Contamination of soil with copper, zinc, nickel, lead, cadmium and chromium in concentrations of 50 mg kg^{-1} was found to have a negative influence on the activity of dehydrogenases, urease, acid phosphatase, alkaline phosphatase and yield of oats. The soil enzymes can be arranged in terms of their sensitivity to heavy metals as follows: dehydrogenases > urease > alkaline phosphatase > acid phosphatase. Higher activity of dehydrogenases was determined in brown soil developed from heavy loamy sand, while urease, acid phosphatase and alkaline phosphatase were more active in brown soil formed from light silty clay. The contamination of copper with other heavy metals was inhibited in heavy loamy sand more than in light silty clay.

Keywords: acid phosphatase, alkaline phosphatase, dehydrogenases, heavy metals, oat yield, urease

Introduction

The biochemical activity of soil is affected by several factors, including the presence of heavy metals, which penetrate the soil from various sources and modify its properties [1-3]. Heavy metals are a serious problem for the whole ecosystem, but particularly for the organisms inhabiting a given environment. Heavy metals are taken up by plants and then passed on to subsequent links in the food chain [4-6]. Their toxicity and bioavailability are conditioned by the chemical form and the amounts in which they occur in the ecosystem [7-9], as well as some external factors such as temperature, oxidation-reduction potential, the presence of anions and cations of other metals and soil pH. Lower soil pH causes increased mobility and availability of heavy metals [10].

Soil contamination by heavy metals results in some changes in the counts of microorganisms and enzymatic activity which, according to Dick et al. [11], Kucharski [12] and Tresar-Cepeda et al. [13], is an objective measure of the soil's microbiological status. The negative influence of most of the heavy metals on the activity of soil enzymes was reported by Leiros et al. [7], Nowak and Smolik [14], Nowak et al. [15] and Wyszkowska and Kucharski [16].

Although there are many reports which prove that single metals are toxic to the biological activity of soil [11-13, 17, 18], few papers deal with the issue of interaction

---

*Corresponding author; e-mail: jan.kucharski@uwm.edu.pl

between metals and their effect on the enzymatic activity of soil. The present study has been conducted in order to determine the effect of soil contamination with copper interacting with other heavy metals on the biochemical properties of soil. Phytotoxic influence of heavy metals was determined on the basis of the biomass production of oats. Jurkowska et al. [19], Wyszkowska [20], and Wyszkowski and Wyszkowska [21] have demonstrated the negative effects of single heavy metals on oat yields but they did not study their interaction.

Materials and Methods

The experiments were carried out four times in polyethylene pots kept in a plant house. Two types of soil collected from the arable humus horizon were used for the trials. Under natural conditions these two types of soil are classified as Eutric Camisols according to WRB [22]. A more detailed description of the soils is presented in Table 1. The treated plant was cv. Bajka oats (12 plants per pot). Prior to the commencement of the trials studied, soil samples (each weighing 3 kg) were mixed with mineral fertilizers and heavy metals. The same ferilization level with macro- and microelements was applied to all the objects. Expressed as pure elements, the following quantities of fertilizers were added: 100 mg N as $CO(NH_2)_2$, 44 mg P as K_2HPO_4, 83 mg K as K_2HPO_4 + KCl, 20 mg Mg as $MgSO_4 \cdot 7H_2O$, 5 mg Zn as $ZnCl_2$, 5 mg Mn as $MnCl_2 \cdot 4H_2O$, 5 mg Mo as $Na_2MoO_4 \cdot 2H_2O$ and 0.33 mg B as H_3BO_4 per kg of soil. The soil samples were then contaminated with copper added at concentrations of 50 and 450 mg kg^{-1}. Copper like the second dose may be present in soils from the industrial district of Poland. The samples which received 450 mg kg^{-1} were treated with other heavy metals (Zn, Ni, Pb, Cd, Cr), each added at concentrations of 50 mg of pure element per kg of soil. Copper was used as $CuCl_2$, nickel as $NiCl_2 \cdot 6H_2O$, cadmium as $CdCl_2 \cdot 2.5H_2O$, chromium as $K_2Cr_2O_7$, lead as $PbCl_2$ and zinc as $ZnCl_2$. The following metals or combination of metals were compared in the experiment: Cu_{50}; Cu_{450}; Zn; Ni; Pb; Cd; Cr; Cu, Zn; Cu, Ni; Cu, Pb; Cu, Cd; Cu, Cr; Ni, Cr; Cu, Zn, Ni; Cu, Zn, Pb; Cu, Zn, Cd; Cu, Zn, Cr; Cu, Zn, Ni, Pb; Cu, Zn, Ni, Cd; Cu, Zn, Ni, Cr; Cu, Zn, Ni, Pb, Cd; Cu, Zn, Ni, Pb, Cr; and Cu, Zn, Ni, Pb, Cd, Cr. The effect produced by the heavy metals was verified by the control (unpolluted) samples.

The experiment was curried out in spring 2005 (May and June). Average daily temperature of these months were 11.5°C (from 7.5 to 19.6°C) and 13.1°C (from 10.1 to 17.7°C), respectively. Throughout the entire experimental

Table 1. Some physiochemical properties of the soils in the experiments.

Type of soil*	Granulometric composition (mm)			pH_{KCl}	$C_{organic}$ g kg^{-1}	CEC** mmol(+) kg^{-1}	BS %
	1-0.1	0.1-0.02	<0.02				
hls	66	17	17	6.9	7.5	100.5	88.9
lsc	42	32	26	7.0	11.2	167.8	94.7

* hls – heavy loamy sand, lsc – light silty clay, ** CEC – cation exchange capacity, BS – base saturation

Table 2. Activity of dehydrogenases (n = 96) in 1 kg d-m. of soil contaminated by heavy metals (cm^3 H_2 d^{-1}).

No	Elements*	Type of soil		Average
		hls	lsc	
1.	0	10.10	8.68	9.39
2.	Cu_{50}	7.89	6.24	7.07
3.	Zn	7.77	5.52	6.65
4.	Ni	9.08	5.22	7.15
5.	Pb	7.23	6.70	6.97
6.	Cd	5.99	4.35	5.17
7.	Cr	3.09	2.68	2.89
8.	Cu_{450}	0.00	0.00	0.00
$LSD_{0.01}$		a – 0.12; b – 0.04; a x b – 0.17		

* 50 mg rates of heavy metals per kg d.m. of soil in combinations 2 to 7; combination 8 contained 450 mg Cu; LSD for: a – contaminated object, b – type of soil

period, a constant soil humidity of 60% capillary water capacity was maintained.

On days 28 and 56 (oat harvest) the following measurements were performed three times on the soil samples: activity of dehydrogenases with TTC substrate [23], activity of urease according to Alef and Nannepieri [24] and the activities of acid phosphatase (Pac) and alkaline phosphatase (Pal) by the method described by Alef et al. [25]. The activity of dehydrogenases was expressed in cm^3 of H_2 needed to reduce TTC do TFP. The activity of urease in mg of N-NH_4 generated from the hydrolyzed urea and that of the phosphatases in mmols of p-nitrophenole (PNP) produced from sodium 4-nitrophenylophosphate. The results of the biochemical given in this paper as averages of the results obtained from two days of analyses.

The results were elaborated statistically using a two-factor analysis of variance Anova. Statistical analysis was performed with the aid of Statistica software [26].

Results

The activity of dehydrogenases in heavy loamy sand was significantly higher than in light silty clay (Table 2). In both soils the heavy metals, applied separately at a rate of 50 mg per kg d-m., reduced the activity of dehydrogenases. Complete inhibition of these enzymes occurred in an additional sample contaminated with 450 mg copper. This situation existed as well in the soil contaminated with copper (450 mg) together with other heavy metals

Table 3. Activity of urease (n = 276) in 1 kg d-m. of soil contaminated by heavy metals (mg N-NH_4 h^{-1}).

No	Elements*	Type of soil		Average
		hls	lsc	
1.	0	20.18	49.00	34.59
2.	Cu_{50}	13.69	31.52	22.61
3.	Zn	11.43	48.63	30.03
4.	Ni	14.15	27.48	20.82
5.	Pb	14.95	50.83	32.89
6.	Cd	15.72	41.05	28.39
7.	Cr	9.43	11.02	10.23
8.	Cu_{450}	4.54	5.83	5.19
9.	Cu, Zn	4.74	8.31	6.53
10.	Cu, Ni	5.78	6.92	6.35
11.	Cu, Pb	4.87	8.19	6.53
12.	Cu, Cd	4.66	8.56	6.61
13.	Cu, Cr	4.47	6.80	5.64
14.	Cu, Zn, Ni	4.57	13.07	8.82
15.	Cu, Zn, Pb	4.50	11.59	8.05
16.	Cu, Zn, Cd	4.84	10.16	7.50
17.	Cu, Zn, Cr	3.07	10.41	6.74
18.	Cu, Zn, Ni, Pb	4.48	15.13	9.81
19.	Cu, Zn, Ni, Cd	4.13	15.01	9.57
20.	Cu, Zn, Ni, Cr	4.31	9.90	7.11
21.	Cu, Zn, Ni, Pb, Cd	3.41	16.25	9.83
22.	Cu, Zn, Ni, Pb, Cr	2.90	11.16	7.03
23.	Cu, Zn, Ni, Pb, Cd, Cr	7.45	10.85	9.15
$LSD_{0.01}$		a – 0.48; b – 0.14; a x b – 0.67		

* 450 mg Cu per 1 kg d.m. of soil in combinations 8 to 23; the remaining heavy metals at the rate of 50 mg each in all the combinations; LSD for: a – contaminated object, b – type of soil

(50 mg each). Due to 100% inhibition of the activity of dehydrogenases in the other samples, these results were not presented in the table.

Regardless of soil type, the strongest inhibitors of dehydrogenases were hexavalent chromium and cadmium, while nickel produced the weakest effect on these enzymes. With regards to their inhibitory effect on the activity of dehydrogenases, the elements can be arranged as follows: Cr(VI)>Cd>Zn>Pb>Cu>Ni. Although there were some small differences between the two types, e.g. in heavy loamy sand the activity of dehydrogenases was least inhibited by nickel, whereas in light silty clay – the weakest inhibitory effect was produced by lead.

Unlike dehydrogenases, the activity of urease was 2.4 times higher in light silty clay than in heavy loamy sand (Table 3). Depending on the type of soil, the heavy metals applied at a dose of 50 mg per kg d-m. produced varied effects on the urease. In light silty clay neither zinc nor lead affected the activity of this enzyme. In contrast, all the metals influenced the activity of urease in heavy loamy sand, although the effect produced by cadmium was weakest. Nonetheless, the average results for both soils indicate that the heavy metals used for the experiment can be ranked in the following order: Cr(VI)>Ni>Cu>Cd>Zn>Pb in terms of their inhibitory effect on urease.

The inhibitory effect of copper on urease was stronger when the contamination dose was increased from 50 to 450 mg per kg d-m., thus exciding even the effect produced by chromium applied in the amount of 50 mg per kg d-m. of soil.

The higher dose of copper applied in conjunction with another heavy metal, i.e. zinc, nickel, cadmium or chro-

Table 4. Activity of acid phosphatase (n = 276) in 1 kg d-m. of soil contaminated by heavy metals (mmol PNP h^{-1}).

No	Elements*	Type of soil		Average
		hls	lsc	
1.	0	1.73	1.89	1.81
2.	Cu_{50}	1.55	2.08	1.82
3.	Zn	1.62	2.07	1.85
4.	Ni	1.66	1.94	1.80
5.	Pb	1.61	2.06	1.84
6.	Cd	1.47	2.18	1.83
7.	Cr	1.30	1.55	1.43
8.	Cu_{450}	0.71	1.04	0.88
9.	Cu, Zn	0.71	0.87	0.79
10.	Cu, Ni	0.69	0.82	0.76
11.	Cu, Pb	0.68	0.97	0.83
12.	Cu, Cd	0.69	0.84	0.77
13.	Cu, Cr	0.72	0.93	0.83
14.	Cu, Zn, Ni	0.67	1.06	0.87
15.	Cu, Zn, Pb	0.71	0.96	0.84
16.	Cu, Zn, Cd	0.67	0.93	0.80
17.	Cu, Zn, Cr	0.67	1.16	0.92
18.	Cu, Zn, Ni, Pb	0.67	1.18	0.93
19.	Cu, Zn, Ni, Cd	0.66	0.77	0.72
20.	Cu, Zn, Ni, Cr	0.67	1.03	0.85
21.	Cu, Zn, Ni, Pb, Cd	0.71	1.13	0.92
22.	Cu, Zn, Ni, Pb, Cr	0.64	1.11	0.88
23.	Cu, Zn, Ni, Pb, Cd, Cr	0.76	1.11	0.94
$LSD_{0.01}$		a – 0.48; b – 0.14; a x b – 0.67		

[*] explanations given under Table 3

mium, did not result in any further inhibition of the activity of urease. In contrast to this, the addition of nickel to heavy loamy sand polluted with 450 mg copper per kg d-m. of soil reduced the inhibitory effect produced by the copper on the activity of urease. In light silty clay all the heavy metals added to copper contaminating soil resulted in such effects. In light silty clay the activity of urease was inhibited to a lesser extent when copper was added in combination with two or even three other heavy metals. In light silty clay where the soil was polluted with 450 mg Cu and 50 mg of Zn, Ni, Pb and Cr, the urease was even more active than in the same type of soil contaminated exclusively by copper. No such interaction was observed in heavy loamy sand and the total contamination with Cu, Zn, Cr; Cu, Zn, Ni, Pb, Cd or Cu, Zn, Ni, Pb, Cr strengthened the adverse effect of copper on urease.

Acid phosphatase and alkaline phosphatase turned out to be the most tolerant to soil contamination by copper or other heavy metals. Although these enzymes were also adversely affected by the heavy metals used in the trials, the inhibition of their activity was not as strong as in the case of dehydrogenases or urease.

The activity of acid phosphatase in light silty clay, like that of urease, was higher than in heavy loamy sand (Table 4). Enzyme activity in light silty clay was stimulated by all the heavy metals (at concentrations of 50 mg per kg d-m.) except chromium, which strongly inhibited it under the same conditions. In heavy loamy sand all the metals tested produced an inhibitory effect on acid phosphatase, with hexavalent chromium being the strongest inhibitor.

When the level of soil contamination by copper was raised from 50 to 450 mg per kg d-m. of soil, some sig-

Table 5. Activity of alkaline phosphatase (n = 276) in 1 kg d-m. of soil contaminated by heavy metals (mmol PNP h^{-1})

No	Elements*	Type of soil		Average
		hls	lsc	
1.	0	1.90	2.81	2.36
2.	Cu_{50}	1.71	2.73	2.22
3.	Zn	1.90	2.86	2.38
4.	Ni	1.58	2.49	2.04
5.	Pb	1.86	3.49	2.68
6.	Cd	1.13	2.40	1.77
7.	Cr	1.81	2.93	2.37
8.	Cu_{450}	0.45	0.69	0.57
9.	Cu, Zn	1.46	0.85	1.16
10.	Cu, Ni	0.44	0.66	0.55
11.	Cu, Pb	0.43	0.84	0.64
12.	Cu, Cd	0.39	0.68	0.54
13.	Cu, Cr	0.48	1.07	0.78
14.	Cu, Zn, Ni	0.39	1.40	0.90
15.	Cu, Zn, Pb	0.40	0.96	0.68
16.	Cu, Zn, Cd	0.34	0.85	0.60
17.	Cu, Zn, Cr	0.40	1.34	0.87
18.	Cu, Zn, Ni, Pb	0.38	1.38	0.88
19.	Cu, Zn, Ni, Cd	0.31	1.17	0.74
20.	Cu, Zn, Ni, Cr	0.37	1.39	0.88
21.	Cu, Zn, Ni, Pb, Cd	0.32	1.20	0.76
22.	Cu, Zn, Ni, Pb, Cr	0.41	1.38	0.90
23.	Cu, Zn, Ni, Pb, Cd, Cr	0.54	1.05	0.80
$LSD_{0.01}$		a – 0.48; b – 0.14; a x b – 0.67		

[*] explanations given under table 3

nificant inhibition of the activity of acid phosphatase was observed (Table 4), though this was weaker than in the case of the dehydrogenases or urease. The response of acid phosphatase to aggregated contamination by copper and zinc, copper and nickel, copper and cadmium or copper and chromium was completely different than that of urease. Soil pollution with copper in conjunction with one of the above-mentioned elements increased the inhibitory effect in light silty clay, but had no such effect in heavy loamy sand. In the latter type of soil the activity of acid phosphatase was not modified even when the pool of polluting elements increased. There were only two mixtures of heavy metals (Cu, Zn, Ni, Cd and Cu, Zn, Ni, Pb, Cd) that increased the inhibition of acid phosphatase relative to copper which was applied at 450 mg per kg d-m. On the other hand, when more heavy metals were added to the pool of pollutants to that which the light silty clay soil samples were subjected to, in some cases the inhibitory effect of soil contamination by copper was lessened by Cu, Zn, C; Cu, Zn, Ni, Pb; Cu, Zn, Ni, Pb, Cd; Cu, Zn, Ni, Pb, Cr and Cu, Zn, Ni, Pb, Cd, Cr.

The activity of alkaline phosphatase (Table 5), like that of acid phosphatase and urease, was higher in light silty clay than in heavy loamy sand. In both types of soil the activity of this enzyme was significantly depressed by Cu, Ni and Cd applied separately at a level of 50 mg per kg d-m. The strongest negative effect was produced by cadmium, and the weakest by copper. Zinc and lead did not inhibit the activity of alkaline phosphatase, and hexavalent chromium did so only in the lighter soil.

When the amounts of the contaminants were raised from 50 to 450 mg per kg d-m., a nearly four-fold decrease

Table 6. Oat yields (n = 184) on soils contaminated by heavy metals (g d-m. per pot).

No	Elements*	Type of soil		Average
		hls	lsc	
1.	0	18.61	19.57	19.09
2.	Cu_{50}	16.03	14.96	15.50
3.	Zn	14.01	15.36	14.69
4.	Ni	14.24	16.16	15.20
5.	Pb	16.18	13.30	14.74
6.	Cd	13.25	14.35	13.80
7.	Cr	15.71	10.40	13.06
8.	Cu_{450}	1.74	1.12	1.43
9.	Cu, Zn	1.62	0.71	1.17
10.	Cu, Ni	0.95	0.57	0.76
11.	Cu, Pb	1.52	0.88	1.20
12.	Cu, Cd	2.57	0.80	1.69
13.	Cu, Cr	1.76	1.58	1.67
14.	Cu, Zn, Ni	0.79	0.71	0.75
15.	Cu, Zn, Pb	1.09	1.01	1.05
16.	Cu, Zn, Cd	1.65	0.89	1.27
17.	Cu, Zn, Cr	1.58	2.08	1.83
18.	Cu, Zn, Ni, Pb	0.71	3.37	2.04
19.	Cu, Zn, Ni, Cd	0.52	4.68	2.60
20.	Cu, Zn, Ni, Cr	1.10	2.66	1.88
21.	Cu, Zn, Ni, Pb, Cd	0.92	3.83	2.38
22.	Cu, Zn, Ni, Pb, Cr	1.27	2.92	2.10
23.	Cu, Zn, Ni, Pb, Cd, Cr	0.56	1.96	1.26
$LSD_{0.01}$		a – 9.87; b – 2.91; a x b – 13.96		

* explanations given under Table 3

in the activity of alkaline phosphatase was recorded. Further addition of subsequent heavy metals to soil decreased the negative effect of copper on the alkaline phosphatase in light clay, and this mitigating influence was more evident as the number of heavy metals applied together increased. In the heavy loamy sand a similar effect occurred only when Cu, Zn or Cu, Zn, Ni, Pb, Cr were applied. In the remaining samples copper used with other elements attenuated the alkaline phosphatase activity to on even greater extent.

Soil contamination by copper and other heavy metals had an unfavourable effect on the enzymatic activity of soil as well as the growth and development of oats (Table 6). It was observed that the copper began to exhibit a negative effect on the soils at a concentration of 50 mg kg^{-1}, and when this rose to 450 mg kg^{-1} the oat yield dropped by 90% in both soils. All the heavy metals applied together with copper decreased the oat yield. In samples where copper was applied together with only one more heavy metal, the toxicity of the applied mixtures decreased in the following order: Cu, Ni >Cu, Pb >Cu, Zn>Cu, Cr>Cu, Cd. When two more metals were added, the decreasing order was as follows: Cu, Zn, Ni >Cu, Zn, Pb>Cu, Zn, Cd >Cu, Zn, Cr. Using three additional metals, the combinations can be arranged in decreasing order as Cu, Zn, Ni, Cr>Cu, Zn, Ni, Pb>Cu, Zn, Ni, Cr.

Discussion

The negative effect of heavy metals on the activity of enzymes, which was observed in the present trials, had also been confirmed in our previous studies [16, 21, 27] and verified in literature [14]. Furthermore Welp [17] claims that copper, along with chromium VI and III belong to metals which have a strong adverse effect on the activity of enzymes.

Differences between the soils in the response to heavy metals stem mainly from soil grain-size distribution and sorptive complex exchange capacity (Table 1). Both of these soil characteristics point to higher buffer properties for the light silty clay than for the heavy loamy sand, which is not indifferent to the effect produced by the heavy metals used in the experiments on the activity of soil enzymes.

Such reverse influence of urease and phosphatases contamination by copper and other heavy metals in the two types of soil analyzed may be as a result of different sorptive properties of the soils. This assumption can be inferred from earlier reports [3]. The energy of sorption and desorption of particular cations may be involved, which may possibly explain the lack of aggregate influence on the enzymes by the combined heavy metals, especially in light soil. This, however, is not always confirmed by the results reported in some references [28].

Leiros et al. [7] found out that copper needed to be applied at concentrations as high as 800 mg kg^{-1} to produce an unfavourable effect on the activity of urease. This is in contrast to the present study, where a concentration of 50 mg of copper per kg d-m. of soil was able to inhibit the urease, irrespective of soil type.

The effect that heavy metals actually exert depends on their quantities in soil. When occurring at amounts close to naturally occurring levels, they can stimulate soil enzymes. However, if present in excessive amounts heavy metals become inhibitors of soil enzymatic activity. The inhibition of soil enzymes by heavy metals is not a cumulative total of their separate effects but rather a combined influence, and indeed some effects of the heavy metals can partly cancel each other out. As a result, a higher number and variation of heavy metals could be less toxic than a single metal, as has been demonstrated in our experiment with copper contamination.

Conclusions

1. Soil contamination by copper, zinc, nickel, lead, cadmium and chromium added in at concentration of 50 mg kg^{-1} produced negative effects on the activity of dehydrogenases, urease, acid phosphatase, alkaline phosphatase and oat yields.
2. Soil contamination by copper only may be much more inhibitory to soil enzyme activity than in conjunction with other heavy metals.
3. With regards to the tolerance of soil enzymes to heavy metals, the former may be arranged in the following order: dehydrogenases > urease > alkaline phosphatase > acid phosphatase.
4. Higher activity of dehydrogeanses was determined in brown soil developed from heavy loamy sand, while the activity of urease, acid phosphatase and alkaline phosphatase was higher in brown soil developed from light silty clay.

References

1. HUANG Q., SHINDO H. Effect of copper on the activity and kinetics of free and immobilized acid phopshatase. Soil Biol. Biochem. **32**, 1885, **2000**.
2. KHAN M., SCULLION J. Effect of metal (Cd, Cu, Ni, Pb or Zn) enrichment of sewage – sludge on soil micro – organism and their activities. Appl. Soil Ecol. **20**, 145, **2002**.
3. KUCHARSKI J., WYSZKOWSKA J. Intern-relationship between number of microorganisms and spring barley yield and degree of soil contamination with copper. Plant Soil Environ. **50**(6), 243, **2004**.
4. BARAŁKIEWICZ D., SIEPAK J. Chromium, nickel and cobalt in environmental samples and existing legal forms. Polish J. Environ. St. **8**(4), 4, 201, **1999**.
5. CIEĆKO Z., KALEMBASA S., WYSZKOWSKI M., ROLKA E. Effect of soil contamination by cadmium on potassium uptake by plants. Polish J. Environ. St. **13**(2), 333, **2004**.
6. KRÓLAK E. Accumulation of Zn, Cu, Pb and Cd by dandelion (Taraxacum officinale Web.) in environments with various degrees of metallic contamination. Polish J. Environ. St. **12**(6), 713, **2003**.

7. LEIROS M.C., TRASAR – CEPEDA C., GARCIA – FERNANDEZ F., GIL – SOTRES F. Defining the validity of a biochemical index of soil quality. Biol. Fertil. Soils. **30**, 140, **1999**.
8. LOSKA K., WIECHUŁA D. Effects of pH and aeration on copper migration in above – sediment water. Pol. J. Environ. St. **9(**5), 433, **2000**.
9. WYSZKOWSKA J., WYSZKOWSKI M. Effect of cadmium and magnesium on enzymatic activity in soil. Polish J. Environ. St. **12**(4), 473, **2003**.
10. SŁABA M., DŁUGOŃSKI J. Microbiological removal and recovery of heavy metals. Post. Mikrobiol. **41(**2), 167, **2002**. (in Polish)
11. DICK W.A., CHENG L., WANG P. Soil acid and alkaline phosphatase as pH adjustment indicators. Soil Biol. Biochem. **32**, 1915, **2000**.
12. KUCHARSKI J. Relacje między aktywnością enzymów a żyznością gleby. Relationships between enzymatic activity and soil fertility. In, Microorganisms in the environment, occurrence, activity and meaning. AR Kraków (red. W. Barabasz), 327, **1997** (in Polish).
13. TRASAR – CEPEDA C., LEIROS M.C., SEOANE S., GIL – SOTRES F. Limitations of soil enzymes as indicators of soil pollution. Soil Biol. Biochem. **32**, 1867, **2000**.
14. NOWAK J., SMOLIK B. Influence of cuprum (II) nitrate (V) and lead (II) nitrate (V) on the soil enzymes activity. Rocz. Gleb. **53**, (3/4), 85, **2002** (in Polish).
15. NOWAK J., SZYMCZAK J., SŁOBODZIAN T. An attempt at the determination of a 50% toxicity threshold for rates of different heavy metals towards soil phosphatases. Zesz. Prob. Nauk Rol. **492**, 241, **2003** (in Polish).
16. WYSZKOWSKA J., KUCHARSKI J. Biochemical and physicochemical properties of soil contaminated with the heavy metals. Zesz. Prob. Nauk Rol. **492**, 435, **2003** (in Polish).
17. WELP G. Inhibitory effects of the total and water – soluble concentrations of nine different metals on the dehydrogenase activity of a loess soil. Biol. Fertil. Soils. **30**, 132.1**999**.
18. WYSZKOWSKA J., KUCHARSKI J., LAJSZNER W. Enzymatic activities in different soils contaminated with copper. Polish J. Environ. Stud., **14**(5), 119, **2005**.
19. JURKOWSKA H., ROGOŻ A., WOJCIECHOWICZ T. Interactive influence of big doses of Cu, Zn, Pb and Cd on their uptake by plants. Pol. J. Soil Sc. **29(**1), 73, **1996**.
20. WYSZKOWSKA J. Soil contaminatiom by chromium and its enzymatic activity and yielding. Polish J. Environ. Stud. **11**, 79, **2002**.
21. WYSZKOWSKI M., WYSZKOWSKA J. The effect of soil contamination with zinc, nickel, copper and lead on the yield and macroelements distribution in oats. J. Elementol., **9**(1), 61, **2004**. (in Polish).
22. WORD REFERENCE BASE FOR SOIL RESOURCES FAO. Rome. World Soil Resources. Reports **84**, pp 91, **1998**.
23. ÖHLINGER R. Dehydrogenase Activity with the Substrate TTC. [In] Methods in Soil Biology. (Eds) Schinner F., öhlinger R., Kandeler E., Margesin R., Springer Verlag Berlin Heidelberg, pp. 241, **1996**.
24. ALEF K., NANNIPIERI P. Urease activity. in: Methods in Applied Soil Microbiology and Biochemistry. ALef K., Nannipieri P. (eds), Academic press. Harcourt Brace & Company, Publishers, London, pp. 316, **1998**.
25. ALEF K., NANNIPIERI P., TRASAR-CEPEDA C. Phosphatase activity. in: Methods in Applied Soil Microbiology and Biochemistry. ALef K., Nannipieri P. (eds), Academic Press. Harcourt Brace & Company, Publishers, London, pp. 335, **1998**.
26. STATSOFT, INC. STATISTICA (data analysis software system), version 6. www.statsoft.com, **2003**.
27. WYSZKOWSKA J. Biological properties of soil contaminated with hexavalent chromium. Wyd. UWM, Rozprawy i monografie, **65**, 1, **2002** (in Polish).
28. PASZKO T. Studies of competitive Cu^{2+}, Co^{2+} and Cr^{3+} sorption in grey-brown podzolic soils. Polish J. Environ. St. **12** (4), 439, **2003**.