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SENSITIVITY OF SOIL ENZYMES TO EXCESSIVE ZINC CONCENTRATIONS*

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Abstract

The sensitivity of soil enzymes to soil contamination with zinc was analyzed. A laboratory experiment was performed on sandy loam at pH 7.0, sampled from arable land at a depth of 0 to 20 cm. Soil samples were passed through a sieve with 2 mm mesh size and contaminated with the following zinc doses: 0, 300, 600, 1200 and 2400 mg Zn^{2+} kg^{-1} soil. Zinc was applied in the form of aqueous solution of $ZnCl_2$. Soil was mixed thoroughly with zinc, and its moisture content was brought to 50% capillary water capacity. The samples were incubated at 25°C. Beakers with soil samples were weighed once a week to replenish evaporated water. The activity of soil enzymes: dehydrogenases, urease, acid phosphatase, alkaline phosphatase, catalase, arylsulfatase and β-glucosidase, was determined after 15, 30, 60 and 120 days of the experiment. The results were used to calculate soil resistance (RS), ED_{20} and ED_{50} values.

The results of the study indicate that soil enzymes are characterized by varied sensitivity to excessive zinc concentrations, and that the RS index is a reliable measure of enzymatic responses to zinc pollution. The analyzed enzymes were classified in the following decreasing order in terms of their resistance to zinc: β-glucosidase> acid phosphatase > urease >arylsulfatase = alkaline phosphatase> catalase > dehydrogenases. Zinc continued to exert a negative effect on soil enzymes throughout the experiment (120 days). ED_{20} values for the analyzed enzymes in mg Zn^{2+} kg^{-1} DM soil were determined at: 103 for dehydrogenases, 184 for alkaline phosphatase, 233 for urease, 247 for arylsulfatase, 416 for acid phosphatase, 419 for catalase and 1373 for β-glucosidase.

Key words: soil enzymes, zinc, ED_{20}, ED_{50}, soil resistance (RS) index, soil contamination.

OPORNOŚĆ ENZYMÓW GLEBOWYCH NA NADMIERNE ILOŚCI CYNKU

Abstrakt

Celem badań było określenie wrażliwości enzymów na zanieczyszczenie gleby cynkiem. Doświadczenie przeprowadzono w warunkach laboratoryjnych na glinie piaszczystej o pH 7,0, pobranej z użytku rolnego z warstwy od 0 do 20 cm. Przed rozpoczęciem badań glebę przesiano przez sito o oczkach 2 mm i zanieczyszczono następującymi dawkami cynku: 0, 300, 600, 1200, 2400 mg Zn^{2+} kg^{-1} gleby. Cynk stosowano w postaci

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wodnego roztworu $ZnCl_2$. Następnie po dokładnym wymieszaniu gleby jej wilgotność doprowadzono do 50% kapilarnej pojemności wodnej i poddano inkubacji w cieplarce w temp. 25°C. Jeden raz w tygodniu zlewki przeważano w celu uzupełnienia ewentualnych ubytków wody. Po 15, 30, 60 i 120 dniach inkubacji część zlewek likwidowano i oznaczono aktywność enzymów glebowych: dehydrogenaz, ureazy, fosfatazy kwaśnej, fosfatazy alkalicznej, katalazy, arylosulfatazy i β-glukozydazy. Na podstawie wyników obliczono indeks oporności enzymów (RS) oraz wskaźniki ED_{20} i ED_{50}.

Stwierdzono, że enzymy glebowe mają zróżnicowaną oporność na nadmiar cynku w glebie, a wskaźnik oporności RS jest dobrą miarą ich reakcji na zanieczyszczenie tym metalem. Pod względem zmniejszającej się oporności można je uszeregować następująco: β-glukozydaza> fosfataza kwaśna > ureaza >arylosulfataza = fosfataza kwaśna > katalaza > dehydrogenazy. Negatywne działanie cynku na enzymy glebowe utrzymywało się przez cały okres badań (120 dni).Wartość ED_{20} dla poszczególnych enzymów w mg Zn^{2+} kg^{-1}s.m. gleby wynosiła: dehydrogenazy – 103, fosfataza alkaliczna – 184, ureaza – 233, arylosulfataza – 247, fosfataza kwaśna – 416, katalaza – 419, β-glukozydaza – 1373.

Słowa kluczowe: enzymy glebowe, cynk, ED_{20},ED_{50}, wskaźnik oporności (RS), zanieczyszczenie.

INTRODUCTION

There is a growing body of evidence that intensive farming (Wang et al. 2013) and industrial development (Kucharski et al. 2011) contribute to soil contamination with heavy metals (Kucharski et al. 2000, Wyszkowska et al. 2001, Gillet, Ponge 2002, Kucharski, Wyszkowska 2004, Seifert, Domka 2005, Wyszkowska et al. 2007, Kucharski et al. 2009). In recent years, Roca-Perez et al. (2010) have observed increasing levels of heavy metal contamination in farmland. Trace element deposition in soil can result from anthropogenic factors and from natural accumulation. Polluted wastewater and air are potential sources of heavy metal contamination in the soil environment (Chary et al. 2008, Huang et al. 2009, Sienkiewicz, Czarneckaet al. 2012, Glina, Bogacz 2013, Wang et al. 2013).

Soil enzymes, in particular intracellular enzymes produced by microorganisms (Nielsen, Winding 2002, Kucharski et al. 2011), are robust indicators of changes in soil quality. They participate in the biogeochemical cycling of elements (Nielsen, Winding 2002, Kucharski, Wyszkowska 2004, Wyszkowska et al. 2009, Wyszkowski, Wyszkowska 2009), including organic matter transformation (Wang et al. 2013). Carbon, nitrogen and phosphorus cycling enzymes as well as oxidoreductases are important indicators of soil quality (Bielińska, Żukowska 2002, Rodriguez et al. 2004, Bielińska et al. 2005, Wyszkowska et al. 2010, Qu et al. 2011).

Heavy metals are slowly transformed in the environment (Adriano et al. 2004). High concentrations of hazardous substances and microelements in soil can exert toxic effects on soil microorganisms (Bruins et al. 2000, Jonak et al. 2004) by inducing changes in enzyme activity or the population size of soil-dwelling microbes (Szymańska-Pulikowska 2012, Wyszkowska et al. 2013), which are the main source of soil enzymes. Enzyme activity levels are indicative of the structure and function of microbial communities (Brockett et al. 2012). Wang et al. (2012) observed that in contaminated environments, some bacteria regulate gene expression inside a molecule, which leads to rapid exchange and reduces free zinc concentrations in soil.

Heavy metal absorption by plants and microorganisms is determined by soil's sorption capacity, organic matter content and pH. HUANG et al. (2009) and ZHAO et al. (2010) observed elevated zinc concentrations in industrial regions as well as in rice fields, which could have very serious implications for consumer health.

The objective of this study was to determine the sensitivity of soil enzymes to soil contamination with zinc.

MATERIALS AND METHODS

Experimental design

A laboratory experiment was performed on samples of sandy loam at pH 7.0, which are characterized in Table 1. Soil was passed through a sieve with 2 mm size mesh before analysis. 100 cm^3 beakers were filled with 100 g of air-dried soil each. Soil samples were contaminated with zinc doses of 0, 300, 600, 1200 and 2400 mg Zn^{2+} kg^{-1} soil, applied in the form of $ZnCl_2$. The soil was mixed thoroughly with zinc, and its moisture content was brought to 50% capillary water capacity. The samples were incubated at 25°C. Beakers were weighed once a week to replenish evaporated water. After 15, 30, 60 and 120 days of the experiment, three beakers from every treatment were emptied to determine the activity of soil enzymes: dehydrogenases, urease, acid phosphatase, alkaline phosphatase, catalase, arylsulfatase and β-glucosidase. The results were used to calculate ED_{20} and ED_{50} values, and the indicators of soil resistance (RS) and soil resilience (RL).

Soil enzymes

Activity of dehydrogenases was determined by the method proposed by ÖHLINGER (1996). Soil samples were incubated at 37°C for 24 hours using 3% aqueous solution of

Table 1

The physicochemical and chemical properties of soil

Property	Loamy sand
Soil texture (μm)	g kg^{-1}
2000 -50	720
50 - 2	210
2 < 0	70
pH_{KCl}	7.0
	mmol(+) kg^{-1}
Hydrolytic acidity (HAC)	16.05
Sum of exchangeable bases Ca^{++}, Mg^{++}, K^+, and Na^+ (TEB)	75.0
Cation exchange capacity (CEC)	91.05
Base saturation (BS) %	82.30
	g kg^{-1}
$C_{organic}$	7.05
N_{total}	0.67
	mg kg^{-1}
Zn_{total}	16.60

2,3,5-triphenyl tetrazolium chloride (TTC) as the substrate. The absorbance of triphenylformazan (TPF) was measured in a Perkin-Elmber Lambda 25 spectrophotometer at 485 nm. The activity of dehydrogenases was expressed in μmol TPF kg^{-1} d.m. h^{-1}.

The catalase activity was determined according to ALEF and NANNPIERI (1998) by measuring the volume of potassium permanganate which was used up during titration by the decomposition of hydrogen peroxide to water and oxygen. The results were given in mol O_2 kg^{-1} d.m. soil h^{-1}.

The activity of urease, acid phosphatase, alkaline phosphatase, β-glucosidase and arylsulfatase was determined in accordance with the procedures proposed by ALEF and NANNPIERI (1998). In the analysis of urease activity, soil was incubated with 10% aqueous solution of urea as the substrate. The quantity of produced N-NH_4 was determined with the use of the Nessler's reagent. The absorbance of ammoniated mercury iodide was measured in a Perkin-Elmber Lambda 25 spectrophotometer at 410 nm. Urease activity was expressed in mmol N-NH_4 kg^{-1} d.m. soil h^{-1}. Soil samples were incubated with 4-Nitrophenyl β-D-glucopyranoside(PNG) to determine β-glucosidase activity, with 4-Nitrophenyl phosphate disodium to measure the activities of acid phosphatase and alkaline phosphatase, and with potassium 4-Nitrophenyl sulfate (PNS) to determine arylsulfatase activity. 4-Nitrophenol (PNP) was the catalysis product for all enzymes, and its absorbance was measured in the Perkin-Elmber Lambda 25 spectrophotometer at 400-420 nm. The results were expressed in mmol PNP kg^{-1} d.m. soil h^{-1}.

Soil resistance (RS)

The soil resistance (RS) index was calculated using the formula proposed by ORWIN and WARDLE (2004):

$$RS = 1 - \frac{2\left|D_0\right|}{C_0 + \left|D_0\right|},$$

$D_0 = C_0 - P_0$,
C_0 – parameter value in control (uncontaminated) soil over time t_0,
P_0 – parameter value in disturbed (contaminated) soil over time t_0.

Statistical analysis

The homogeneity of variance was estimated by the Tukey's test at $p = 0.01$. Pearson's correlation coefficients between the degree of soil contamination with zinc and enzyme activity were calculated. The effect of zinc on the activity of soil enzymes was also evaluated by principal component analysis (PCA). Statistical analyses were performed with the use of Statistica 10.0 software (StatSoft, 2012). Zinc doses which induced a 20% (ED_{20}) and 50% (ED_{50}) decrease in the activity levels of dehydrogenases, catalase, urease, β-glucosidase, acid phosphatase, alkaline phosphatase and arylsulfatase were determined.

RESULTS AND DISCUSSION

According to many authors (Quet al. 2011, Melgar-Ramírez et al. 2012), a heavy metal concentration is the most important indicator of soil quality. The main anthropogenic sources of environmental pollution are mining, transportation, heavy and light industries which contaminate local ecosystems. Qu et al. (2011) observed that heavy metals exert a negative effect on the activity of soil enzymes. In the cited study, the activities of all examined enzymes decreased with an increase in pollution levels. A similar trend was noted in our study (Table 2), where the activities of all enzymes were negatively corre-

Table 2

Enzyme activity in soil contaminated with zinc (kg^{-1} d.m. h^{-1})

Dose Zn^{2+} (mg kg^{-1} of soil d.m.)	Enzyme*						
	Deh (µmol TFF)	Kat (mol O_2)	Glu (mmol PNP)	Ure (mmol N-NH_4)	Pac (mmol PNP)	Pal (mmol PNP)	Aryl (mmol PNP)
15 day							
0	20.291^{b}	0.210^{a}	0.755^{a}	0.725^{b}	0.812^{ab}	1.678^{a}	0.191^{a}
300	11.232^{d}	0.183^{c}	0.716^{a}	0.579^{c}	0.691^{cd}	1.131^{c}	0.153^{b}
600	5.903^{f}	0.157^{e}	0.646^{b}	0.388^{d}	0.632^{de}	0.827^{e}	0.118^{d}
1200	1.790^{h}	0.096^{gh}	0.588^{bc}	0.176^{gh}	0.501^{fg}	0.590^{f}	0.070^{hi}
2400	0.412^{ij}	0.060^{k}	0.505^{d}	0.175^{f}	0.328^{hi}	0.325^{g}	0.030^{ll}
r	-0.834	-0.965	-0.973	-0.864	-0.984	-0.895	-0.958
30 day							
0	21.494^{a}	0.195^{b}	0.575^{c}	0.808^{a}	0.893^{a}	1.481^{b}	0.137^{c}
300	12.057^{c}	0.178^{c}	0.536^{cd}	0.577^{c}	0.743^{bcd}	0.956^{d}	0.085^{efg}
600	5.877^{f}	0.155^{e}	0.494^{de}	0.384^{d}	0.476^{fg}	0.827^{e}	0.075^{gh}
1200	1.877^{h}	0.092^{hi}	0.414^{f}	0.114^{h}	0.399^{gh}	0.523^{f}	0.041^{kl}
2400	0.437^{ij}	0.027^{l}	0.392f^{gh}	0.164^{fg}	0.315^{hi}	0.318^{g}	0.031^{ll}
r	-0.829	-0.99^{l}	-0.928	-0.827	-0.866	-0.898	-0.868
60 day							
0	20.660^{b}	0.204^{a}	0.490^{de}	0.822^{a}	0.832^{ab}	1.455^{b}	0.120^{d}
300	11.977^{c}	0.168^{d}	0.539^{cd}	0.545^{c}	0.762^{bc}	0.964^{d}	0.091^{ef}
600	5.837^{f}	0.131^{f}	0.440^{ef}	0.332^{e}	0.564^{ef}	0.828^{e}	0.080^{fgh}
1200	1.624^{h}	0.083^{j}	0.410^{fg}	0.109^{h}	0.495^{fg}	0.517^{f}	0.052^{jk}
2400	0.368^{ij}	0.018^{l}	0.352^{gh}	0.153^{fgh}	0.178^{j}	0.315^{g}	0.040^{kl}
r	-0.835	-0.982	-0.897	-0.806	-0.983	-0.906	-0.922
120 day							
0	9.429^{e}	0.135^{f}	0.416^{f}	0.567^{c}	0.757^{bc}	1.441^{b}	0.098^{e}
300	6.049^{f}	0.101^{g}	0.482^{de}	0.294^{e}	0.751^{bcd}	0.817^{e}	0.059^{ij}
600	2.480^{g}	0.086^{hj}	0.406^{fg}	0.299^{e}	0.535^{ef}	0.544^{f}	0.051^{jk}
1200	0.694^{i}	0.055^{k}	0.331^{h}	0.119^{gh}	0.353^{hi}	0.320^{g}	0.021^{lm}
2400	0.113^{j}	0.015^{l}	0.265^{i}	0.143^{fgh}	0.24^{ij}	0.32^{g}	0.014^{m}
r	-0.837	-0.975	-0.909	-0.776	-0.937	-0.774	-0.875

* Deh – dehydrogenases, Kat – catalase, Glu–β-glucosidase, Ure – urease, Pac – acid phosphatase, Pal – alkaline phosphatase, Aryl – arylosulphatase;
Enzymes marked with the same letter form a homogeneous group.

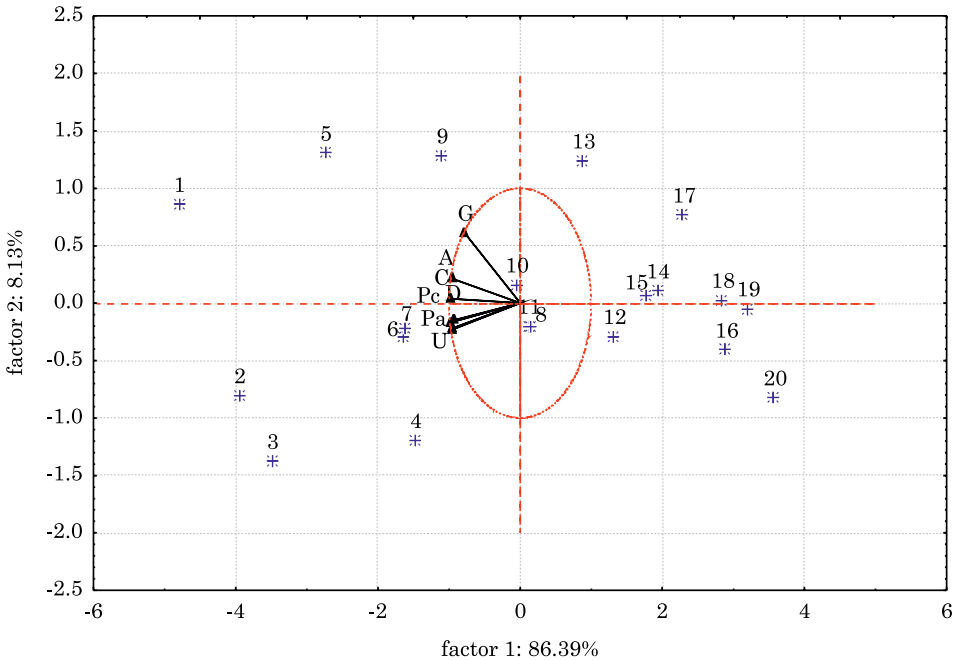

Fig. 1. Enzyme activity in loamy sand contaminated with zinc – the PCA method.

Vectors represent the analyzed variables: D – dehydrogenases, C – catalase, U – urease, Pc – acid phosphatase, Pa – alkaline phosphatase, G – b-glucosidase, a – arylosulphatase

1 - 0 mg Zn^{2+} on 15 day; 2 - 0 mg Zn^{2+} on 30 day; 3 - 0 mg Zn^{2+} on 60 day; 4 - 0 mg Zn^{2+} on 120 day; 5 - 300 mg Zn^{2+} on 15 day; 6 - 300 mg Zn^{2+} on 30 day; 7 - 300 mg Zn^{2+} on 60 day; 8 - 300 mg Zn^{2+} on 120 day; 9 - 600 mg Zn^{2+} on 15 day; 10 - 600 mg Zn^{2+} on 30 day; 11 - 60 mg Zn^{2+} on 60 day; 12 - 600 mg Zn^{2+} on 120 day; 13 - 1200 mg Zn^{2+} on 15 day; 14 - 1200 mg Zn^{2+} on 30 day; 15 - 1200 mg Zn^{2+} on 60 day; 16 - 1200 mg Zn^{2+} on 120 day; 17 - 2400 mg Zn^{2+} on 15 day; 18 - 2400 mg Zn^{2+} on 30 day; 19 - 2400 mg Zn^{2+} on 60 day; 20 - 2400 mg Zn^{2+} on 120 day

lated with the level of zinc contamination. Our observations corroborate the findings of Lee et al. (2009) and Kucharski et al. (2011).

The distribution of vectors around the axis representing the first principal component indicates that the activity of all analyzed enzymes, excluding β-glucosidase, was negatively correlated with this variable (Figure 1). Only the vector representing the first principal component of β-glucosidase was positively correlated with the second principal component. The projection of data onto component space indicates that the activity levels of all enzymes decreased with an increase in zinc concentrations. The highest levels of enzymatic activity were observed on day 15, and the lowest – on day 120, regardless of the degree of soil contamination. The effects of zinc on soil enzymes were also analyzed by Boros et al. (2011) and Wyszkowska et al. (2013), whereas Borowik et al. (2013) evaluated its influence on nitrification.

The examined enzymes were classified in the following decreasing order in terms of their average sensitivity to soil pollution with 2400 mg Zn^{2+} kg^{-1}: dehydrogenases (98% decrease in enzyme activity) > catalase (84%) > alkaline phosphatase = arylsulfatase (79%) > urease (78%) > acid phosphatase (68%) >β-glucosidase (32%).

Soil resistance indicators are effective measures of microbial and enzymatic responses to environmental stress (Borowik et al. 2013, Orwin, Wardle 2004). The data shown in Table 3 demonstrates that the soil resistance (RS) index is highly useful for evaluations of soil quality in zinc-contaminated environments. Low RS values are indicative of long-term toxic effects of zinc. In this study, low RS values were reported on soil incubation days 15 and 120. The resistance of the analyzed enzymes to zinc decreased with an increase in zinc concentrations in soil. Significant negative correlations were observed between RS values and zinc doses. β-glucosidase was most resistant to all concentrations of zinc, whereas dehydrogenases activity was characterized by the lowest resistance to the analyzed pollutant. Based on the data presented in Tables 3 and 4, the analyzed enzymes were arranged in the following decreasing order in terms of their resistance to zinc: β-glucosidase> catalase > acid phosphatase >arylsulfatase> urease >

Table 3

Soil resistance (RS) to zinc on days 15 and 120, determined according to the levels of enzyme activity

Dose Zn^{2+} (mg kg^{-1} of soil d.m.)	Enzyme*						
	Deh	Kat	Ure	Glu	Pac	Pal	Aryl
15 day							
300	0.383^{b}	0.772^{a}	0.665^{a}	0.902^{a}	0.740^{b}	0.508^{a}	0.672^{a}
600	0.170^{c}	0.598^{b}	0.364^{b}	0.748^{bc}	0.638^{c}	0.327^{c}	0.445^{b}
1200	0.046^{e}	0.296^{d}	0.138^{cde}	0.637^{d}	0.446^{e}	0.214^{de}	0.224^{d}
2400	0.010^{g}	0.167^{f}	0.137^{cde}	0.502^{e}	0.253^{f}	0.107^{f}	0.084^{f}
Average	0.152	0.458	0.326	0.697	0.519	0.289	0.356
r	-0.829	-0.929	-0.796	-0.950	-0.983	-0.950	-0.925
120 day							
300	0.472^{a}	0.597^{b}	0.350^{b}	0.726^{bc}	0.982^{a}	0.395^{b}	0.432^{b}
600	0.151^{d}	0.468^{c}	0.358^{b}	0.952^{a}	0.546^{d}	0.233^{d}	0.351^{e}
1200	0.038^{f}	0.254^{e}	0.117^{de}	0.662^{cd}	0.303^{f}	0.125^{f}	0.121^{e}
2400	0.006^{g}	0.058^{g}	0.144^{cd}	0.469^{e}	0.189^{g}	0.125^{f}	0.078^{f}
Average	0.167	0.344	0.242	0.702	0.505	0.220	0.246
*r**	-0.773	-0.975	-0.787	-0.813	-0.852	-0.802	-0.898

* key under Table 2

Table 4

Zinc dose (mg kg^{-1} d.m. soil) responsible for 20% and 50% decrease in enzyme activity, ED_{20} and ED_{50}

% decrease in enzyme activity	Enzyme*						
	Deh	Kat	Ure	Glu	Pac	Pal	Aryl
20	103	419	233	1373	416	184	247
50	689	1255	928	3184	1514	991	1082

* key under Table 2

alkaline phosphatase > dehydrogenase. The highest value of ED_{20} was determined for β-glucosidase at 1373 mg Zn^{2+} kg^{-1} d.m. soil, and the lowest one – for dehydrogenases at 103 mg Zn^{2+} kg^{-1} d.m. soil. ED_{50} values were determined at 3184 mg Zn^{2+} for the former and 689 mg Zn^{2+} for the latter enzyme.

Renella et al. (2005) and Lee et al. (2009) attributed the observed decrease in enzyme activity in soils contaminated with heavy metals to their adverse effects on microbial counts. Other authors (Schwartz et al. 2001, Kizilkaya 2004, Gülser, Erdogan 2008, Qu et al. 2011) observed that heavy metals affect enzymes not only by inducing changes in microbial populations, but also by modifying their diversity. The extent to which enzyme activity was inhibited by zinc could have been determined by both of the above factors. Similarly to other elements, excessive concentrations of zinc induce changes in electron transport, cell membrane permeability and contribute to oxidative stress (Wang et al. 2009, Cui, Zhao 2011). Zinc could also lead to enzyme denaturation (Jonak et al. 2004). The noted reduction in enzyme activity could have also resulted from the negative effect of zinc on the physicochemical properties of soil (Lestan et al. 2003, Boros et al. 2011, Cui, Zhao 2011).

CONCLUSIONS

1. Soil enzymes are characterized by varied sensitivity to excessive zinc concentrations in soil. The analyzed enzymes were classified in the following decreasing order in terms of their resistance to zinc: β-glucosidase> acid phosphatase > urease >arylsulfatase = alkaline phosphatase > catalase > dehydrogenases.

2. Zinc continued to exert negative effect on soil enzymes throughout the entire experiment (120 days).

3. The values of ED_{20} for the analyzed enzymes (in mg Zn^{2+} kg^{-1} d.m. soil) were determined at: 103 for dehydrogenases, 184 for alkaline phosphatase, 233 for urease, 247 for arylsulfatase, 416 for acid phosphatase, 419 for catalase and 1373 for β-glucosidase.

REFERENCES

Adriano D. C., Wenzel W. W., Vangronsveld J., Bolan N. S. 2004. *Role of assisted natural remediation in environmental cleanup*. Geoderma. 122: 121-142. DOI:10.1016/j.geoderma.2004.01.003.

Alef K., Nannipieri P. 1998. *Urease activity* In: *Methods in applied soil microbiology and biochemistry*. Alef K., Nannipieri P. (eds), Acad. Press. Harcourt Brace & Company, Publishers, London, 316-320.

Bielińska E.J., Baranowska E., Jędruch M., Gostkowska K. 2005. *Appraisal of allotment soil environment from areas under the varying influence of anthropopressure through analysis of phosphate activity*. Environ.Engineer.,13: 26-28.

Bielińska E.J., Żukowska G. 2002. *Activity of protease and urease in light soil fertilized with sewage sludge*. Acta Agroph., 70: 41-47. (in Polish)

Boros E., Baćmaga M., Kucharski J., Wyszkowska J. 2011. *The usefulness of organic substances*

and plant growth in neutralizing the effects of zinc on the biochemical properties of soil. Fresen. Environ. Bull., 20(12): 3101-3109.

Borowik A. Wyszkowska J., Kucharski J., Baćmaga M., Tomkiel M. 2014. *Pressure exerted by zinc on the nitrification process.* J. Elem., 19(2): 327-338. DOI: 10.5601/jelem.2014.19.2.646.

Brockett F. T., Prescott C. E., Grayston S.J. 2012. *Soil moisture is the major factor influencing microbial community structure and enzyme activities across seven biogeoclimatic zones in western Canada.* Soil Biol. Biochem., 44: 9-20. DOI:10.1016/j.soilbio.2011.09.003

Bruins M. R., Kapil S., Oehme F. W. 2000. *Microbial resistancetometals in the environment.* Ecotox. Environ. Safe., 45: 198-207. DOI:10.1006/eesa.1999.1860

Chary N. S., Kamala C. T., Raj D. S. S. 2008. *Assessing risk of heavy metals from consuming food grown on sewage irrigated soils and food chain transfer.* Ecotox. Environ. Safe., 69: 513-524. DOI:10.1016/j.ecoenv.2007.04.013

Cui Y., Zhao N. 2011. *Oxidative stress and change in plant metabolism of maize (Zea mays* L.*) growing in contaminated soil with elemental sulfur and toxic effect of zinc.* Plant Soil Environ., 57(1): 34-39.

Gillet S., Ponge J. F. 2002. *Humus forms and metal pollution in soil.* Eur. J. Soil Sci., 53: 529. DOI:10.1046/j.1365-2389.2002.00479.x

Glina B., Bogacz A. 2013. *Concentration and pools of trace elements in organic soils in the Izera Mountains.* J. Elem., 18(2): 199-209. DOI: 10.5601/jelem.2013.18.2.01

Gülser F., Erdogan E. 2008. T*he effects of heavy metal pollution on enzyme activities and basal soil respiration of roadside soils.* Environ. Monit. Assess., 145(1-3): 127-133. DOI: 10.1007/s10661-007-0022-7

Huang S. S., Tu J., Liu H. Y., Hua M., Liao Q. L., Feng J. S., Weng Z. H., Huang G. M. 2009. *Multivariate analysis of trace element concentrations in atmospheric deposition in the Yangtze River Delta, East China.* Atmos. Environ., 43: 5781-5790. DOI:10.1016/j.atmosenv.2009.07.055

Jonak C., Nakagami H., Hirt H. 2004. *Heavy metal stress. Activation of distinct mitogen-activated protein kinase pathawahys by copper and cadmium.* Plant Physiol., 136: 3276-3283. DOI/10.1104/pp.104.045724

Kizilkaya R. 2004. *Cu and Zn accumulation in earthworm Lumbricus terrestris L. in sewage sludge amended soil and fractions of Cu and Zn in casts and surrounding soil.* Ecol. Eng., 22 (2): 141-151. DOI:10.1016/j.ecoleng.2004.04.002

Kucharski J., Boros E., Wyszkowska J. 2009. Biochem*ical activity of nickel-contaminated soil.* Polish. J. Environ., St., 18(6):1039-1044.

Kucharski J., Wieczorek K., Wyszkowska J. 2011. *Changes in the enzymatic activity in sandy loam soil exposed to zinc pressure.* J. Elem., 16(4): 577-589. DOI: 10.5601/jelem.2011.16.4.07

Kucharski J., Wyszkowska J. 2004. *Inter-realtionship between number of microorganisms or spring barley yield and degree of soil contamination with copper.* Plant Soil Environ., 50(6): 243-249.

Kucharski J., Wyszkowska J., Nowak G., Harmś H. 2000. *Activity of enzymes in soils treated with sewage sludges.* Pol. J. Soil Sci., 33(1): 29-36.

Lee S. H., Lee J. S., Choi Y. J., Kim J. G. 2009. *In situ stabilization of cadmium-, lead-and zinc-contaminated soil using various amendments.* Chemosphere., 77(8): 1069-1075. DOI: 10.1016/j.chemosphere.2009.08.056

Lestan D., Grcman H., Zupan M., Bacac N. 2003. *Relationship of soil properties to fractionation of Pb and Zn in soil and their uptake into Plantago lanceolata.* Soil Sediment Contam., 12 (4): 507-522. DOI: 10.1080/713610986.

Melgar-Ramírez R., González V., Sánchez J. A., Garcíai. 2012. *Effects of application of organic and inorganic wastes for restoration of sulphur-mine soil.* Water Air Soil Pollut., 223: 6123--6131. DOI: 10.1007/s11270-012-1345-8

Nielsen, M. N., Winding, A. 2002. *Microorganisms as indicators of soil health*. Nat. Environ. Res. Inst., Denmark. Technical Report No. 388.

Öhlinger R. 1996. *Dehydrogenases activity with the substrate TTC*. In: *Methods in soil biology*. Schinner F., Öhlinger R., Kandeler E., Margesin R. (eds). Springer Verlag Berlin Heidelberg, 241-243.

Orwin K.H, Wardle D.A. 2004. *New indices for quantifying the resistance and resilience of soil biota to exogenous disturbances*. Soil Biol. Biochem., 36: 1907-1912. DOI:10.1016/j.soilbio.2004.04.036

Qu J., Ren G., Chen B., Fan J., E Y. 2011. *Effects of lead and zinc mining contamination on bacterial community diversity and enzyme activities of vicinal cropland*. Environ. Monit. Assess., 182: 597-606. DOI: 10.1007/s10661-011-1900-6

Renella G., Mench M., Landi L., Nannipieri P. 2005. *Microbial diversity and hydrolase synthesis in longterm Cd-contaminated soils*. Soil Biol. Biochem., 37(1): 133-139. DOI: 10.1016/j.soilbio.2004.06.015

Roca-Perez L., Gil C., Cervera M.L., Gonza´lvez A., Ramos-Miras J., Pons V., Bech J., Boluda R. 2010. *Selenium and heavy metals content in some Mediterranean soils*. J Geochem. Explor., 107: 110-116. DOI:10.1016/j.gexplo.2010.08.004

Rodriguez B. B., Bolbot J. A., Tothill I. E. 2004. *Development of urease and glutamic dehydrogenase amperometric assay for heavy metals screening in polluted samples*. Biosens. Bioelectron., 19(10): 1157-1167. DOI: 10.1016/j.bios.2003.11.002

Schwartz Ch., Gerard E., Perronnet K., Morel J. L. 2001. *Measurement of in situ phytoextraction of zinc by spontaneous metallophytes growing on a former smelter site*. Sci. Total. Environ., 279: 215-221.

Seifert K., Domka F. 2005. *Inhibiting effect of surfactants and heavy metal ions on the nitrification process*. Pol. J. Environ. Stud., 14(1): 87-93.

Sienkiewicz S., Czarnecka M.H. 2012. *Content of available Cu, Zn and Mn in soil amended with municipal sewage sludge*. J. Elem., 17(4): 649-657. DOI: 10.5601/jelem. 2012.17.4.08

STATSOFT Inc. 2012. *Statistica (data analysis software system), version 10.0*. www.statsoft.com

Szymańska-Pulikowska A. 2012. *Changes in the content of selected heavy metals in groundwater exposed to the impact of a municipal landfill site*. J. Elem., 17(4): 689-702, DOI: 10.5601/jelem.2012.17.4.11

Wang Ch., Yang Z., Yuan X., Browne P., Chen L., Ji J. 2013. *The influences of soil properties on Cu and Zn availability in soil and their transfer to wheat (Triticum aestivum* L.*) in the Yangtze River delta region, China*. Geoderma, 193-194:131-139. DOI.org/10.1016/j.geoderma.2012.10.004

Wang D., Hosteen O., Fierke C. A. 2012. *ZntR-mediated transcription of ZntA responds to nanomolar intracellular free zinc*. J. Inorg. Biochem., 111: 173-181. DOI:10.1016/j.jinorgbio.2012.02.008

Wang H., Liu R.L., Jin J.Y. 2009. *Effects of zinc and soil moisture on photosynthetic rate and chlorophyll fluorescence parameters of maize*. Biol. Plantarum., 53: 191-194.

Wyszkowska J., Boros E., Kucharski J. 2007. *Effect of interactions between nickel and other heavy metals on the soil microbiological properties*. Plant Soil Environ., 53(12): 544-552.

WyszkowskaJ., Borowik A., Kucharski M., Kucharski J. 2013. *Applicability of biochemical indices to quality assessmnet of soil pulluted with heavy metals*. J. Elem., 18(4): 733-756, DOI: 10.5601/jelem.2013.18.4.504.

Wyszkowska J., Kucharski J., Jastrzębska E., Hłasko A. 2001. *The biological properties of the soil as influenced by chromium contamination*. Pol. J. Environ. Stud. St., 10(1): 37-42.

Wyszkowska J., Kucharski M., Kucharski J. 2010. *Activity of β-glucosidase, arylsuphatase and phosphatases in soil contaminated with copper*. J Elem., 15(1): 213-226. DOI: 10.5601/jelem.2010.15.1.213-226.

Wyszkowska J., Kucharski M., Kucharski J., Borowik A. 2009. *Activity of dehydrogenases, catalase and urease in copper polluted soil*. J. Elem., 14(3): 605-617.

Wyszkowski M., Wyszkowska J. 2009. *The effect of soil contamination with cadmium on the growth and chemical composition of spring barley (Hordeum vulgare L.) and its relationship with the enzymatic activity of soil*. Fresen. Environ. Bull., 18 (7):1046-1053.

Zhao K. L., Liu X. M., XuJ. M., Selim H. M. 2010. *Heavy metal contaminations in a soil- rice system: identification of spatial dependence in relation to soil properties of paddy fields*. J. Hazard. Mater., 181: 778-787. DOi:10.1016/j.jhazmat.2010.05.081.