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ACTIVITY OF SELECTED ENZYMES IN SOIL LOADED WITH VARIED LEVELS OF HEAVY METALS

Aleksandra Nadgórska-Socha, Iwona Łukasik, Ryszard Ciepał, Sylwia Pomierny

Ecology Department, Silesian University, ul. Bankowa 9, 40-007 Katowice
e-mail: olan@hoga.pl

A b s t r a c t. The investigations were performed using soil samples which came from the vicinity of the non-ferrous metallurgical plant Szopienice, from zinc wastes heap, and from a former calamine site. The aim of the experiments was to find relations between the activity of dehydrogenase, acid and alkaline phosphomonoesterase (phosphatases), urease, and the concentration of bioavailable metals: Zn, Cd, Pb (0.01M $CaCl_2$ was used as the extractant). The soil enzyme activities were compared in soils with varied heavy metals levels. The results of the study showed the highest activity of dehydrogenase, phosphatases and urease in soil samples taken at the calamine site, where the lowest heavy metals content was found. Lower activities of the investigated enzymes were noted for 50 and 250 meter distances from the non-ferrous metallurgical plant Szopienice, where the highest content of heavy metals and lower organic matter content in soil was found. Soil enzyme activity (phosphatases and dehydrogenase especially) can be used as a highly sensitive indicator of the effect of heavy metals pollution.

K e y w o r d s: soil enzyme activity, heavy metals

INTRODUCTION

Soils located nearby urban-industrial agglomerations are usually enriched in heavy metals. Steady heavy metals inflow and accumulation in soil can lead to irreversible reduction of soil microbial process rate [1-3]. Extreme metal contamination in the vicinity of smelting plants causes clearly visible effects such as accumulation of organic matter layers on the soil surface through inhibition of the activity of soil microorganisms and soil fauna [4]. On the other hand, organic matter content is one of the factors which limit soil microbial activity – the main source of soil enzymes [5]. Many enzymes are sensitive to pH. Moreover, enzyme activity decreases with increasing heavy metal pollution, using heavy metals salts, but the amount of decrease differs among the enzymes [6]. Wyszkowska *et al.* [7]

suggested that soil enzymatic activity can be used as a reliable indicator of the impact of toxic metals on the soil biochemical activity. The aim of this study was the estimation of acid and alkaline phosphatase, urease and dehydrogenase activities in the surface layer of soil located around a non-ferrous smelting plant in Katowice Szopienice, at a zinc wastes heap located in Katowice, and at a former calamine site in Dąbrowa Górnicza (southern Poland). The smelting plant has emitted metal-rich dusts for many years (from 1834) [8]. The heap occupies 25 ha and it is composed of floss from muffle furnaces, ash, waste materials from distillation and roasting furnaces, and chamotte bricks. Presently the area is abandoned [9]. The third stand was a former calamine mine in the 19th century [10]. The relationships between the activity of enzymes and heavy metal bioavailability were investigated. The relation between soil organic content, pH and soil enzymes activity was determined, too.

MATERIAL AND METHODS

Soil samples from the layer of 0-10cm were collected from the vicinity of the non-ferrous smelting plant "Szopienice" in Katowice (at distances of 50 m, 250 m, and 450 m from the emitter), from the heap (H), and from the former calamine site (C). The soil samples were collected in May (V) and in September (IX), in 2002 and 2003. Heavy metal contents were estimated according to the methods of Bouwman *et al.* [11], Lock *et al.* [12] and Ostrowska *et al.* [13], in air dry soil samples which were sieved through a 0.25 mm sieve. Heavy metals were extracted using 0.01M $CaCl_2$ (bioavailable fraction), 10 % HNO_3. The analyses were performed using furnace atomic absorption (AAS UNICAM 939/959). Soil pH was measured in water (1:2.5 soil: water ratio) using a pH meter [13]. Organic matter content (soil was treated with a mixture of $K_2Cr_2O_7$ and H_2SO_4) was estimated by the method of Schinner *et al.* [14]. The soil enzymes activity was determined in soils samples at field moisture, sieved through a 2 mm sieve. The activity of alkaline and acid phosphatase was measured according to the method of Schinner *et al.* [14]. The p-nitrophenol (NP) released by phosphomonoesterase activity was extracted and coloured with sodium hydroxide and determined photometrically at 400 nm. The urease activity estimation was based on the colorimetric determination of ammonium formation after enzymatic urea hydrolysis (10% solution, λ – 630 nm) [14]. Triphenyltetrazolium chloride was the substrate which was used for the dehydrogenase activity determination. The Triphenyltetrazolium formazan (TPF) produced was extracted with acetone and measured photometrically at 546 nm [14]. The results are presented as mean values from six replicates of each treatment, together with standard error of the means. The statistical significant differences between soil enzymes activity, heavy metals contents, pH values, organic matter contents at investigated stands were marked with the * sign. The data were processed using the software Statistica 5.0 PL to compute the Pearson correlation coefficients.

RESULTS

Figures 1-4 present the mean soil enzyme activities in the soil samples during the investigation period. Figures 5-7 present bioavailable heavy metals contents in the soil, Figure 8 the percentage content of organic matter, and Figure 9 the soil pH values. Tables 1 and 2 show the heavy metals contents, extracted with 10 % HNO_3. Generally, the highest enzyme activity was found in soil samples collected from the calamine site. However, high mean activity of urease was observed in soil samples collected at the distance of 450 m from the emitter, and higher activity of phosphatases in soil samples from the heap as compared to the smelting plant "Szopienice". The lowest enzyme activity values were found in soils located at the distance of 250 m from the emitter (Figs 1-4).

At the distance of 250 m from the emitter, the highest bioavailability of Pb, Zn and Cd was noted (Figs 5-7). The Zn concentration ranged from 34.5 to 495 mg kg^{-1}, that of Pb from 14 to 94.3 mg kg^{-1}, and of Cd from 1.1 mg kg^{-1} to above 18 mg kg^{-1} in the analysed soil samples (Figs 5-7). The Zn concentration was the highest among the trace metals in the analysed soil samples (Fig. 5). The heavy metals bioavailability was low in comparison to heavy metals content in the soil extracted with 10 % HNO_3 (Tab. 1 and 2). This content is called contamination fraction by Ostrowska et al. [13]). For example, bioavailable Zn content (in soil samples collected at the distance of 250 m from the emitter in May) was 24 times lower, Pb 16.8 and Cd 3.8 times lower than these metals concentrations in the soil samples extracted with 10 % HNO_3 (Tab. 1, Figs. 5-7). The lowest organic matter content was noted for soil samples collected 250 m from the emitter (Fig. 8).

The lowest activity of acid and alkaline phosphatase was noted for 50 m and 250 m distances. At the end of the investigation period the activity of acid phosphatase decreased in most of the investigated stands (Fig. 1). The lowest activity of dehydrogenase was noted at the distance of 250 m and for the waste heap soil, too. The activity of dehydrogenase was the highest in soil samples collected at the calamine site, in which the lowest Zn and Cd content and the highest organic matter content was found. At the distance of 250 m from the emitter the lowest activity of urease was observed. However, there was little variation in the urease activity in the soil of the investigated stands.

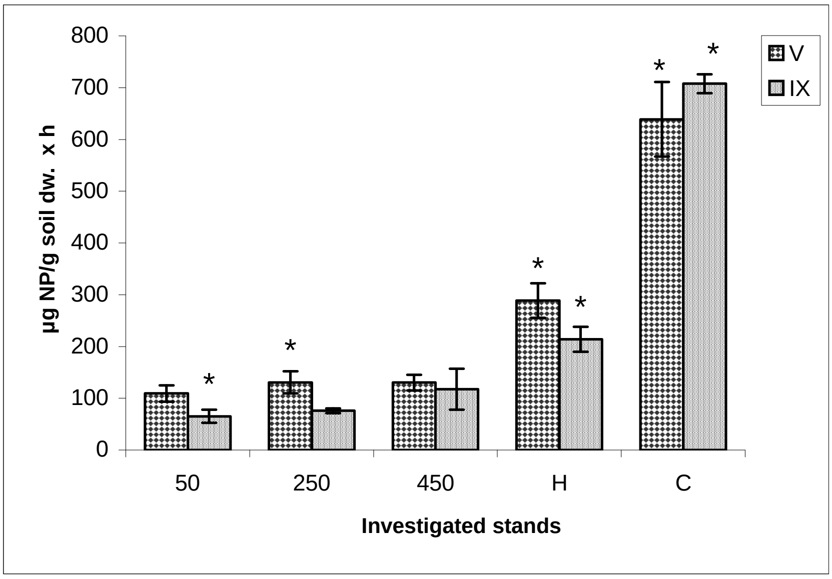

Fig. 1. Mean activities of acid phosphatase in 2002 and 2003 in soil of investigated stands (V- May, IX- September)

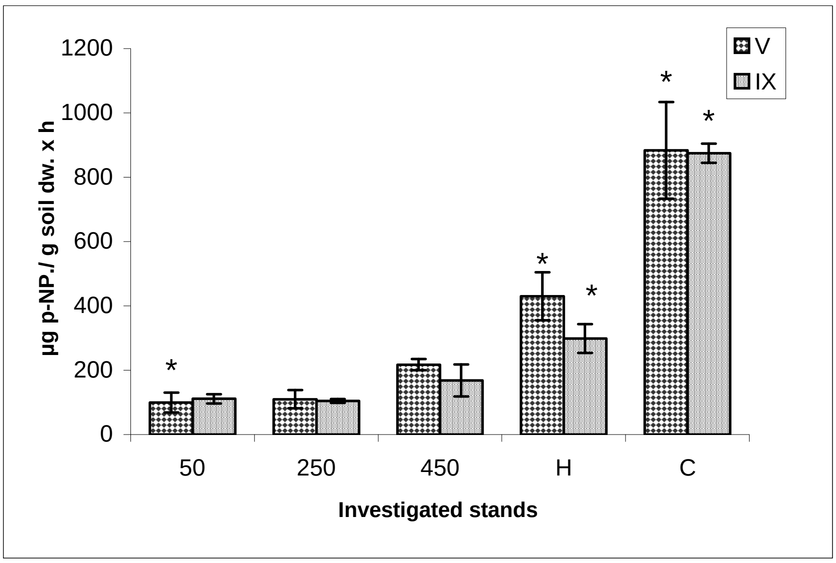

Fig. 2. Mean activities of alkaline phosphatase in 2002 and 2003 in soil of investigated stands

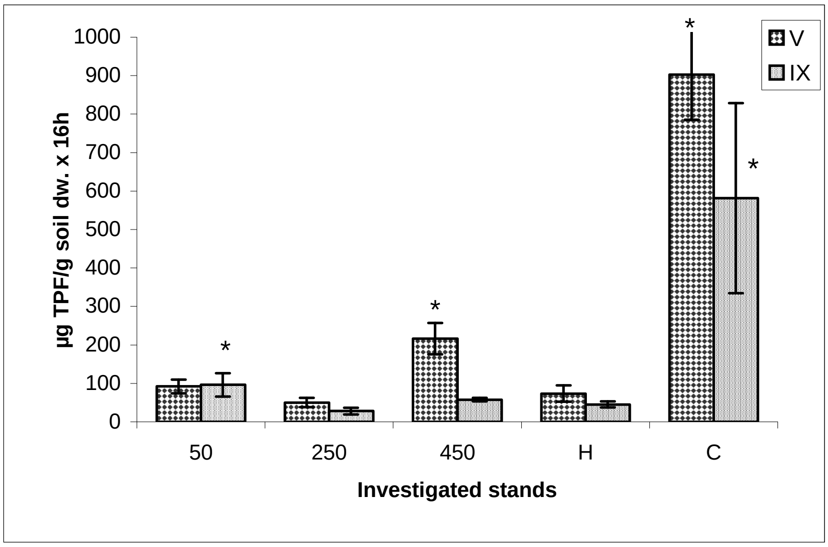

Fig. 3. Mean activity of dehydrogenase in 2002 and 2003 in soil of investigated stands

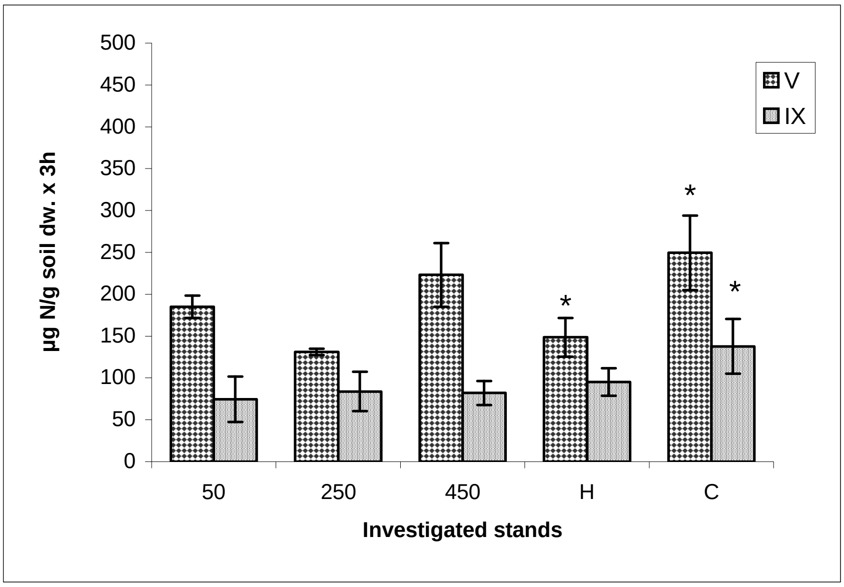

Fig. 4. Mean activities of urease in 2002 and 2003 in soil of investigated stands

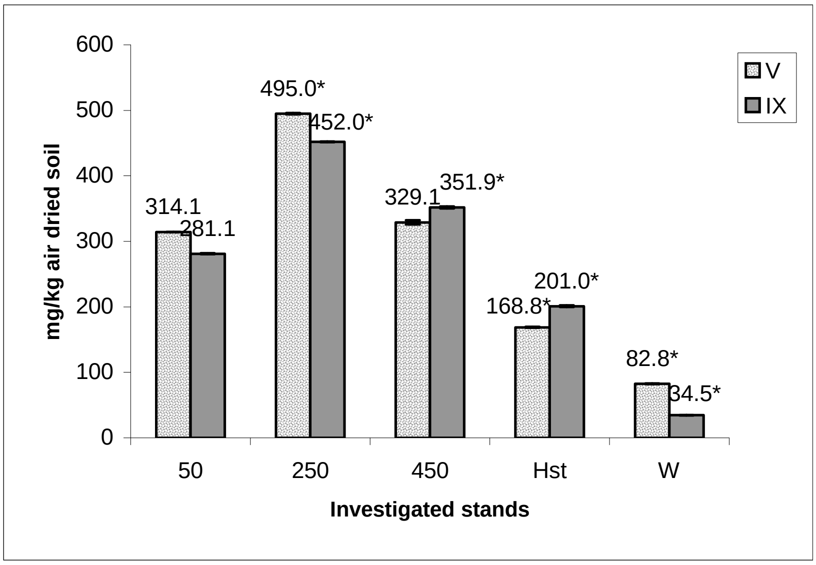

Fig. 5. Mean content of bioavailable Zn in 2002 and 2003 in soil of investigated stands

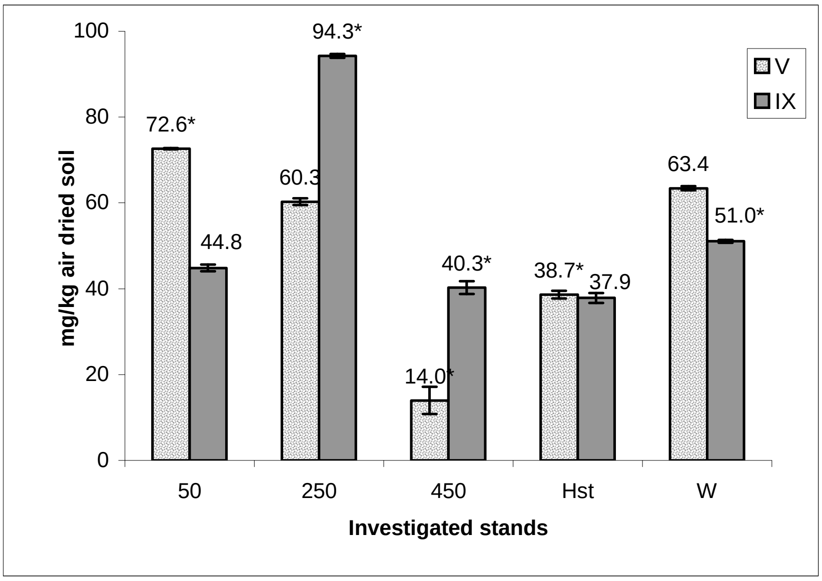

Fig. 6. Mean content of bioavailable Pb in 2002 and 2003 in soil of investigated stands

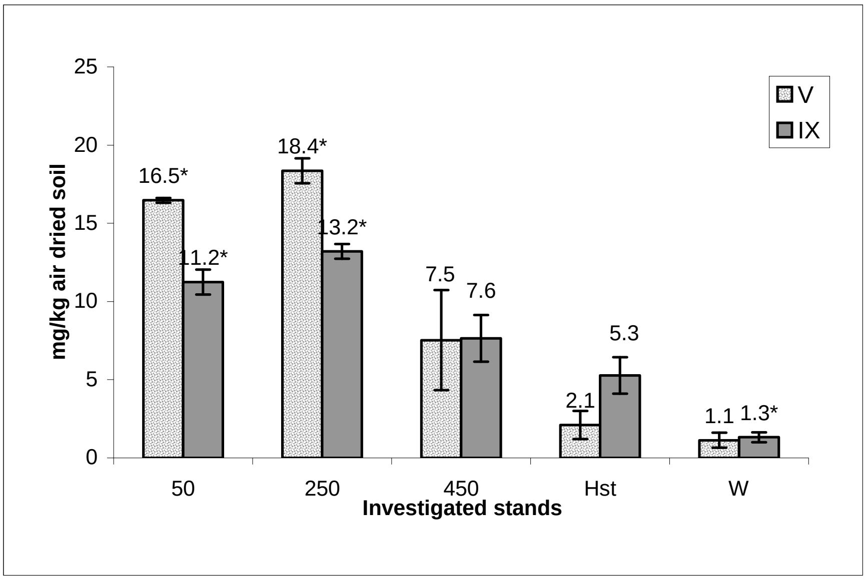

Fig. 7. Mean content of bioavailable Cd in 2002 and 2003 in soil of investigated stands

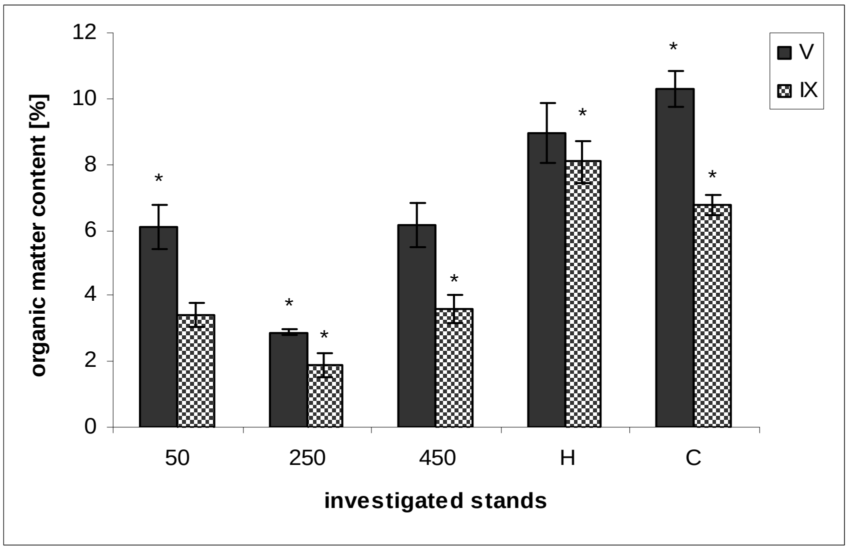

Fig. 8. Mean content of organic matter content in 2002 and 2003 in soil of investigated stands

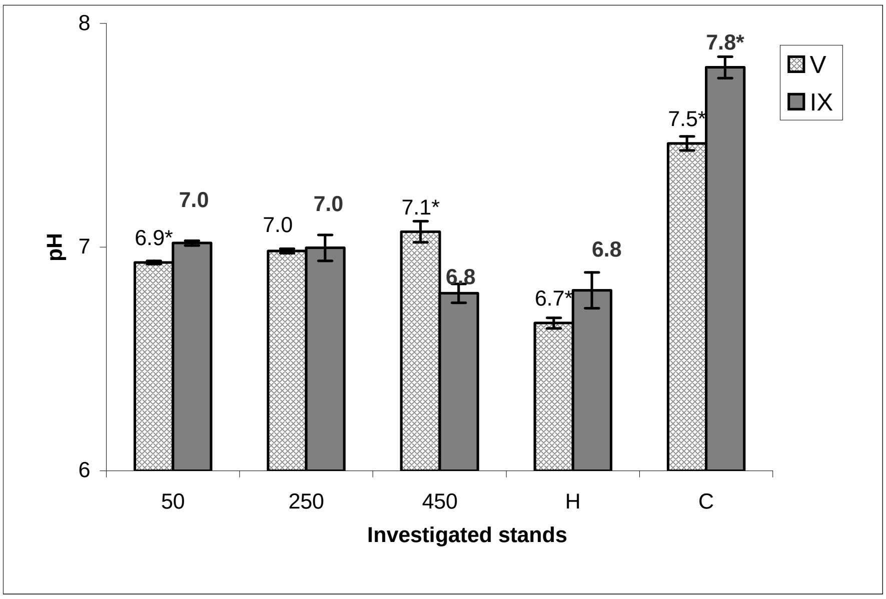

Fig. 9. Mean pH value in 2002 and 2003 in soil of investigated stands

Table 1. Zn, Cd, Pb content (10 % HNO_3 was used for the extraction) in soils of investigated stands in May in 2002 and 2003. The values are means ± SE of six replicates

Metal (mg kg^{-1})	50 m	250 m	450 m	Heap	Calamine site
Zn	65710 ± 15818*	11913.9 ± 1919	13995.9 ± 4524	46663.1 ± 3066	24687.1± 1980.4*
Cd	123 ± 38*	71.2 ± 22	43.3 ± 8*	79.4 ± 23	61.2 ± 11
Pb	1350 ± 520	1019 ± 248	1180 ± 299	1769 ± 327	1167.2 ± 204

Table 2. Zn, Cd, Pb content (10 % HNO_3 was used for the extraction) in soils of investigated stands in September in 2002 and 2003. The values are means ± SE of six replicates

Metal (mg kg^{-1})	50 m	250 m	450 m	Heap	Calamine site
Zn	66639 ± 12184*	8513.7 ± 626	6454.79 ± 1234	45732 ± 3454*	24579 ± 1965*
Cd	119 ± 31	42 ± 10	39 ± 9	101 ± 36	54.7 ± 9
Pb	1280 ± 558	1011.2 ± 245	1157 ± 115	1788 ± 430	1139 ± 135

DISCUSSION

The higher activities of the investigated enzymes at the former calamine site are likely due to higher soil organic matter content and better plant cover (Fig. 8, Tab. 1, 2). Pascual et al. [15] noted that decrease in enzyme activity may be due to progressive erosion of the soil as a consequence of low levels of plant cover (low plants biomass level) and low levels of organic matter in the soil. This situation was observed especially in the vicinity of the emitter – the non-ferrous metallurgical plant “Szopienice”. Garcia *et al*. [16] reported that enzymatic activity increased as the organic matter content increased. Higher concentration of organic matter causes higher amount of humus-enzyme complexes formation. Bounded enzymes are less sensitive to temperature, humidity and pH changes [17-20]. No increased accumulation of soil organic matter was observed in comparison to increased accumulation of soil organic matter in the litter layer in contaminated forest soils around smelting plants [21].

The enzyme activity in the soil decreases with the increase of heavy metals ions concentration in the soil [2]. Our investigations confirmed these previous findings, the enzyme activities decreased at points located in soils at the distances of 50 m and 250 m from the emitter “Szopienice” (Figs 5-7) (mostly negative correlation coefficients between bioavailable Zn and Cd contents in soil and soil enzyme activities – Tab. 3-4). About 100 mg kg^{-1} of Zn in the soil can reduce nitrification processes, and about 1000 mg kg^{-1} inhibits most of the microbial processes [22]. Soil microorganisms are sensitive to higher amount of Cd which inhibits the activity of microbial processes, too. Increase of Pb content in surface soil layer affects soil microorganisms. Reduction of enzymatic soil microorganism activity can cause inhibition of organic matter decomposition, especially cellulose. This effect leads to soil degradation [21,22].

Table 3. Correlation coefficients between activities of soil enzymes and heavy metals contents, organic matter content and pH value in May (*statistically significant for p<0.05)

Enzyme	Bioavailable Zn	Bioavailable Cd	Bioavailable Pb	Organic matter content	pH
Acid phosphatase	–0.65*	–0.52*	0.14	0.40*	0.53*
Alkaline phosphatase	–0.53*	–0.57*	0.07	0.66*	0.39
Dehydrogenase	–0.58*	0.01	0.01	0.47*	0.82*
Urease	–0.40*	–0.27	–0.21	0.09	0.65*

Table 4. Correlation coefficients between activities of soil enzymes and heavy metals contents, organic matter content and pH value in September (* statistically significant for p<0,05)

Enzyme	Bioavailable Zn	Bioavailable Cd	Bioavailable Pb	Organic matter content	pH
Acid phosphatase	–0.79*	–0.72*	–0.11	0.37*	0.73*
Alkaline phosphatase	–0.82*	–0.75*	0.02	0.41*	0.82*
Dehydrogenase	–0.42*	–0.03	–0.03	0.18	0.51*
Urease	–0.48*	–0.20	0.20	0.09	0.16

Kobus [5], Dick [20], and Dick and Tabatabai [23], pointed out that phosphatases are sensitive enzymes which are pH dependent, which confirms our results (Tab. 3-4). Increase of metal bioavailability upon soil acidification is well known [24-26]. In our investigation this statement was confirmed only in those soil samples which were collected at the distance of 450 m from the emitter. Kieliszewska-Rokicka [27] also reported lower activity of phosphatases in the vicinity of the aluminium smelting plant "Konin" (0.4 km) in comparison with points located at greater distances from the emitter (2.5-3 km). Similar results were recorded in the vicinity of the non-ferrous metal smelting plant "Szopienice" (Figs 1-2).

The activity of dehydrogenase was the highest at the calamine site (Fig. 3). This site was the best covered by plants and the lowest heavy metals bioavailabity was observed there. Dehydrogenase activity reflects the total oxidative activities of the soil microflora, important in oxidation of soil organic matter [20]. Dehydrogenases are active inside living cells and denaturation of these enzymes follows after the cell death. The activity of dehydrogenase is similar to that of a number of active microorganisms [19,26,27].

Heavy metals at the concentration of 5 $\mu M\ g^{-1}$ inhibit urease activity, and Ag, Hg and Cu are more toxic than Zn and Cd [2]. Pascual *et al.* [15] indicated that the study of different hydrolases enzyme activity (including phosphatases and urease) is important because they indicate the potential of a soil to carry out specific biochemical reactions and are important in maintaining soil fertility. Basing on the content of organic carbon in soil and soil enzymatic activity (urease, acid and alkaline phosphatase, dehydrogenase), potential biochemical index of soil fertility can be calculated [3].

Olszowska [21] and Kucharski *et al.* [3] noted that heavy metals pollution of soil environment caused a reduction of most biochemical reactions in soil, including soil enzyme activity, and it leads to the reduction of organic matter decomposition rate pointed above. Our study confirmed that soil enzymes activity (especially phosphatases and dehydrogenase) can be used as a sensitive and early indicator of stress caused by chemical pollution of the ecosystem [21]. The heavy metals source and organic matter content have an important effect on soil enzymes activity. The authors' investigations showed a negative effect of heavy metals accumulation from the non-ferrous emitter dusts in the soil on enzymes activities.

Studies of soil enzyme activities should be conducted in conjunction with other microbial, chemical and physical measurements to fully assess and improve chances of correctly diagnosing soil health [20,23,28,29].

CONCLUSIONS

1. The soil enzyme activities were affected by heavy metals present in soil. The lowest activities of the investigated enzymes were found in the nearest vicinity of the nonferrous metallurgical plant "Szopienice"– 50 and 250 m (in most negative correlation coefficients between bioavailable Zn and Cd contents in soil and soil enzyme activities).
2. The soil enzyme activities were connected with soil organic matter content (positive correlation coefficients). Low plant cover and low organic matter contents could be the cause for the decreased soil enzyme activities observed in the vicinity of the emitter, too.
3. Soil enzymes (acid and alkaline phosphatase and dehydrogenese) activity is a sensitive indicator of the heavy metals pollution effect. The urease activity measurement is not good when the bioavailability in the soil is concerned.

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AKTYWNOŚĆ WYBRANYCH ENZYMÓW W GLEBIE O ZRÓŻNICOWANYM POZIOMIE OBCIĄŻENIA METALAMI CIĘŻKIMI

Aleksandra Nagórska-Socha, Iwona Łukasik, Ryszard Ciepał, Sylwia Pomierny

Katedra Ekologii, Wydział Biologii i Ochrony Środowiska, Uniwersytet Śląski
ul. Bankowa 9, 40-007 Katowice
e-mail lukasik@us.edu.pl

Streszczenie. Badano aktywność fosfatazy kwaśnej, alkalicznej, dehydrogenazy i ureazy w glebie najbliższego sąsiedztwa Huty Metali Nieżelaznych w Katowicach Szopienicach, hałdy pocynkowej i terenu po eksploatacji galmanu. Celem pracy była próba znalezienia zależności między aktywnością fosfataz (kwaśnej i alkalicznej), dehydrogenazy i ureazy i biodostępnością metali: Zn, Cd, Pb w glebie. Porównano aktywność enzymatyczną w glebie o różnym poziomie zanieczyszczenia. Najwyższą aktywność dehydrogenazy, fosfataz i ureazy stwierdzono w próbkach gleby pobieranych w terenie po eksploatacji galmanu, gdzie zanotowano również najniższą biodostępność badanych metali. Niższą aktywność enzymów glebowych obserwowano w próbkach gleby pobieranych w odległości 50 i 250 m od Huty Metali Nieżelaznych w Katowicach, gdzie zanotowano wyższą zawartość metali ciężkich i niższą zawartość materii organicznej. Aktywność enzymatyczna gleby (a szczególnie dehydrogenazy i fosfataz) może być uznana za wskaźnik zanieczyszczenia gleby metalami ciężkimi.

Słowa kluczowe: aktywność enzymatyczna gleby, metale ciężkie