Polish J. of Environ. Stud. Vol. 18, No. 3 (2009), 421-427

Original Research

Effect of Mineral Xenobiotics on the Enzymatic Activity of Anthropogenically Changed Soils

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Received: 17 March 2008
Accepted: 29 January 2009

Abstract

This paper presents dependences between the contents of Cu and Pb, and the enzymatic activity of dehydrogenases, acid phosphatase, urease and protease. Studies were carried out in soil samples taken from upper horizons of black earth and grey-brown podsolic soils in the neighbourhood of the Legnica Copper Works. The analyzed soils showed silt texture (SiC) and a differentiated content of organic matter. Upper humus horizons were characterized by high contamination by Cu and Pb, but their amounts decreased with an increasesd distance from the emission source. Significant amounts of the mentioned mineral xenobiotics caused a distinct decrease of biological life activity in the analyzed soils.

Keywords: soil, enzymatic activity, copper, lead

Introduction

Enzymes are the driving force of all transformations and processes taking place in living organisms. Many authors regard enzymatic activity as the most important indicator of biological activity of soil [1-3]. Soil enzymes are very sensitive to anthropopressure and, therefore, are frequently used in the estimation of anthropogenic changes in the environment. Contamination of soils with heavy metals decreases, as a rule, the intensity of biochemical reactions, which in consequence lead to a decrease of the number of microorganisms and a drop in the enzymatic activity of soil pedons [4]. Heavy metals occurring in small amounts in the environment can have a stimulating effect on the biological activity of soil, while in greater amounts they exert an inhibiting effect [4-8].

The objective of the presented studies was the determination of the effect of heavy metals – in uncontrolled conditions – on the enzymatic activity of soils in the neighbourhood of the Legnica Copper Works.

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Experimental Procedures

The objects of studies consisted of soil samples taken in the years 2003-05 from humus horizons of two soil types, i.e. black soils (Phaeozems) and grey-brown podzolic soils (Luvisols) anthropogenically changed. The mentioned samples were taken at two depths: 0-10 cm (samples A) and 10-30 cm (samples B). Research points were selected on two transects located in different directions from the source of emission, i.e. from shaft furnaces of the Legnica Copper Works (Fig. 1). The first transect (object I) was situated in the eastern direction, and soil samples were taken in the following distances from the object: 750, 1,000, 1,250, 1,500, 1,800 and 2,100 m from the emission source. The second transect (object II) was in the western direction. Sampling pedons lying in this direction were situated at 600, 1,000 and 1,350 m. A reference point was a control sample (K) taken from the upper horizon of the grey-brown podzolic soil (Luvisols) lying in the western direction from the copper works, i.e. at a distance of about 3,500 m from the source of contamination.

The studied objects of the grey-brown podsolic soil (samples 1, 2, 7-9) were covered by artificially planted forest vegetation – birch and poplar trees. On the other hand, on black earth (samples 3 and 4), there was a cultivation of shrubby willow (*Salix americana* var.). Samples 5 and 6 were taken from black earth constituting wasteland covered by gramineous vegetation. In the collected soil material, the following properties were determined [9]:

- texture of soils by Bouyoucos method modified by Prószyński,
- organic carbon and total nitrogen on Verio-Max analyzer,
- reaction in distilled water and electrolyte 1 mol dm^{-3} HCl by potentiometric method,
- total content of heavy metals, Cu and Pb by atomic absorption spectrometry (AAS) after solution of samples in aqua regia ($3HCl+HNO_3$),
- enzymatic activity of dehydrogenases (Adh) by Thalmann [10] method, acid phosphatase (AF) by Tabatabai and Bremner [11] method, urease (AU) by Zantua and Bremner [12] method and protease (AP) by Ladd and Buttler [13] method.

The obtained resuslts were stastically elaborated the using STATPAKT program at confidence level P=0.95 of correlation coefficient between enzymatic activity and the studied properties of soils.

Results and Discussion

Regarding texture, the analyzed soils belong to silt texture group (SiC), according to FAO [14]. Taking into consideration the agronomic soil categories, this group must be counted to heavy soils-category IV. Organic carbon content in the analyzed samples oscillated from 7.0 to 29.0 g kg^{-1}, while total nitrogen was within 0.65-2.93 g kg^{-1}. C:N proportion was in the interval of 9-14.1, which may be a testimony of rather intensive mineralization processes of organic matter, which penetrate into the soil in the form of falling leaves (leaves of willow, birch or poplar). The reaction of the studied soils was differentiated. The majority of samples belonged to slightly acid soils (pH_{KCl} 5.6-6.5) or neutral soils (pH_{KCl} 6.6-7.2). The total amounts of Cu and Pb in all samples were high (Cu 450-3,465 mg kg^{-1}; Pb 158-874 mg kg^{-1}). Although the highest accumulation was found in the closest vicinity from the source of contamination. It was also observed that a stronger contamination with these elements was shown by sampling pedons localized in the eastern side of the Copper Works than in the western side. In both directions, the amount of Cu in the upper horizons decreased with the increase of the distance from the Works. These observations were backed up by statistical analysis in the form of the calculated linear regression equations (Figs. 2 and 3). Similar study results referring to the discussed

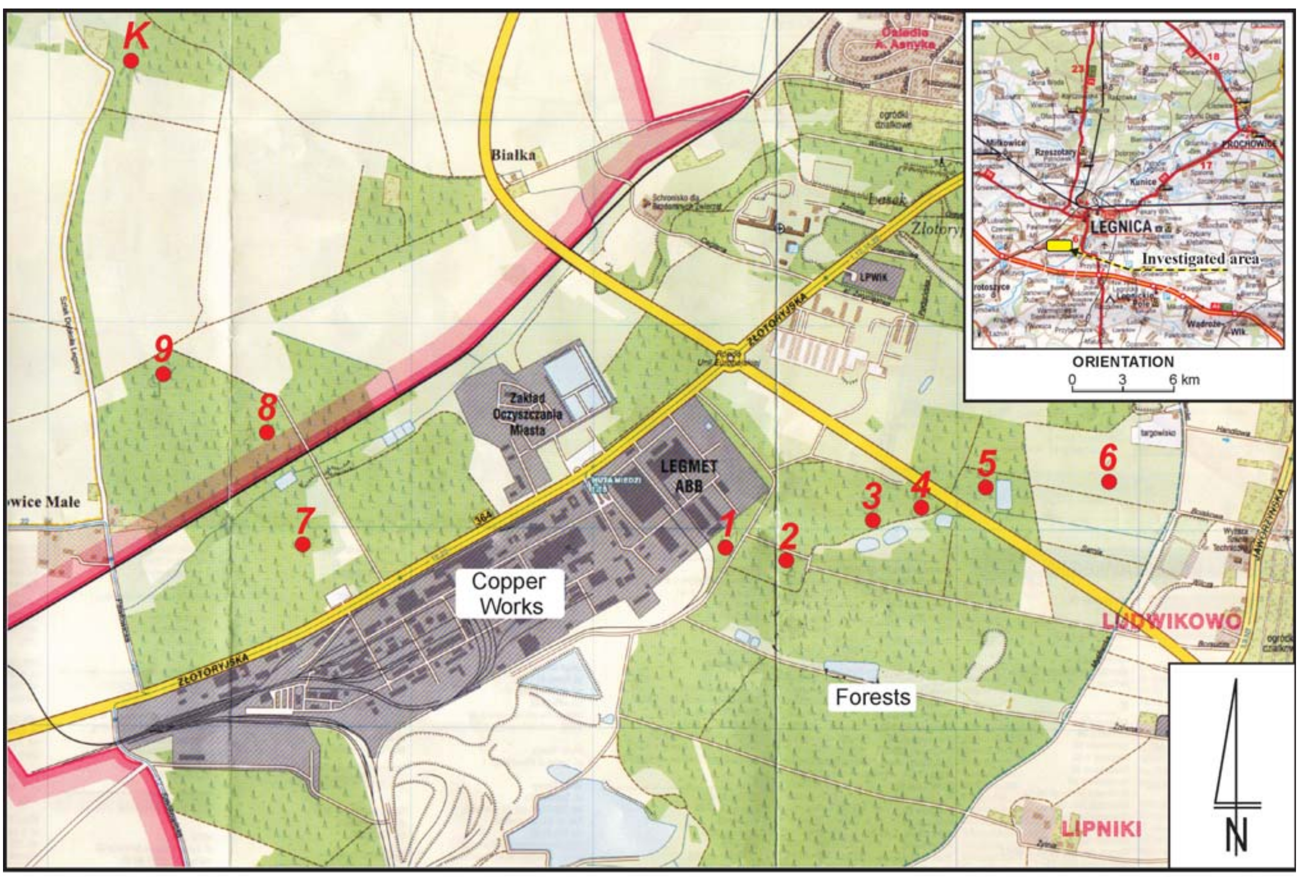

Fig. 1. Localization of soil sampling sites.

Table 1. Coefficient of linear correlation between enzymatic activity and the studied features of soils (samples from the depth of 0 - 10 cm (A).

	Dehydrogenases	Acid phosphatase	Urease	Protease
Organic C	0.46*	0.50**	0.36*	·
Total N	0.53**	0.74**	0.62**	·
Total Cu	-0.38*	-0.65**	·	·
Total Pb	-0.39*	-0.59**	·	·

*Significance at the level $p \leq 0.05$;
** Significance at the level $p \leq 0.01$

Table 2. Coefficient of linear correlation between enzymatic activity and the studied features of soil (samples from the depth of 10 - 30 cm (B).

	Dehydrogenases	Acid phosphatase	Urease	Protease
Organic C	0.44*	0.60**	0.52**	·
Total N	0.56**	0.81**	0.68**	·
Total Cu	·	-0.53**	·	·
Total Pb	·	-0.43**	·	·

*Significance at the level $p \leq 0.05$;
** Significance at the level $p \leq 0.01$

object were also obtained by other researchers [15, 16]. Lead content in soil samples was differentiated, although it was rather high. The highest concentration was found in samples taken from the upper horizon from a depth of 0-10 cm (samples A). The amounts of this element oscillated within the limits from 100 to 500 mg kg^{-1} of soil in the eastern direction and from 100 to 700 mg kg^{-1} of soil in the western direction. Linear regression equations confirmed the drop in lead content with the increased distance from the emission source (Figs. 4 and 5).

A successive analyzed parameter was the enzymatic activity of soil, estimated mainly on the basis of the activity of dehydrogenases, phosphatases, ureases and proteases.

In the period of studies (2003-05), an intensification of the activity of hydrolases in the studied pedon was observed, as the distance from the copper Works increased. It was particularly visible in the case of acid phosphatase and urease (Figs. 6 and 7). In turn, the activity of dehydrogenases ranged on a very low level in all research sites, also in the soil of the control object (Fig. 8). Dehydrogenases are enzymes that are particularly sensitive to the action of toxic compounds [17]. Therefore, in our studies, the very low activity of dehydrogenases in the soils is a testimony to decreased General Microbiological Activity (GMA) in the environment. A high inactivation of dehydrogenases in soils anthropogenically transformed was also observed by other researchers [17-19]. In the studied soils, the activity of acid phosphatase and urease showed a wide spectrum: acid phosphotase ranged from 13.45 to 119.85 mmol PNP kg^{-1} h^{-1}, urease from 0.15 to 6.40 mg $N\text{-}NH_4^+$ kg^{-1} $h^{-1}$and protease from 4.94 to 24.12 mg of tyrosine kg^{-1} h^{-1}. Results obtained for acid phosphatase and urease indicate that these enzymes

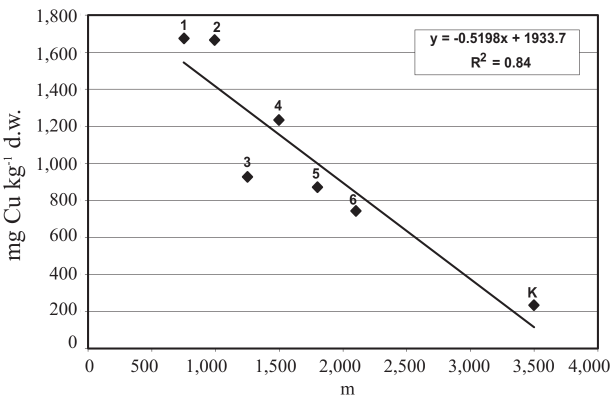

Fig. 2. Dependence between copper content (Cu) and the distance of sampling site from the emitter (object I).

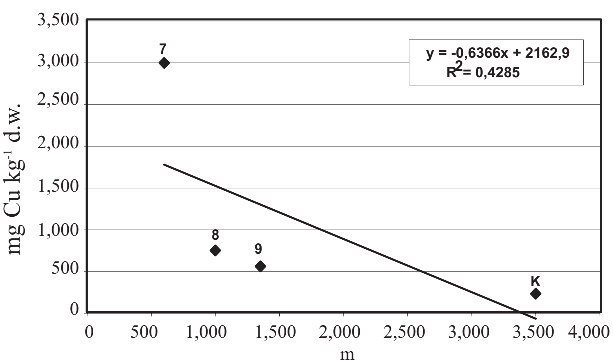

Fig. 3. Dependence between copper content (Cu) and the distance of sampling site from the emitter (object II).

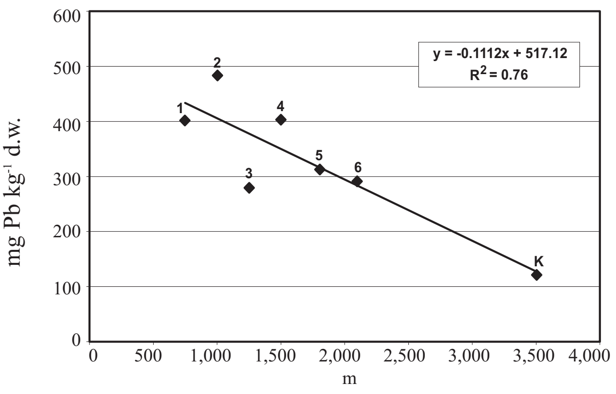

Fig. 4. Dependence between lead content (Pb) and the distance of the sampling site from the emitter (object I).

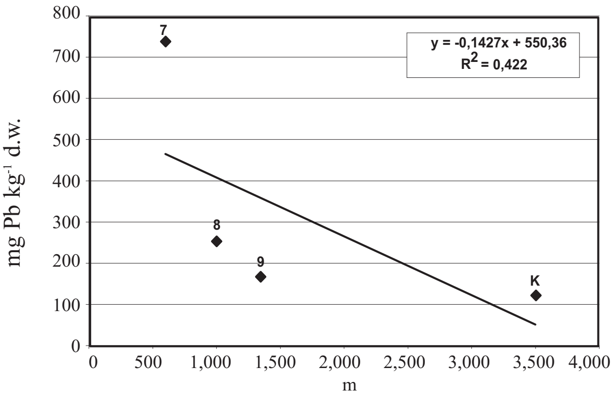

Fig. 5. Dependence between lead content (Pb) and the distance of the sampling site from the emitter (object II).

show the highest usefulness for the estimation of changes taking place in soil environment under the influence of industrial contamination. Also, Kucharski [20] underlines the particular usefulness of urease and phosphatase for the estimation of changes in soil properties in conditions of many years of industrial emission. The low level of urease activity in the analyzed samples could have been the effect of the limited accessibility of the substrate, urea [21], because urea is synthetized only in its presence.

The magnitude of the observed changes in the intensity of enzymatic activity together with the increase of the distance from the source of emission depended also on the enzyme type and term of studies, as well as on the properties of the studied soils. The obtained results were reflected in the values of the coefficients of linear correlation between the activity of the studied enzymes and the content of heavy metals in the soils (Tables 1 and 2). The observed stimulation of enzymatic soil activity was connected with the relatively high content of organic C and the total amount of nitrogen in the soil (Tables 1 and 2). Activity level of enzymes in the soil closely depends on the presence of carbon substrates [22, 23]. High enzymatic inactivation of soils in points lying in the closest distance from the Copper Works was connected with the highest intensity of soil environmental pollution by heavy metals. It found a confirmation in the analysis of the dependence of enzyme activity on the content of heavy metals in the studied soils (Tables 1 and 2), because enzymes are characterized by high susceptability to unfavourable factors of the environment [24, 25]. In laboratory studies carried out by Nowak et al. [26], a significant decrease of enzymatic activity was found in samples incubated with extremely high doses of metals. In the interpretation of the obtained results, the fact must be taken into consideration that the effect of the action of metals is connected both with the content of humus compounds, enzyme type and the given metal [27].

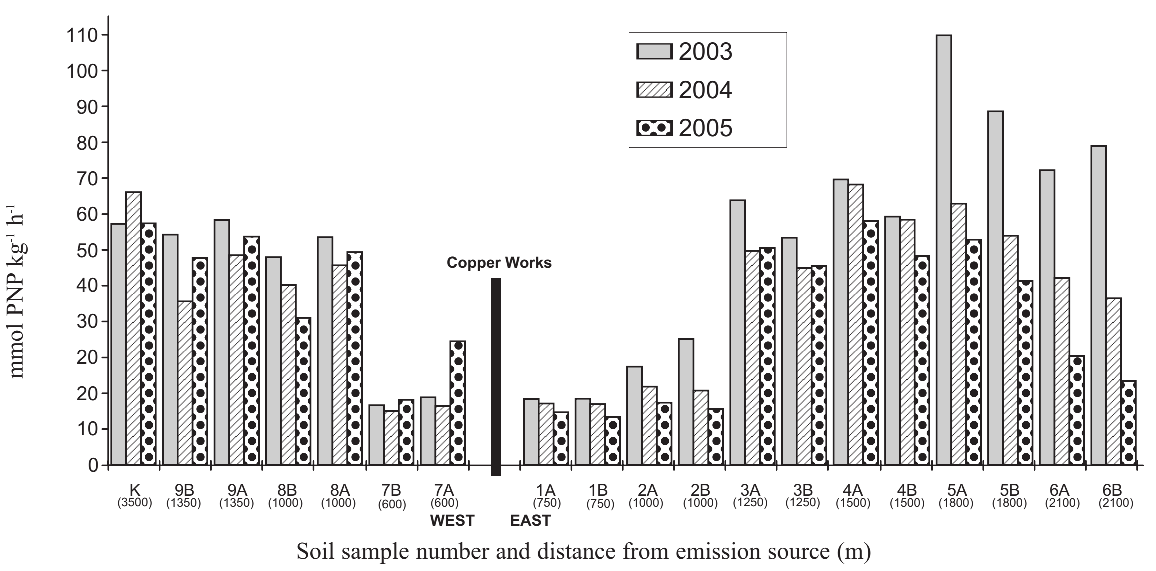

Fig. 6. Activity of acid phosphatase in the analyzed soil samples.
A – soil samples from the depth of 0 – 10 cm
B - soil samples from the depth of 10 – 30 cm
Soil sample number and distance from emission source (m).

Mocek and Owczarzak [28] call attention to the fact that a high content of organic matter in the soil 'buffers' the effect of external factors. Seasonal changes in the enzymatic activity of the studied soils resulted from a differentiated resistance and sensitivity of the particular enzymes both to natural and anthropogenic factors [19, 29]. In these studies, a significant increase of acid phosphatase and urease was visible in autumn, while protease increased its activity in spring (Figs. 6, 7, 9). Studies of many authors [1, 30, 31, among others] indicate that the factors limiting the biological activity of soils are temperature or soil moisture level. Kramer and Green [32] have proven that the activity of phosphatase significantly depends on seasonal changes of temperature and soil moisture. A successive factor modifying the activity in the studied soils could be the differentiated species composition of the vegetative cover that influences the accumulation in the soil of substrates specific of the enzymatic reactions. Dahm and Rydlak [33] noted that the synthesis of protease is impeded by root secretions of plants, particularly by arborescent ones. In the root zone of poplars, the presence of proteolytic bacteria was not found at all, on the other hand, in case of pine, those bacteria occurred only sporadically.

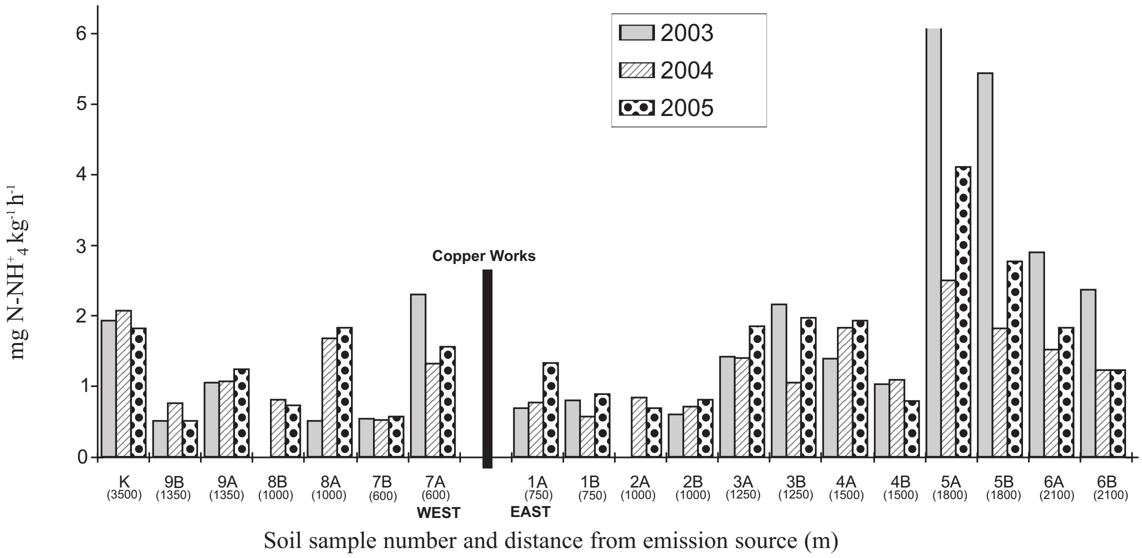

Fig. 7. Activity of urease in the analyzed soil samples.
A – soil samples from the depth of 0 – 10 cm
B - soil samples from the depth of 10 – 30 cm
Soil sample number and distance from emission source (m).

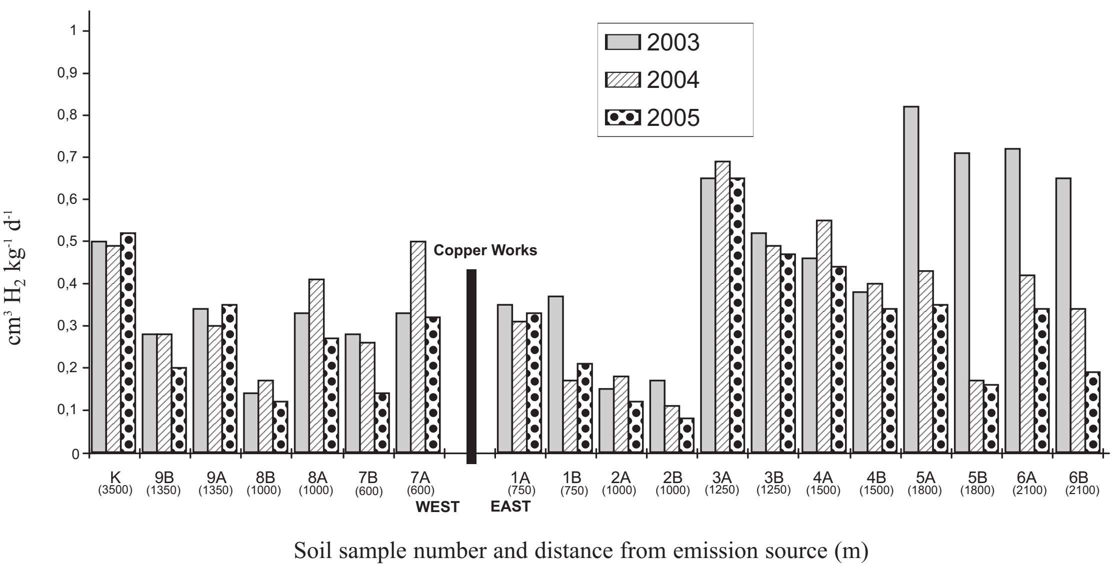

Fig. 8. Activity of dehydrogenases in the analyzed soil samples.
A – soil samples from the depth of 0 – 10 cm
B - soil samples from the depth of 10 – 30 cm
Soil sample number and distance from emission source (m).

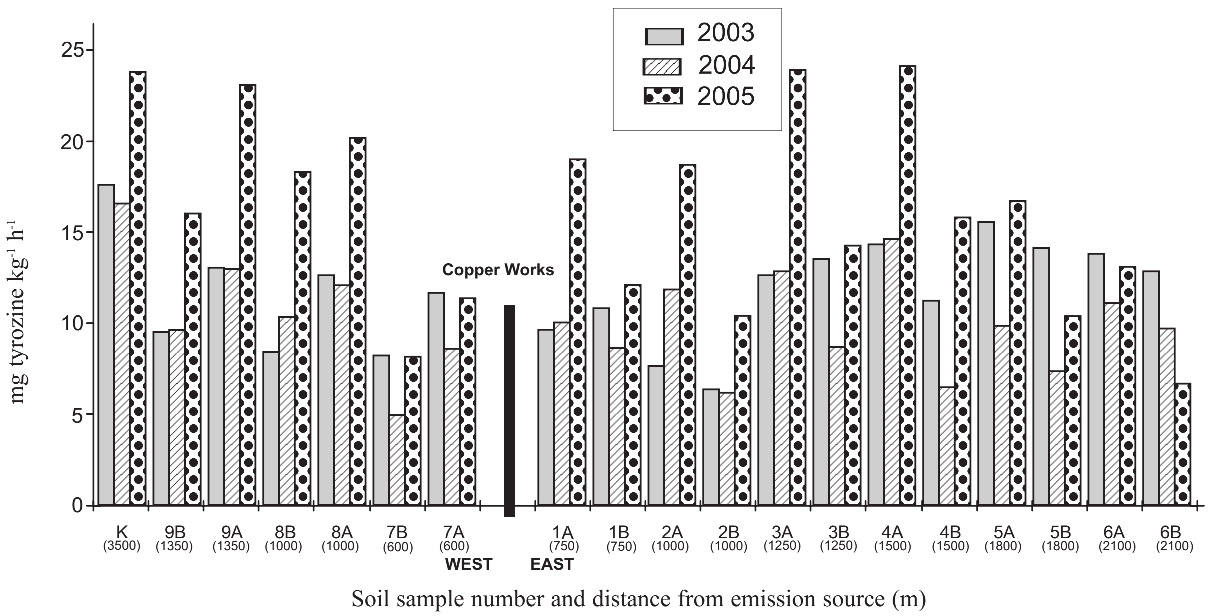

Fig. 9. Activity of protease in the analyzed soil samples.
A – soil samples from the depth of 0 – 10 cm
B - soil samples from the depth of 10 – 30 cm
Soil sample number and distance from emission source (m).

Among all studied enzymes, the activity of acid phosphatase was the most sensitive indicator of the analyzed soil contamination by heavy metals. This was confirmed by highly significant values of the coefficient of negative correlation between the particular features of soils (Tables 1 and 2). Similar results were obtained by Bielińska and Domżał [34], who found a reverse linear relation between the activity of phosphatases and the content of Cd, Cu, Pb and Ni. The mentioned authors also showed that phosphatase was the most sensitive enzyme to soil pollution by heavy metals. Similar regularities were found in the present work. Numerous data from the subjective literature [5, 35-37] also confirm the negative effect of heavy metals on the activity of phosphatases.

In our own studies, also a negative correlation was obtained between the activity of dehydrogenases and the content of Cu and Pb in soils (Table 1) The phenomenon of the increase of dehydrogenase activity in soils contaminated by heavy metals was also observed by Januszek [19], while Welp [36] or Rogers and Li [37] found a high sensitivity of dehydrogenases to the content in the soil of Cr, Cu, Zn, Hg and Pb.

Heavy metals doubtlessly belong to compounds with a high degree of threat for the regular functioning of live organisms. Their high harmfulness results mainly from the action on protein – by impeding the activity of enzymes, damaging the chains of nucleic acids and by a tendency to accumulate in the tissues of live organisms. According to Hoffmann [2], enzymatic activity is a better measure of fertility and productivity of soil than other biological indicators (among others, respiratory activity or the number of microorganisms), since it reflects the present and the past biological status of soil [38].

Conclusions

1. Upper horizons of soils covered by the studies were characterized by high contamination by Cu and Pb, however the amounts of these metals decreased with the distance from the emitters of gases and metal-carrying dusts.
2. In the analyzed soil material it has been confirmed that there exist significant correlations between enzymatic activity of dehydrogenases, acid phosphotase and urease, on the one hand, and the content of organic carbon and total nitrogen, on the other. With the increase of heavy metals content, the enzymatic activity of soil decreased.
3. Acid phosphatase has proven to be the best indicator permitting to estimate the drop of biological life activity in the humus horizons of soils strongly contaminated by Cu and Pb in both depth zones (0-10, 10-30 cm).
4. From an ecological point of view, it is significant that low enzymatic activity of soils in the vicinity of copper works occurred in the period of three successive years of studies, which testified that this status of the soil has been fixed.

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