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EFFECTS OF HEAVY METALS ON THE ACTIVITY OF DEHYDROGENASES, PHOSPHATASES AND UREASE IN NATURALLY AND ARTIFICIALLY CONTAMINATED SOILS

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Abstract

Most of the processes occurring in soil are catalysed by enzymes. As a result of their sensitivity towards heavy metals, enzymes in contaminated soils are usually less active. The purpose of this paper was to assess the influence of bioavailable forms of Cd, Cu, Pb and Zn on the activity of dehydrogenases, urease, acid and alkaline phosphatase, and to compare the results obtained from naturally and artificially contaminated soils. A pot experiment was carried out on two loamy sand soils, naturally and artificially contaminated with Cd, Cu, Pb and Zn. The total content of heavy metals classified these soils as very heavily contaminated with Cu, heavily contaminated with Pb and contaminated with Cd and Zn, all according to the IUNG system (1995). One of the following organic materials: swine manure or triticale straw, was added to the soil batches. The experiment was carried out in three replications, in two pH ranges: slightly acid and acid. Soil samples for analyses were taken after 14, 28, 165 and 450 days of incubation. The results of the experiment showed that the activity of soil enzymes depended on the content of bioavailable heavy metals; the total concentration of trace elements and H^+ were less important. However, considerable differences were found in enzyme activity between naturally and artificially contaminated soils. This indicates that results obtained from other research conducted on freshly contaminated soils cannot be easily transferred to field conditions. The analysed enzymes responded differently to the concentration of bioavailable forms of heavy metals. Alkaline phosphatase was the least tolerant to bioavailable forms of heavy metals, unlike urease, which was the most tolerant soil enzyme. A similar pattern of sensitivity toward trace elements, which could be ordered as Zn > Cd > Cu > Pb, was noticed for dehydrogenases, acid and alkaline phosphatases. Urease was found to be more tolerant to Zn.

Keywords: enzyme activity, dehydrogenases, phosphatase, urease, soil contamination, bioavailable forms of trace elements.

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INTRODUCTION

An increasing concentration of active pools of heavy metals in soil, caused mainly by industries and agriculture, distorts naturally occurring processes in this environment (Tiller 1989). Among the consequences are disturbances in organic matter transformations, which lead to the accumulation of physically uncomplexed organic matter. A decreased rate of litter decomposition near the source of a pollutant has been reported in numerous studies (Baath 1989, Cotrufo et al. 1995, Wiatrowska et al. 2013), where it is linked to the inhibitory influence of heavy metals on soil biota, affecting their diversity, abundance and activity.

The biogeochemical circulation of elements, including decomposition and transformation of organic matter, is catalysed by soil enzymes, which are very sensitive to heavy metal accumulation in the environment (Kucharski, Wyszkowska 2004). It should be emphasised that relationships between heavy metal concentrations and soil enzymatic activity are not as simple as could be expected. The influence of trace elements on soil biota depends not only on their total concentration but also on the chemical form of a given element (speciation). Numerous investigations on interactions between heavy metals and soil enzymes have been conducted on artificially contaminated soils. In such conditions, the dominant form of elements in soil matrix are free ions, which are most toxic to living organisms (Stephan et al. 2008). Additionally, the reported data usually refer to total metal concentration, not to active forms. The results obtained from such research projects cannot be directly translated into field conditions, where the pollution process has lasted for a long time and aging processes have occurred, leading to a decreasing content of active forms of trace elements.

The aim of this study was to assess the influence of bioavailable forms of Cd, Cu, Pb and Zn on the activity of selected soil enzymes (dehydrogenases, urease, acid and alkaline phosphatase). Another purpose of this project was to establish to what extent results obtained from experiments run on freshly metal-spiked soil could be applied to field conditions. For this purpose, the research project was conducted both on naturally contaminated soil, which since early the 1970s has been receiving huge amounts of dust rich in trace elements, and on artificially metal-spiked soil.

MATERIAL AND METHODS

Soil characteristic and experiment design

The research project was set up as a pot experiment, where two loamy sand soils, classified as Haplic Albeluvisol, sampled from the surface horizon (0-20 cm), were used. The characteristics of the soils are given in Table 1.

Table 1

Selected physicochemical properties of the soils studied

Sampling site	Soil texture			pH_{H_2O}	C_{org}	CEC	Total concentration			
	sand	silt	clay				Cd	Cu	Pb	Zn
	(%)				(g kg^{-1})	(mmol(+) kg^{-1})	(mg kg^{-1})			
Naturally contaminated soil	82	17	1	6.92	59.60	143.9	4.06	2500	1267	414.5
Artificially metal-spiked soil	82	17	1	6.65	16.10	34.80	2.01	24.65	40.53	21.12

C_{org} – organic carbon, CEC – cation exchange capacity

The first batch of naturally contaminated soil was sampled from Wróblin Głogowski, an area affected by metallurgical fallout from the Głogów Copper Smelter. The Głogów Smelter began production in 1971, emitting into the atmosphere dust containing large amounts of trace elements, mainly Cu, Pb, Zn, Cd and As (Grzebisz et al. 2001, Jaworska, Dąbkowska-Naskręt 2012). The second soil batch originated from an area unaffected by industrial emission (Granowo village) and was artificially contaminated with $Cd(NO_3)_2$, $Cu(NO_3)_2 \cdot 6H_2O$, $Pb(NO_3)_2$ and $Zn(NO_3)_2 \cdot 6H_2O$. Trace elements were incorporated in such doses as to achieve a similar level of contamination as detected in the soil sampled from Wróblin Głogowski. Both soils used in the pot experiment showed the 5th degree contamination (very heavily contaminated) for Cu, 4th degree (heavily contaminated) for Pb and 3rd degree (contaminated) for Cd and Zn, all according to the Polish standard (Kabata-Pendias et al. 1995). Artificially metal-spiked soil was pre-incubated for 2 weeks, in room temperature (20-23°C) and soil moisture set at 50-60% of field capacity. Then the soil pH of both soils was corrected to achieve slightly acidic (pH 6.4±0.4) and acidic conditions (pH 5.5±0.4). In the case of artificially contaminated soil, adequate doses of $Ca(OH)_2$ were added to increase soil pH, as the hydrolysis of the added salts lowered the soil pH. The other soil was added HCl to decrease its pH down to the established level of soil acidity. After an additional 4 days, one of the two organic materials chosen for the tests was added to the soils: swine manure (at a dose of 40 Mg ha^{-1}) or triticale straw (at a dose of 4.5 Mg ha^{-1}). The experiment was set up in three replicates.

The combinations were incubated for 450 days, during which time the soil moisture was monitored and water was replenished by irrigation. Soil samples for analyses were taken after 14, 28, 165 and 450 days of incubation.

Total organic carbon was determined by dry combustion in a Multi N/C 3100 (Analytikjena). Soil pH was measured in 1:1 soil to water suspension. Cation exchange capacity (CEC) was determined by a modified Mehlich method as the sum of extractable bases with a mixture of $BaCl_2$-triethanolamine and extractable acidity with sodium acetate (Thomas 1982). Total concentrations of Cu, Cd, Pb and Zn were determined by atomic absorption

spectrometry after acid digestion, in *aqua regia* followed by hydrofluoric and boric acids (Lim, Jackson 1982). The bioavailable pool of heavy metals was analysed according to 5ISO/TS 21268-1 protocol (2007).

Determination of the enzymes activity

The activity of phosphatases were determined according to the protocol described by Tabatai and Bremmer (1969) with 4-nitrophenylophosphate disodium as a substrate. The activity of dehydrogenases was assessed as described by Tabatabai (1982) with 2,3,5-triphenyltetrazolium chloride. The substrate used for determination of the urease activity was urea (Zantua, Bremner 1975).

Statistical analysis

A principal component analysis and data clustering were carried out for the soils' properties (pH, moisture, temperature), heavy metal concentration (total, bioavailable forms) and enzyme activity in order to indentify factors altering the activity of soil enzymes.

RESULTS AND DISCUSSION

The results indicate that the activity of acid and alkaline phosphatases as well as urease and dehydrogenases depended on the content of the bioavailable pool of heavy metals, whereas the others factors, like soil pH and the total content of trace elements, were less important. A significant reduction in enzyme activity was observed in samples with a high concentration of bioavailable (active) forms of heavy metals (Figures 1-4). Additionally, the activity of soil enzymes varied throughout the experiment in line with the concentration of the bioavailable pool of trace elements. The enzymes tested showed the highest sensitivity to the concentration of active forms of Zn and were the least sensitive to the content of Pb. Taking into consideration our current knowledge suggesting that Pb is not an essential element for living organisms, it was expected to obtain a stronger relation between this metal and enzyme activity. However, Pb ions in soil show higher affinity to a sorption site than the other elements studied (McBride 1989, Li, Shuman 1997, wiatrowska el al. 2011). As Pb is strongly attached to the soil surface, the concentration of its bioavailable forms is significantly lower than that of Zn and Cd. This pattern was specifically noticeable in artificially contaminated soil. The better solubility of Zn and Cd compounds and their weaker affinity to the solid soil phase ensured their high mobility (Komisarek, Wiatrowska, 2009). The share of the bioavailable Cd form in artificially contaminated soil reached around 30% in acidic and 25% in slightly acidic conditions (Table 2). In the case of Zn, this share was 22% and 20%, respectively. On the basis of

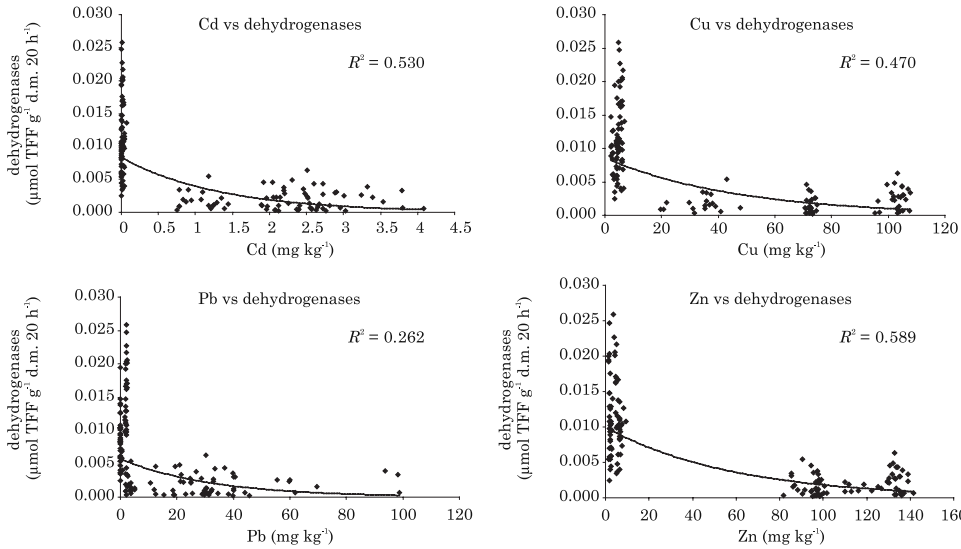

Fig. 1. Activity of dehydrogenases versus the bioavailable heavy metal content

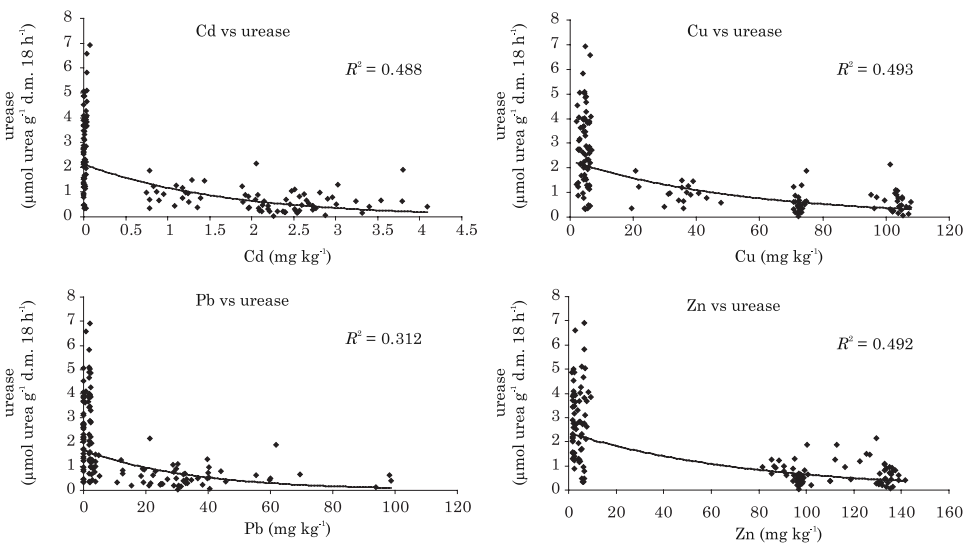

Fig. 2. Activity of urease versus the bioavailable heavy metal content

these results, a stronger inhibitory effect on soil enzymes was expected from Cd. Nonetheless, the results obtained from naturally contaminated soil showed a higher mobility of Zn than Cd, which could partially explain the higher values of the R^2 factor for zinc. Similar results for zinc were reported by STEPHAN et al. (2008), who pointed out to the high toxicity of Zn^{2+} ions to biota, similar to Al^{3+} and Mn^{2+}.

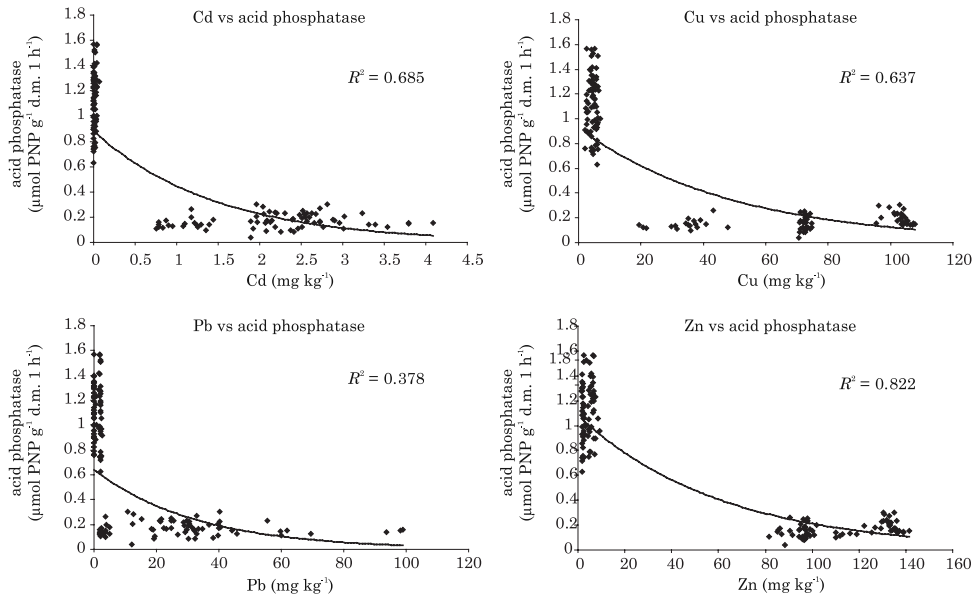

Fig. 3. Activity of acid phosphatase versus the bioavailable heavy metal content

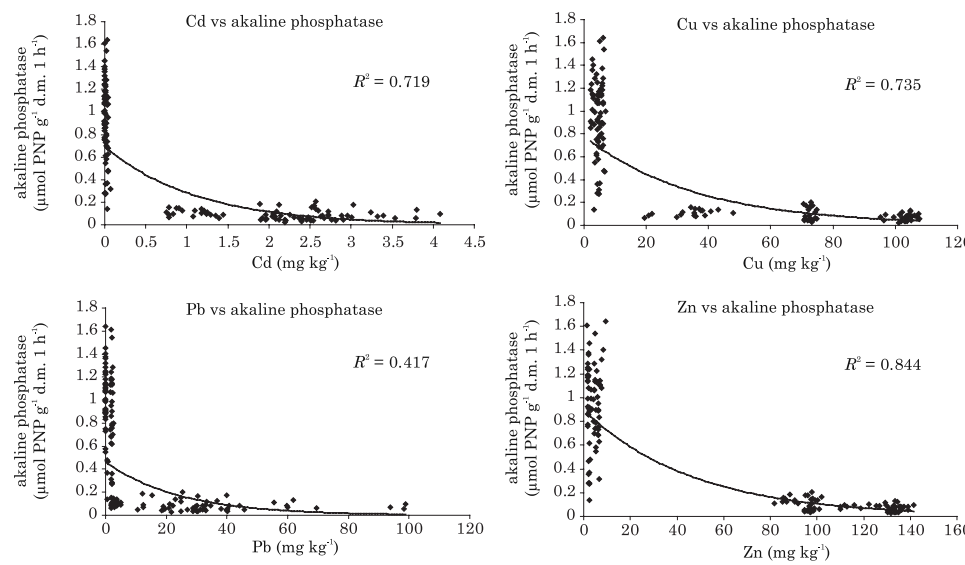

Fig. 4. Activity of basic phosphatase versus the bioavailable heavy metal content

Our comparison of the activity of particular enzymes between naturally contaminated and artificially metal-spiked soils showed statistically significant differences ($p > 0.05$). The activity of dehydrogenases was 5.4 and 5.9 times higher in naturally contaminated soil, in slightly acidic and acidic

Table 2

Content of bioavailable forms of heavy metals in soil in respect to their total content (%)

Combinations	14	28	165	450
	days of incubation			
Cd				
N-A	0.000	0.345	1.281	0.345
NM-A	0.324	0.288	0.647	0.000
NS-A	0.196	0.539	0.735	0.049
N-SA	0.000	0.197	0.837	0.000
NM-SA	0.000	0.000	0.144	0.000
NS-SA	0.000	0.147	0.343	0.000
A-A	43.92	48.44	50.56	47.84
AM-A	26.98	28.79	22.08	26.79
AS-A	37.52	40.53	41.34	40.35
A-SA	32.69	35.92	16.02	14.73
AM-SA	24.95	25.25	11.83	10.39
AS-SA	37.13	38.83	19.19	17.34
Cu				
N-A	0.193	0.232	0.185	0.190
NM-A	0.183	0.208	0.175	0.129
NS-A	0.213	0.243	0.203	0.112
N-SA	0.147	0.200	0.239	0.111
NM-SA	0.227	0.209	0.176	0.124
NS-SA	0.191	0.225	0.160	0.090
A-A	4.439	6.411	6.361	4.444
AM-A	4.308	6.116	5.909	4.282
AS-A	4.382	6.287	6.264	4.368
A-SA	4.372	6.205	1.709	2.355
AM-SA	4.244	5.962	1.551	2.136
AS-SA	4.366	6.206	2.111	2.430
Pb				
N-A	0.000	0.170	0.150	0.000
NM-A	0.000	0.183	0.129	0.000
NS-A	0.000	0.186	0.159	0.000
N-SA	0.000	0.144	0.051	0.000
NM-SA	0.000	0.169	0.167	0.000
NS-SA	0.000	0.152	0.125	0.000
A-A	8.720	12.50	10.40	8.071
AM-A	4.331	3.656	3.302	2.886
AS-A	6.350	5.685	5.921	4.790
A-SA	5.556	4.894	0.465	0.564
AM-SA	3.273	2.922	0.403	0.360
AS-SA	5.475	5.416	0.662	0.547

cont. Table 2

Zn				
N-A	1.547	1.188	1.574	1.934
NM-A	1.136	0.854	1.075	1.429
NS-A	1.471	1.096	1.410	1.791
N-SA	0.468	0.406	0.618	0.555
NM-SA	0.555	0.393	0.510	0.534
NS-SA	0.443	0.409	0.580	0.528
A-A	18.50	25.50	25.98	18.72
AM-A	16.39	22.46	22.77	15.97
AS-A	18.21	25.04	25.47	18.21
A-SA	17.95	24.65	21.14	16.25
AM-SA	16.26	22.15	19.80	15.23
AS-SA	18.16	24.96	22.75	16.59

N-A – naturally contaminated soil without any organic material – acid soil reaction
NM-A – naturally contaminated soil with manure – acid soil reaction
NS-A – naturally contaminated soil with triticale straw – acid soil reaction
N-SA – naturally contaminated soil without any organic material – slightly acid soil reaction
NM-SA – naturally contaminated soil with manure – slightly acid soil reaction
NS-SA – naturally contaminated soil with triticale straw – slightly acid soil reaction
A-A – artificially contaminated soil without any organic material – acid soil reaction
AM-A – artificially contaminated soil with manure – acid soil reaction
AS-A – artificially contaminated soil with triticale straw – acid soil reaction
A-SA – artificially contaminated soil without any organic material – slightly acid soil reaction
AM-SA – artificially contaminated soil with manure – slightly acid soil reaction
AS-SA – artificially contaminated soil with triticale straw – slightly acid soil reaction

conditions, respectively. The organic materials applied to soil did not have any notable impact on the differences observed between the above soils, except the variant with triticale straw in acidic condition, where a large difference was observed, namely the activity of dehydrogenases was 11 times lower in artificially than in naturally contaminated soil. This was caused by a lower activity of dehydrogenases in artificially contaminated soil with triticale straw under acid soil pH. In this combination, neither an increase in the concentration of bioavailable forms of heavy metals (Table 2), nor a drop in pH in comparison to artificially contaminated soil without organic material occurred that could explain the reduced activity of dehydrogenases, indicating that other factors inhibited the activity of dehydrogenases in this combination. In the case of acid phosphatase, an almost 8-fold higher activity was detected in naturally contaminated soil (Table 3). It was only in the combinations of artificially contaminated soil with one of the organic materials added that an increased activity of this enzyme and, consequenly, smaller differences between the soils were observed. This pattern was even more visible in slightly acid soil. Major differences were also noted between the soils in terms of alkaline phosphatase. For this type of phosphatase, a small influence of soil pH on enzyme activity was observed. Artificially contaminated soil exhibited 13 and 11 times lower activity of alkaline phosphatase in

Table 3

Enzyme activity at particular sampling days

Combinations	14	28	165	450
	days of incubation			
Dehydrogenases (μmol TFF g^{-1}d.m. 20 h^{-1})				
N-A	0.008	0.018	0.011	0.010
NM-A	0.004	0.023	0.011	0.008
NS-A	0.009	0.018	0.013	0.009
N-SA	0.009	0.015	0.006	0.009
NM-SA	0.010	0.015	0.014	0.010
NS-SA	0.006	0.017	0.007	0.012
A- A	0.002	0.004	0.001	0.001
AM-A	0.004	0.005	0.001	0.001
AS- A	0.001	0.002	0.001	0.001
A-SA	0.002	0.003	0.002	0.001
AM-SA	0.001	0.003	0.001	0.003
AS-SA	0.001	0.003	0.001	0.003
Urease (μmol urea g^{-1}d.m. 18 h^{-1})				
N-A	0.735	2.073	5.804	3.205
NM-A	1.900	2.188	4.260	2.696
NS-A	0.388	1.744	2.806	4.074
N-SA	1.945	2.436	4.867	2.376
NM-SA	2.447	2.777	4.264	1.749
NS-SA	2.895	2.734	4.040	3.103
A- A	0.402	0.417	0.495	0.905
AM- A	0.545	0.397	0.828	0.572
AS- A	0.423	0.223	0.715	0.789
A-SA	0.455	0.407	0.387	0.824
AM-SA	0.524	0.930	1.540	0.786
AS-SA	0.302	1.037	1.193	1.006
Acid phosphatase (μmol PNP g^{-1}d.m. 1 h^{-1})				
N-A	1.289	1.080	1.469	0.985
NM-A	1.167	1.202	1.226	1.004
NS-A	1.254	0.924	1.227	1.049
N-SA	1.210	1.004	1.134	1.257
NM-SA	0.894	0.820	1.308	0.964
NS-SA	1.328	1.209	0.934	0.934
A- A	0.106	0.162	0.150	0.196
AM- A	0.117	0.226	0.167	0.174
AS- A	0.102	0.224	0.183	0.208
A-SA	0.143	0.192	0.122	0.119
AM-SA	0.142	0.256	0.127	0.155
AS-SA	0.197	0.239	0.171	0.187

cont. Table 3

Alkaline phosphatase (μmol PNP g^{-1}d.m. $1h^{-1}$)				
N-A	0.827	1.275	0.631	1.353
NM-A	0.676	0.896	0.981	1.075
NS-A	0.952	1.015	0.720	1.219
N-SA	1.062	1.075	0.439	1.233
NM-SA	0.999	0.890	0.416	1.164
NS-SA	1.256	1.345	0.234	0.887
A- A	0.071	0.085	0.089	0.138
AM- A	0.049	0.085	0.074	0.189
AS- A	0.052	0.070	0.080	0.156
A-SA	0.047	0.066	0.072	0.116
AM-SA	0.057	0.056	0.090	0.149
AS-SA	0.046	0.046	0.096	0.130

Key under Table 2

acidic and slightly acidic conditions, respectively. The organic materials added to soil, except straw in the acid pH range, reduced differences between the soils by raising the activity of alkaline phosphatase in contaminated soil. The urease activity was usually about 5 times lower in artificially than in naturally contaminated soil (Table 3). Similarly to acid phosphatase, smaller differences between the soils were found in combinations with organic materials, which stimulate retention processes of trace elements. Moreover, in the higher pH range, as the concentration of the bioavailable pool of metals decreased, a rise in the urease activity was observed.

Among the analysed enzymes, the strongest relationship with the active forms of heavy metals were exhibited by phosphatases. Alkaline phosphatase was the most sensitive soil enzyme. Urease and dehydrogenases were less sensitive to the concentration of bioavailable heavy metals than phosphatases, which could have been caused by some specific characteristics of the enzymes. The highest resistance to the contamination with heavy metals was presented by urease. Similar results were also reported by Wyszkowska el al. (2009) and Kucharski et al. (2011). The results obtained herein showed that dehydrogenases and acid and alkaline phosphatases demonstrated similar sensitivity to heavy metals: Zn > Cd > Cu > Pb. The sensitivity of urease was slightly different and could be arranged in the following order: Cu > Zn > Cd > Pb. These results contradict the ones presented by Wyszkowska et al. (2013). The discrepancies might have been caused by the fact that the cited authors related enzyme activity to the total content of heavy metals, and not to their bioavailable pool. According to Wyszkowska et al. (2013), differences in the sensitivity of soil enzymes to trace elements reported by various authors could result from their using soil with different texture. Considering the fact that soil texture is one of the most important factors influencing

sorption properties of soils, it could suggest that different amounts of active forms of heavy metals were studied.

Soil pH considerably affects biological processes occurring in soils. On the one hand, soil reaction controls mobility of heavy metals, which are well-known enzyme inhibitors. On the other hand, many soil enzymes respond to the H^+ concentration in soil solution (TABATABAI 1982). KUCHARSKI et al. (2011) demonstrated stronger inhibition of the activity of dehydrogenases at pH 5.5 than at pH 7. At the dose of 500 mg of Zn (3rd degree - contaminated soil), the activity of those enzymes fell by 71% and 65%, respectively to the soil pH. In our study, no influence of soil pH on the activity of dehydrogenases and other enzymes was found. These contradictory results could be partially explained by a relatively narrow pH range (slightly acid and acid soils) analysed in this project.

In order to identify factors which alter the activity of soil enzymes and to verify the results obtained in this study, data clustering and principal component analysis (PCA) were applied. The PCA analysis showed that the

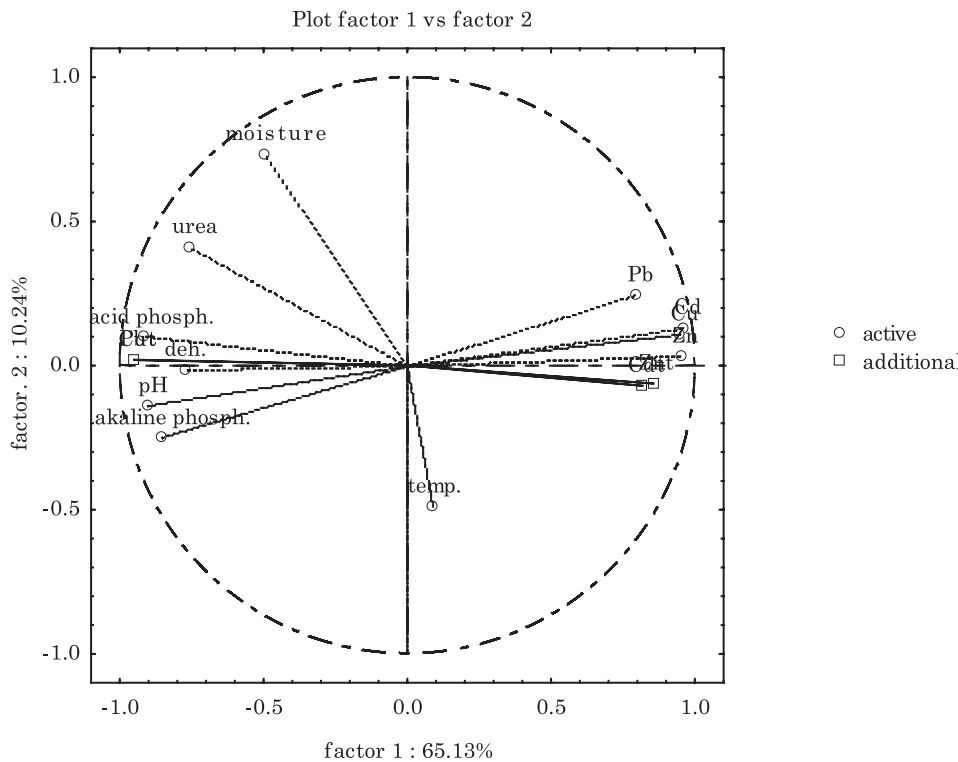

Fig. 5. Principal component analysis: Cd, Cu, Pb, Zn – concentration of bioavailable forms of particulate elements, Cdt, Cut, Pbt, Znt – total content of particulate elements, temp. – soil temperature in °C, moisture – gravimetric water content in soil, alkaline phosph. – activity of alkaline phosphatase, acid phosph. – activity of acid phosphatase, deh. – activity of dehydrogenases, urea – activity of urease

activity of enzymes was negatively correlated with the concentration of the bioavailable pool of heavy metals and the total content of Cd and Zn. In turn, a positive correlation was found with the total content of Pb and Cu (Figure 5). The data clustering results underline the relation between the activity of soil enzymes and amounts of bioavailable forms of Cd, Cu, Pb and Zn (Figure 6) and a weak association with the total content of Zn, Pb and Cu. The statistical analyses confirmed that enzyme activity should not be correlated to the total concentration of trace elements but to their mobile pool, as the behaviour of trace elements in the soil environment depends on their chemical forms and less so on their total content.

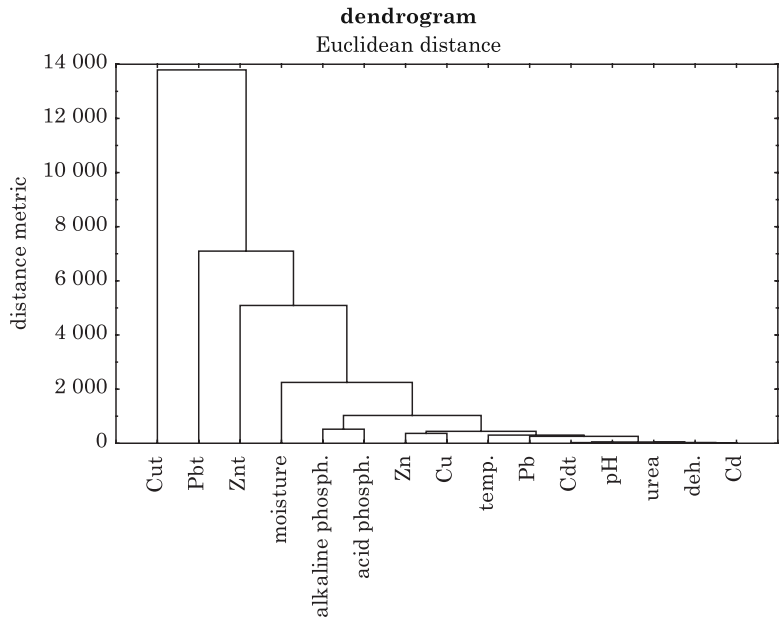

Fig. 6. Dendrogram from data clustering analysis (explantation see Fig. 5)

CONCLUSIONS

1. The activity of soil enzymes was strongly modified by bioavailable pools of heavy metals. Weak correlation was found between the total metal content and enzyme activity.

2. Very large differences between the enzyme activity in artificially metal-spiked and naturally contaminated soil show that results obtained from pot experiment conducted on freshly contaminated soil cannot be directly transferred to field condition, where aging processes occur, leading to a lower mobility of metals.

3. Dehydrogenases, acid and alkaline phosphatases exhibited the highest

sensitivity toward Zn and it decreased in the order of metal concentrations: Zn > Cd > Cu > Pb. In contrast, urease was more tolerant to Zn. The sensitivity of urease was as follows: Cu > Zn > Cd > Pb.

4. For better comparison of the results obtained by different authors, activity of soil enzymes should be related to the mobile forms of heavy metals, not to their total content. This approach would be more appropriate in light of to the current knowledge about trace elements behavior in soils.

5. In respect of their sensitivity to concentrations of the bioavailable pool of Cd, Cu, Pb and Zn, the enzymes can be arranged as follows: alkaline phosphatase > acid phosphatase > dehydrogenases > urease.

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