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ENZYME ACTIVITIES IN SOIL AT INCREASING METAL (CU, NI, PB) DOSES AND TIME-DEPENDENCE IN A MODELL EXPERIMENT

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ABSTRACT

Aim of this paper is to examine the effect of spiked copper (Cu), nickel (Ni) and lead (Pb) metal salts on the dehydrogenase (oxydo-reductase) and phosphatase (hydrolase) enzyme activities in a characteristic Hungarian soil, the pseudomycelliar chernozem. Pot-experiment was performed with a soil, originating from a spot of the Hungarian soil-information-monitoring (TIM) system of Bicserd. The added metal salts were used in water soluble forms and incorporated uniformly to the soil. Soils were treated with increasing metal concentrations to give the following metal amounts: 0, 50, 200, and 800 kg.ha^{-1}. Enzyme activities of the soil were analysed at the 0th, 7 th, 14 th, and 28th days after the metal addition. The laboratory model-experiment has been set up in three replicates.
Effects of metal salts were largely dependent on the chemical and physical properties of pseudomycelliar chernozem soil, the applied heavy metal-types, the doses of used metals and the elapsed time after the pollution. Considering the different metals, the copper prowed to be the most toxic one on the studied enzyme activities, whereas the lead induced those. By comparison with copper the nickel affected a smaller decrease in the soil microbial activity. The dehydrogenase, oxydo-reductase enzyme was found to be more sensitive parameter in comparison with the phosphatase, hydrolase enzyme among the studied condition. Studied enzymes and used methods are suggested, as fast and rather reliable tools for estimating the soil-resilience capacities at heavy metal pollution.

Keywords: soil, metal, contamination, phosphatase, dehydrogenase.

1. INTRODUCTION

Soil is the most important renewable natural resource. Protection of the soils in the Earth is one of the inevitable task nowadays and might be the key-issue of the sustainable agricultural practices [1]. For this reason on the basis of the UNESCO decision, 2015 is the annual year of the soils in the World. It is rather evident for nowadays, that soil-functioning is largely dependent on the soil biota, among them the soil-microorganisms. The activity of rarely measured microbial parts is resulted from the interactions among soil-physical-chemical- and biological parameters.
Metal pollutions can alter those soil-biological activities, and can reduce the soil-fertility and function. The use of soil biological markers related to microbial activity, for instance microbial biomass, enzyme activities are the most wide-spread [2] [3].Those parameters can be correlated with the soil quality and function. The soil-enzymology with other methods can contribute to better understanding of substance circulation in soil. Many studies reported about the effects influences the enzyme activities [4].
Metal contaminations might influence on the quantity indicators of the soil-biological activities and might affecting on the enzyme activities, as well. Since the enzyme activities of the soils are determined by the organisms living in the soil, alterations in the enzyme activities could indicate the biological activity and the real functioning of the soil. Thus, the current biological activity of soils could represent therefore the values of soil-quality, among them the status of metal pollutions.
Severity of metal pollution might be highly dependent on the binding form and availability of metals in the soils. The physico-chemical properties of metals and their affinity to the soil-particles are known to be

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crucial parameters [5]. In case of copper (Cu) for instance the form fixed to the organic substances is known to be the largest. Binded of lead (Pb) to the Fe and Mn oxids is also considerable. In case of the nickel (Ni) on the other hand, the exchangeable form is known to be the biggest.
The effect of increasing heavy metal doses were studied and characterised by the indigenous microbial enzyme activities in a main Hungarian soil, by an in vitro model-experiment. The phosphatase and dehydrogenase activities were representing the hydrolase and oxido-reductase types of enzymes in the soil. The study was concentrating on the interrelations between the physical-chemical soil characteristics, the studied soil-biological parameters and the metal contaminations in a characteristic soil in Hungary.

2. MATERIALS AND METHODS

2.1 Main characteristics of the pseudomycelliar chernozem soil

A characteristic Hungarian soil-type was used in the *in vitro* model-experiment. It is the pseudomycelliar chernozem from Bicsérd. The most frequent physical and chemical parameters of the soil are shown in the Table 1.

Table 1. Main soil physical and chemical properties of the characteristic pseudomyceliar chernozem soil from Hungary, Bicsérd). (Data of the Plant Health and Soil Conservation Service, Fejér county.)

Genetic soiltype	*pH KCl*	*pH H_2O*	*$CaCO_3$ ($mg.kg^{-1}$)*	*Humus (%)*	*K_A*	*P_2O_5 content ($mg.kg^{-1}$)*	*Clay (%)*	*Skilt (%)*	*Sand (%)*
Pseudomycelliar chernozem	7.04	7.84	1.90	2.05	45	22	31.50	33.70	34.90

2.2 Experimental conditions

The water-soluble salt of the copper-sulphate ($CuSO_4*5H_2O$, concentration = 39,29 g/100cm^3), nickel-chlorid ($NiCl_2*6H_2O$, concentration = 40,50 g/100cm^3) and lead-acetate ($(CH_3\text{-}COO)_2Pb*3H_2O$, concentration = 36,62 g/100cm^3) were added to the tested soil in vitro. The test-soil was treated with increasing metal concentrations: 0, 50, 200 and 800 $kg.ha^{-1}$. Enzyme activities of the pseudomycelliar chernozem were analysed on the 0^{th}, 7^{th}, 14^{th}, and 28^{th} days after the metal addition. The experiment was carried out in three replicates.
The heavy metal content of soils depend strongly on the physical type of the soil. Pseudomycelliar chernozem is a loamy soil, and the average Cu-content is 18 $mg.kg^{-1}$, the Ni-content is 20 $mg.kg^{-1}$, Pb-content is 16 $mg.kg^{-1}$ on the basis of Hungarian Soil Monitoring Survey [6]. The soil pH influenced largely the damaging effect of heavy metals. Therefore the pH of metal-solutions have been set up on the neutral pH with KOH. The own pH (KCl) of pseudomycelliar chernozem is 7.04 and the pH (H_2O) 7.84. Organic substances (humus content 2,05 %) increase the mobility of heavy metals in a pH neutral environment.

2.3 Methods used in the study

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The total substrate decomposition of soil-phosphatases has been determined by the method elaborated through Tabatabai and Bremner (1969) [7].

PNP (P-nitro-phenil-phosphate) —(Phosphatase enzymes)→ P-nitro-phenol

The soil-dehydrogenase activity characterizes accuratly the intensity of decomposition –processes of soil-microorganisms [8], and has been specified by the Hungarian standard [9].

TTC (2,3,5-Triphenil-tetrazolium-chlorid —(Dehydrogenase enzymes)→ 1,3,5-Triphenil-formazane

2.4The applied apparatus

The phosphatase and dehydrogenase enzyme activities has been assessed by a semi-automatic spectrophotometer (Type JASCO -530PC UV/VIS). Data were analysed with statistical program using method of min. square error.

3. RESULTS AND DISCUSSION

3.1Time-dependent dehydrogenase and phosphatase enzyme activities

Increasing doses of dehydrogenase and phosphatase enzyme activities were estimated during a monthly affecting periods. Data of the effects of 0 $kg.ha^{-1}$, 50 $kg.ha^{-1}$, 200 $kg.ha^{-1}$ doses are not shown. The effects of 800 $kg.ha^{-1}$ doses is shown in Figure 2. The dehydrogenase and phosphatase enzyme activities of the non-treated pseudomycelliar chernozem showed a similar values against time.
At the Cu doses of 50 $kg.ha^{-1}$ the dehydrogenase enzyme activity remaind about at the same level. Whereas, in case of Pb contamination the dehydrogenase enzyme activity extended until the 14^{th} day, then decreased under the value measured on the day of contamination. By this doses the phosphatase enzyme activity decreased at the Cu and Ni pollution, but increased at Pb pollution.
At the Cu doses of 200 $kg.ha^{-1}$ has been noticed an activation of soil-microorganisms on 7^{th} and 14^{th} day of contamination, then reduced the dehydrogenase enzyme activity. On the other hand the phosphatase enzyme activity decreased continuously.
The dehydrogenase enzyme activity decreased by comparison with control in the event of Cu and Ni pollution at the doses of 800 $kg.ha^{-1}$. This Pb doses increased the activity by 25%. Same tendency has been observed in the change of phosphatase enzyme activity.

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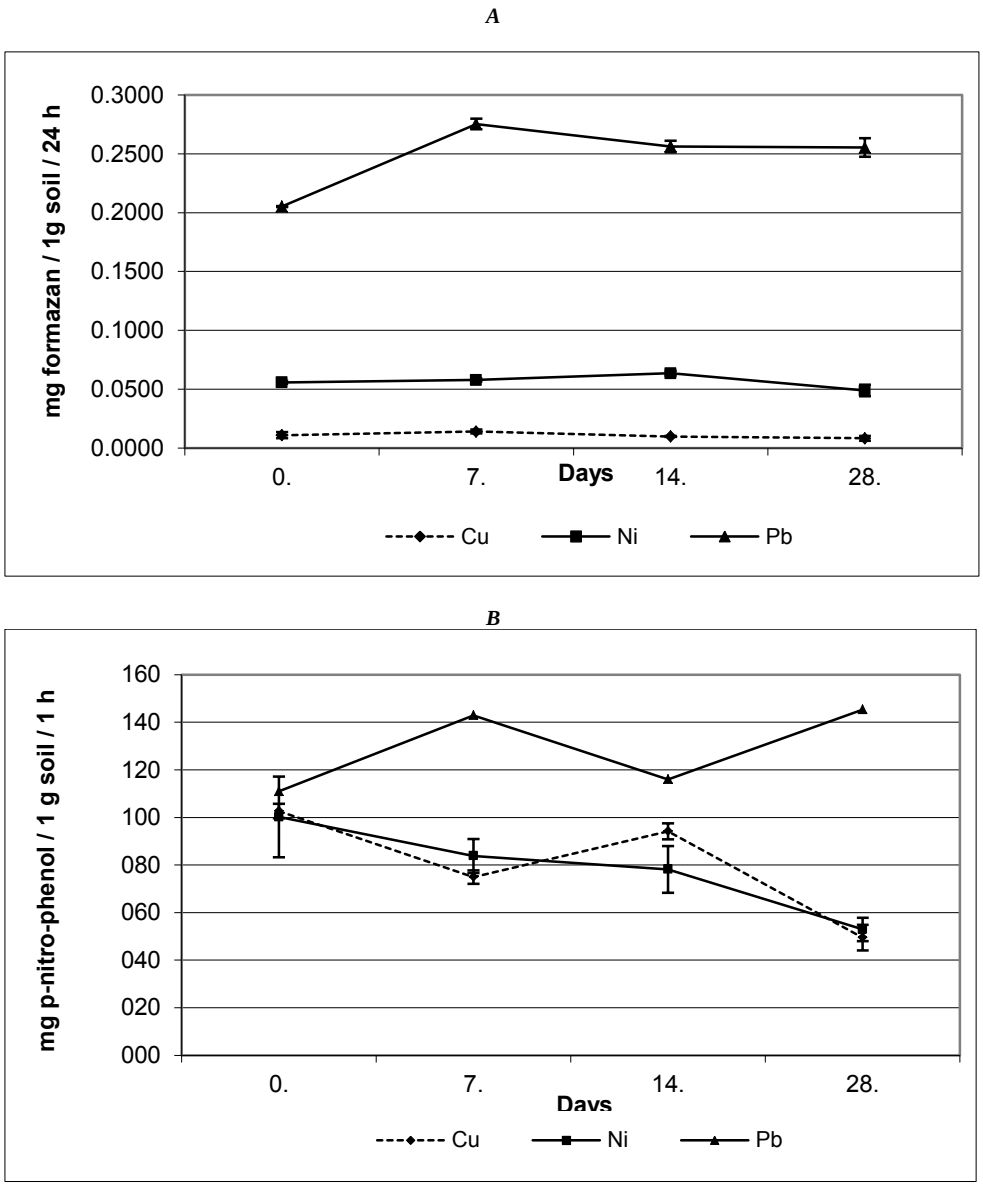

Figure. 2. The dehydrogenase (A) and phosphatase (B) enzyme activities of the tested pseudomycelliar chernozem at the doses of 800 kg.ha^{-1} plotted against the time.

3.2 Dose-dependent dehydrogenase and phosphytase enzyme activities

Figure 3. shows the measured enzyme activities simultaneously with the ratio of used metals and the severity of soil-contamination.

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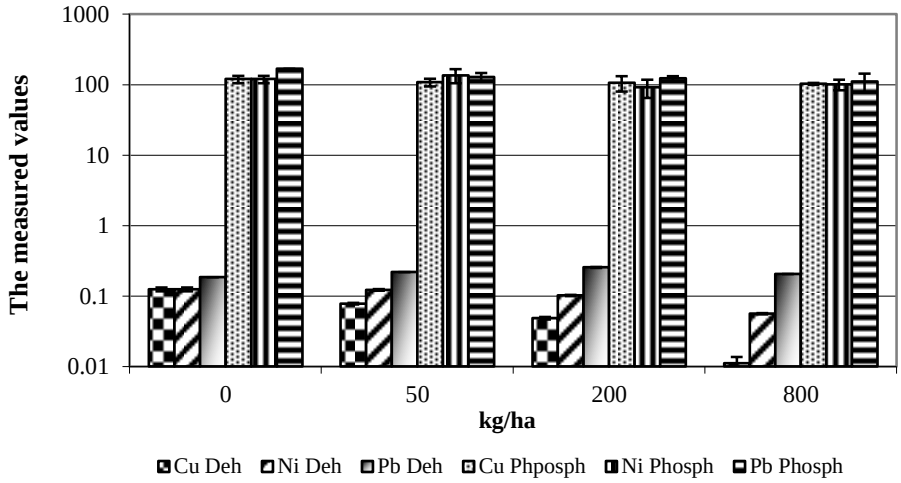
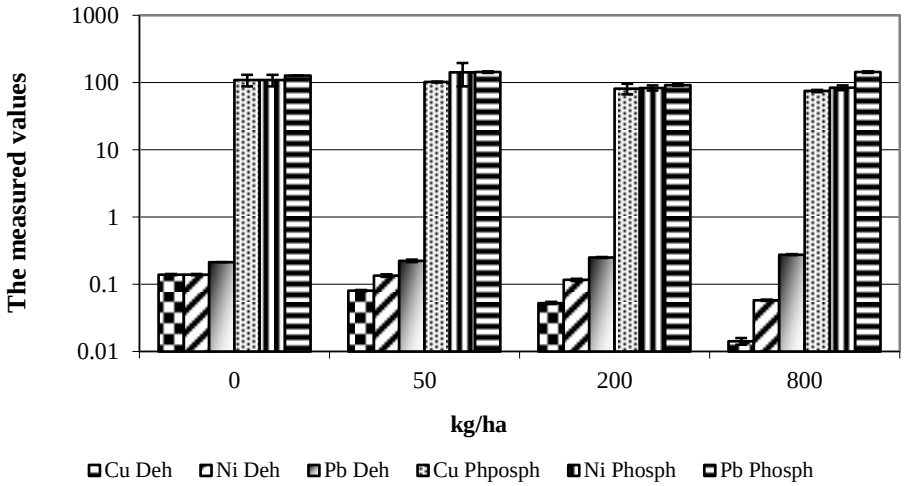
On the day of contamination (A) the phosphatase enzyme activity has been evenly reduced parallel with amount of contamination at all of three metals. Cu and Ni pollutions resulted the similar change of dehydrogenase enzyme activity to the phosphatase enzyme activity.
On the 7 th (B) day a regeneration has been observed. The doses of 800 kg.ha^{-1} has been result the largest effect: at the case of Cu and Ni decreased, while the Pb increased. On the 14th (C) and 28th (D) day of experiment the largest amount of Cu (800 kg.ha^{-1}) reduced the studied enzyme activities to the minimal level. Whereas the Pb increased that by 23%.

A: 0 day



B:7 day



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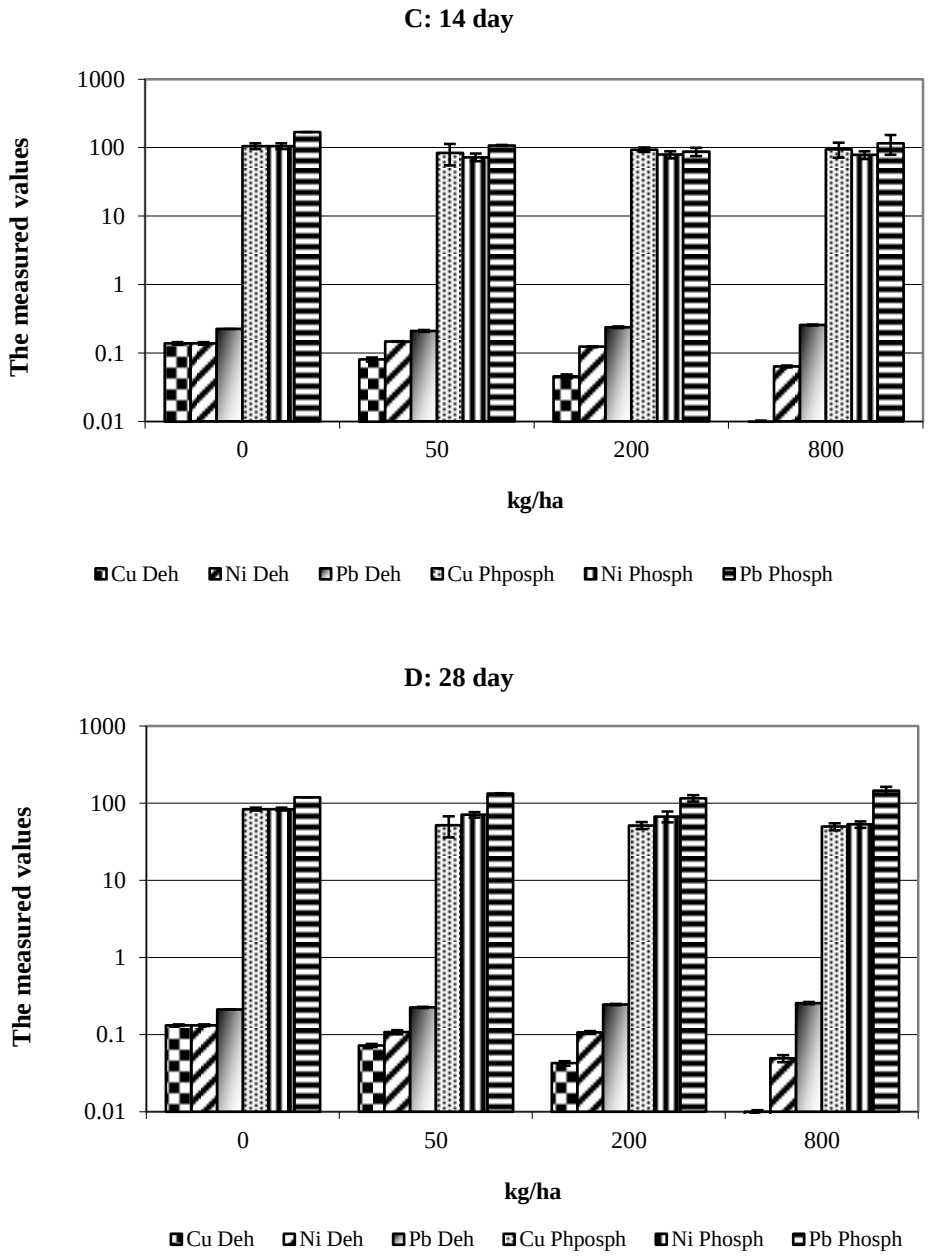

The measured values given: Phosphatase enzyme activity: mg p-nitro-phenol / 1 g soil / 1 h
Dehydrogenase enzyme activity: mg phormazan / 1g soil / 24 h

Figure. 3. The phosphatase and dehydrogenase enzyme activities of the tested pseudomycelliar chernozem at different times versus the extent of contamination.

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Three metals used in the experiment resulted different changes in the activity of soil-microorganisms. The copper was toxic on dehydrogenase and phosphatase enzyme activities in all cases, while the lead increased the two enzyme activities in 90% of variations of exposition times and treatments. The nickel effected a similar, but smaller change in the microbial activity of soil.
Increasing doses of Cu^{2+} and Ni^{2+} extended the dehydrogenase and phosphatase activity-changes, but proportionally to the amount of metals. In the event of Pb^{2+} dose-dependent has been found only by dehydrogenase activity.
The oxido-reductase-type dehydrogenase enzyme system proved to be more sensitive than the hydrolase-type phosphatase enzyme system in case of soils with large adsorption capacity.

4. CONCLUSIONS

The change of the enzyme activities of psedudomycelliar chernozem was very sensitive to the environmental conditions, and much faster to detect, as the quantitative change in the number of the microorganisms. The method of the determination of enzyme activities in case of metal pollutions were suitable to estimate the soil-toxicity. These experiments help us to establish a model for the natural ability of self-cleaning of soils and to detect the efficiency of remediation of the metal pollution.

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