Polish Journal of Environmental Studies Vol. 14, No 5 (2005), 659-664

Enzymatic Activities in Different Soils Contaminated with Copper

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Received: July 29, 2004
Accepted: January 28, 2005

Abstract

The effect of soil contamination with $CuSO_4 \cdot 5H_2O$ applied in the following doses of Cu kg^{-1}: 150, 300, 450 and 600 mg on the activity of dehydrogenases, urease and acid phosphatase and alkaline phosphatase was studied in a pot experiment. Two types of soil were examined: heavy loamy sand and silty sandy loam. The experiment was completed in two series: with yellow lupine cultivation and without plant cultivation. The enzyme activity in the soil samples was determined on days 14, 28, 42 and 56 of the experiment.

Based on the experimental results, soil contamination with $CuSO_4 \cdot 5H_2O$ doses of 150 mg, 300 mg, 450 mg and 600 mg Cu $\cdot kg^{-1}$ significantly inhibited the activity of dehydrogenases, urease and acid and alkaline phosphatases. Dehydrogenases and urease were found to be better indicators of soil contamination with copper than the other enzymes studied. Compared to phosphatases, dehydrogenases and urease appeared to be better indicators of soil contamination with copper, as their activity was more strongly inhibited by copper than the activity of phosphatases. Enzymatic activities were dependent on the type of soil. Dehydrogenases and acid phosphatase exhibited greater activity in heavy loamy sand, while the activity of urease and alkaline phosphatase was greater in the silty sandy loam. Cultivation of yellow lupine stimulated the activity of dehydrogenases and acid phosphatase in both soils as well as that of urease in heavy loamy sand. Soil contamination with copper had a very negative effect on the yield of yellow lupine.

Keywords: dehydrogenases, urease, acid phosphatase, alkaline phosphatase, soil enzyme activity, yellow lupine, soil contamination with copper

Introduction

Copper is an essential element for regular functioning of organisms. However, when present in excessive amounts it contributes to metabolism disturbances. In soil, it is bound to organic matter and clay and occurs in sulphides, sulphates and carbonates and is soluble in soil solution [1]. This element naturally occurs in soil but its concentration is increased resulting from the use of copper due to transfer from other sources such as plant protection agents, organic and mineral fertilizers [1, 2]. Soils are contamined also by copper from industrial and communal waste, sewage sludge [3, 4] and industrial dust emissions [5]. The intensity of the negative effect of copper depends on soil granulometric composition, the content of soil organic matter, pH and exchange capacity [6]. Copper can both inhibit and stimulate the soil enzymes [7]. Its activity is determined by total content as well as on the percentage of the assimilable species. This metal has a mutagenic effect on some microorganisms [5, 8, 9], reduces microbial numbers [10, 11, 12] and inhibits enzyme activity [13, 14, 15].

Considering the wide variety of factors determining the effect of copper excess on soil enzymatic activity, the

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aim of this paper was to determine the effect of soil contamination with copper on the activity of dehydrogenases, urease and acid and alkaline phosphatases in two different types of soil unsown and sown with yellow lupine. A previous study [10, 14] indicated that the extent of heavy metal effect on microorganisms and enzymes is linked to the textural group of soil. The greater the amount of mineral and organic colloids in soil, the lesser the negative effects of contamination with metals. Hence, it is interesting whether these relationships also apply to soil contamination with copper and whether root excretion of yellow lupine is capable of reducing the effects of that contamination, as it did in the case of soil contaminated with chromium [16].

Methods

An experiment was carried out in a vegetation hall in plastic pots filled with 3 kg of soil each. Two types of soil were used in the study: heavy loamy sand (sand – 66%, silt – 17%, silt and clay – 17%, pH – 6.9, C_{org} – 7.5 g kg^{-1} of soil) and silty sandy loam (sand – 42%, silt – 32%, silt and clay – 26%, pH – 7.0, C_{org} – 11.2 g kg^{-1} of soil). Before transferring to pots, the soil was sieved (mesh diameter of 1 cm^2) and contaminated with $CuSO_4 \cdot 5H_2O$, at the following doses per mg Cu kg^{-1} soil: 0, 150, 300, 450 and 600. Such ranges of contamination with copper can be observed in Polish soils in mining areas and areas where stillworks are located. The following macro- and microelement fertilization was used: P – 66 [K_2HPO_4], K – 125 [K_2HPO_4 and KCl], Mg – 20 [$MgSO_4 \cdot 7H_2O$], Zn – 5 [$ZnCl_2$], Mn – 5 [$MnCl_2 \cdot 4H_2O$], Mo – 5 [$Na_2MoO_4 \cdot 2H_2O$] and B – 0.33 [H_3BO_4] (as pure element in w mg kg^{-1}).

The experiment was carried out in two series: soil sown with yellow lupine var. Markiz (5 plants per pot) and soil maintained unsown for 56 days of the experiment. The first series was set up in 6 replications while the second had three replications. On days 14, 28, 42 and 56 soil samples were analyzed for the activity of dehydrogenases with the Lenhard method modified by Casidy et al. [17], urease with the Gorin and Chine Chang method [18] and acid and alkaline phosphatases with the Tabatabai and Bremner method [19]. Soil samples to be used for biochemical analyses were sieved with a mesh diameter of 2 mm^2. A dehydrogenase substrate was a 3% water solution of TTC (2,3,5-triphenyltetrazolium chloride). Soil incubation was carried out for 24 h at 37°C. Extinction of the TFF formed (triphenylformazane) was measured spectrophotometrically at a wavelength of 485 nm. The results were expressed in cm^3 H_2 d^{-1} kg^{-1} d. m. soil. A urease substrate was a 10% water solution of urea. The soil was incubated for 24 h at 37°C. The amount of N-NH_4 produced was determined with the use of Nessler reagent. Extinction of the amide mercuric iodide formed was measured spectrophotometrically at a wavelength of 410 nm and converted into the amount of (mg) N-NH_4 h^{-1} kg^{-1} d. m. soil. A phosphatase substrate was sodium 4-nitrophenylphosphate. The soil was incubated at 37°C for 1 h (acid phosphatase – pH 6.5, alkaline phosphatase – pH 11). After incubation, extinction of the PNP (p-nitrophenol) produced was measured spectrophotometrically at a wavelength of 410 nm. The results were converted into mmoles of PNP h^{-1} kg^{-1} d. m. soil. Throughout the entire experiment, constant soil humidity was maintained at 60% of capillary water capacity. On day 56, yellow lupine was harvested (flowering phase) and the yield of above-ground parts, roots and nodules were determined. All laboratory analyses were carried out in three replications. The results were statistically analyzed with ANOVA variance analysis.

Results and Discussion

Soil enzymatic activity is determined by the total activity of enzymes secreted by microorganisms to the environment and adsorbed by humus or clay minerals. Additionally, microorganisms living in the soil at a given moment contribute to the soil activity [20].

Dehydrogenases could serve as an indirect indicator of the activity of the microorganisms in the soil [21]. These enzymes catalyze oxidation of organic compounds by separating two hydrogen atoms from an organic molecule and transferring them onto co-enzymes which are the first acceptors of electrons in cellular respiration [22]. The inhibition in the activity of dehydrogenases in soil was closely associated with the amount of copper added (Fig. 1, Fig. 2). In both experimental series (in the soil sown with yellow lupine and in the unsown soil), subsequent increasing doses of copper up to the highest dose (600 mg kg^{-1}) inhibited the activity of these enzymes (Fig. 2). The results agree with the results of the previous studies [12, 23, 24].

The inhibition of dehydrogenases was strong in both loamy sand and silty sandy loam regardless of whether it was sown with yellow lupine or not. The decrease in the activity of dehydrogenases in heavy loamy sand ranged from 61% to 99%, while in silty sandy loam it ranged from 59% to 97%. Although the cultivation of yellow lupine increased the activity of these enzymes, the plants did not counteract the inhibitory effect of copper (Fig. 2). Unexpectedly, a higher activity of these enzymes occurred in the lighter (heavy loamy sand) than in the heavier soil (silty sandy loam). Usually, the situation is reversed [25] as with urease (Fig. 3, Fig. 4). In the uncontaminated pots, in the silty sandy loam unsown with yellow lupine, the activity of urease was 3.9-fold higher than that in the heavy loamy sand, while the activity of this enzyme in the sandy loam sown with yellow lupine was 2.2-fold higher than in the other soil. This enzyme was also inactivated by copper. The intensity of the inactivation increased with the increase in copper sulphate dose,

supporting results of some previous studies [12, 14]. The inhibition was stronger in the silty sandy loam than in the heavy loamy sand. In the former, the cultivation of yellow lupine enhanced the toxic effect of copper while in the latter it had a minor effect on the enzymes. In all pots, the decrease in the activity was greater than 50%. The cultivation of yellow lupine had an ambiguous effect on urease. It stimulated the activity in the heavy loamy sand but inhibited the enzyme in the silty sandy loam. Such behaviour is curious because the latter soil contains more organic and mineral colloids adsorbing urease. The adsorbed urease remains active for longer periods even in undesirable conditions [26].

Alkaline phosphatase (Fig. 5, Fig. 6) and acid phosphatase (Fig. 7, Fig. 8) were the most resistant enzymes in soil contaminated with copper. However, they also were affected by the copper concentrations applied. The copper had inhibited the activities of these enzymes, dependent on the applied dose. However, none of the doses inhibited acid phosphatase more than 50%. Only the two highest doses (450 mg and 600 mg kg^{-1}) of copper in the lighter soil and the highest dose (600 mg kg^{-1}) in the heavier soil inhibited the activity of alkaline phosphatase more than 50%. The cultivation of yellow lupine had a positive effect on the activity of phosphatases. The stimulation was higher with acid phosphatase than with alkaline phosphatase. The activity of the latter enzyme, similarly to urease, was higher in the silty sandy loam than in the heavy loamy sand, while the activity of acid phosphatase, similar to dehydrogenases, was higher in the loamy sand than in the sandy loam.

In conclusion, the applied doses of copper strongly inhibited the activity of all the studied enzymes, similar to the previous studies [15, 27]. This element caused remarkable changes in soil enzymatic activity, especially in dehydrogenases and urease. The extent of a negative effect of soil contamination with copper depended, to a slight extent, on the type of soil and the manner of its exploitation (unsown soil, sown soil with yellow lupine). Urease is an exception. Its activity in the lighter soil with cultivation of yellow lupine was inhibited more than in the unsown soil.

Soil contamination with copper also had a negative effect on the growth and development of yellow lupine (Table 1). Toxic activity of that metal on yellow lupine appeared at the lowest dose (150 mg kg^{-1}), and higher

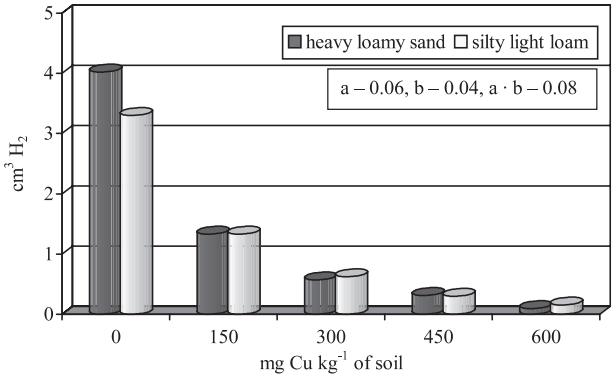

Fig. 1. The activity of dehydrogenases (cm^3 H_2 d^{-1} kg^{-1} d. m. soil), depending on soil contamination with copper.
LSD for: a – copper dose, b – kind of soil

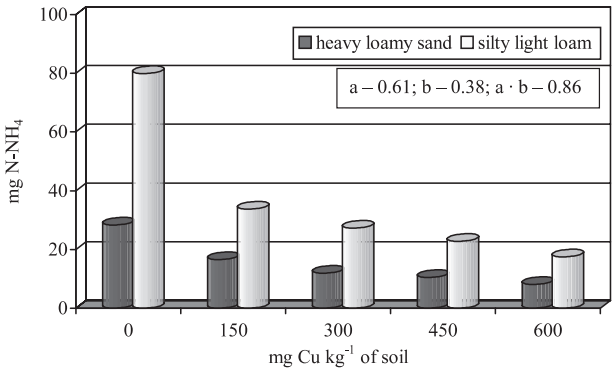

Fig. 3. The activity of urease (mg $N\text{-}NH_4$ h^{-1} kg^{-1} d. m. soil), depending on soil contamination with copper.
* for explanations see Fig. 1

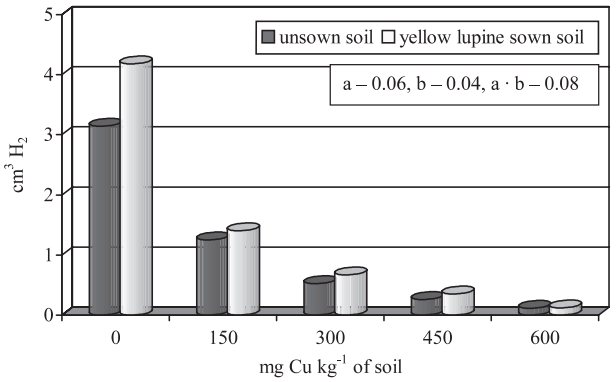

Fig. 2. The activity of dehydrogenases (cm^3 H_2 d^{-1} kg^{-1} d. m. soil), depending on soil management and its contamination with copper.
LSD for: a – copper dose, b – soil management

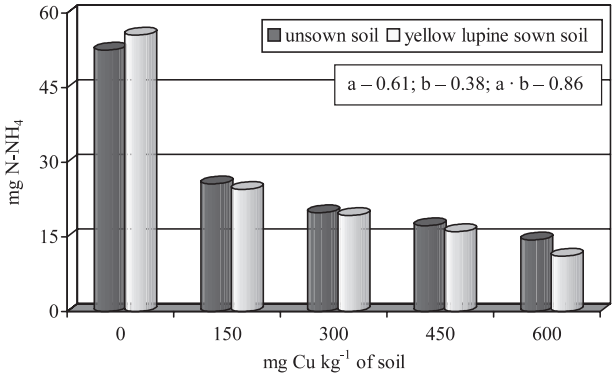

Fig. 4. The activity of urease (mg $N\text{-}NH_4$ h^{-1} kg^{-1} kg^{-1} d. m. soil), depending on soil management and its contamination with copper.
* for explanations see Fig. 2

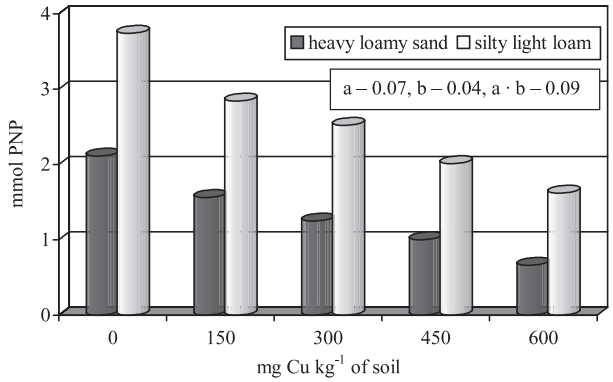

Fig. 5. The activity of alkaline phosphatase (mmol PNP h^{-1} kg^{-1} d. m. soil), depending on soil contamination with copper. * for explanations see Fig. 1

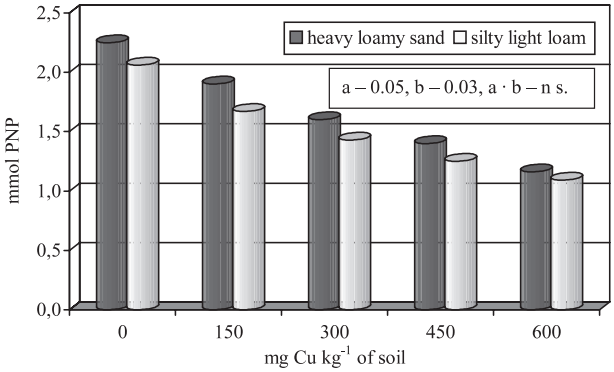

Fig. 7. The activity of acid phosphatase (mmol PNP h-1 kg-1 d.m. soil), depending on soil contamination with copper. * for explanations see Fig. 1

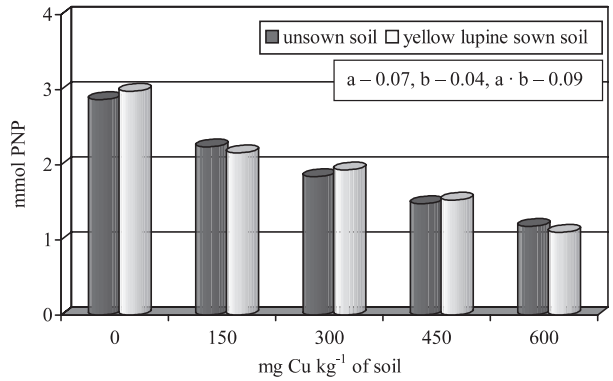

Fig. 6. The activity of alkaline phosphatase (mmol PNP h^{-1} kg^{-1} kg^{-1} d. m. soil), depending on soil management and its contamination with copper. * for explanations see Fig. 2

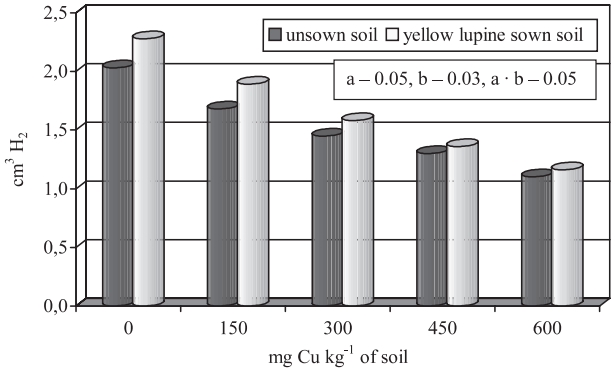

Fig. 8. The activity of acid phosphatase (mmol PNP h^{-1} kg^{-1} kg^{-1} d.m. soil), depending on soil management and its contamination with copper. * for explanations see Fig. 2

Table 1. Effect of soil contamination with copper on the yield of yellow lupine in g d. m. per pot.

Cu dose mg kg^{-1} of soil	Yield (g d.m. pot^{-1})				Nodules yield (g d.m. per root)		Number of nodules on 1 root	
	above-ground		roots					
	hls	sll	hls	sll	hls	sll	hls	sll
0	5.35	2.22	1.09	0.57	0.15	0.12	15.33	15.00
150	3.06	2.44	1.48	0.72	0.12	0.14	20.33	14.00
300	2.91	2.49	1.24	0.76	0.06	0.09	3.33	6.33
450	1.56	2.66	0.49	0.79	0.02	0.04	1.67	2.00
600	0.58	1.67	0.18	0.61	0.00	0.01	0.00	0.33
$\overline{X}$	2.69	2.30	0.89	0.69	0.07	0.08	8.13	7.53
r	-0.97^{**}	-0.36^{*}	-0.82^{**}	0.27	-0.98^{**}	-0.93^{**}	-0.86^{**}	-0.97^{**}
LSD	a – 0.39^{*} b – 0.62^{**} a · b – 0.88^{**}		a – 0.11^{**} b – 0.17^{**} a · b – 0.24^{**}		a – n.s. b – 0.05^{**} a · b – n.s.		a – n.s. b – 4.32^{**} a · b – n.s.	

LSD for: a – type of soil, b – Cu dose

r – correlation co-efficient significant at: ** p $p<0.01$; * $p<0.05$; n = 30; hls –heavy loamy sand, sll – silty light loam; n. s. – non-significant

doses of copper were found to intensify the effect. The application of high doses of copper in different soil does not always lead to the reduction of crop yields. In the previous studies [28] a dose of 100 mg Cu kg^{-1} had a positive effect on the yield of horse bean, which was confirmed by the results of Seliga [29, 30]. However, it was a different plant species, a substantially lower dose of copper and completely different soil.

Conclusions

1. Soil contamination with $CuSO_4 \cdot 5H_2O$ at doses of 150 mg, 300 mg, 450 mg and 600 mg Cu kg^{-1} significantly inhibited the activity of dehydrogenases, urease and acid and alkaline phosphatase. Compared to phosphatases, dehydrogenases and urease appeared to be better indicators of soil contamination with copper, as their activity was more strongly inhibited by copper than the activity of phosphatases.
2. Enzymatic activity was dependent on the type of soil. Dehydrogenases and acid phosphatase exhibited greater activity in heavy loamy sand, while the activity of urease and alkaline phosphatase was greater in the silty sandy loam.
3. Cultivation of yellow lupine stimulated the activity of dehydrogenases and acid phosphatase in both experimental soils as well as that of urease in heavy loamy sand.
4. Soil contamination with copper had a negative effect on the yield of yellow lupine.

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