Dehydrogenase, urease and phosphatase activities as affected by Pb contamination in the Soil

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Abstract

Heavy metals contamination in soil has taken great attention in recent years mainly because of the public awareness as environmental issue. This may adversely affect the soil ecology by microbial loss and changes in enzymatic activities. Soil enzymes are important indicators of soil environmental quality and have been used in the national and international monitoring programs. The present study was conducted to investigate the impact of lead (Pb) on activities of three soil enzymes (dehydrogenase, urease and phosphatase). The soil was spiked with 0, 200, 400, 600, 800 and 1000 mg kg^{-1} Pb and the activities of enzymes were determined at 0, 15, 30, 45 and 60 days after Pb contamination (DAC) in the soil. Results indicated that the declining rate of enzymes activity was comparatively higher from start to 30 DAC, than from 30 to 60 DAC. The significant inhibition (P<0.05) of dehydrogenase activity in Pb contaminated soil started from 15 DAC, while urease and phosphatase declined significantly from start to the end for all Pb treatments except for 200 mg kg^{-1} Pb. Up to 60 DAC, more than 50% decrease in all the three enzymes activity compared to their initial value was observed under 1000 mg kg^{-1} Pb treatment. This study concludes that Pb contamination in soil has adverse effects on the activities of dehydrogenase, urease and phosphatase.

Key words: Dehydrogenase, urease, phosphatase, soil, Pb

Introduction

Heavy metals are toxic at higher levels and may adversely affect soil ecosystem (Giller *et al*., 1998). It is the soil from where heavy metals enter to atmosphere, hydrosphere and biota, and thus it has a fundamental role in overall metal-cycling in the nature. The natural concentrations of most heavy metals in soils vary widely and are mainly related to the soil parent materials. Anthropogenic sources such as smelters, mines, power stations, industry and the application of metal-containing pesticides, fertilizers, and sewage sludge may contribute to and at times exceed those from natural sources (McGrath *et al*., 1995). Soil is widely regarded as an environmental filter ensuring the quality of both water and atmosphere. In the context of sustainability, it is now recognized that soil is an effective de-contaminant of potential pollutants and its chemical, physical and biological quality must be maintained. A high-quality soil is capable of producing healthy and abundant crops; de-contaminating the water passing through it; not emitting gases in quantities detrimental to the environment; and capable of degrading organic input (Brookes, 1995). This view clearly demands that diagnosis of soil pollution should be carried out on the basis of observed alterations in the soil properties controlling the behaviors described above, ideally in a way that allows any loss of soil quality to be quantified as well as identified qualitatively.

A number of soil microbiological parameters, notably microbial biomass, basal respiration, and enzymes activity (Sparling, 1997; Trasar-Cepeda *et al*., 2000; Yao *et al*., 2003) have been suggested as possible indicators of soil environmental quality, and employed in the national and international monitoring programs. Soil enzymes are especially significant in this regard because of their major contribution to recycle essential plant nutrients and degrade organic matter in the soil. As we all know that soil is a living system where all biochemical activities proceed through enzymatic processes. Enzymes accumulated in soils are present as free enzymes, such as exoenzymes released from living cells, endoenzymes released from disintegrating cells, and enzymes bound to cell constituents. It has been reported by Kandeler *et al*. (1996) that the composition of the microbial community determines the potential of that community for enzyme synthesis, and thus any modification of microbial community due to environmental factors should be reflected on the level of soil enzymatic activities.

Lead (Pb) is among the most significant toxic heavy metals. Its concentration in normal agricultural soil is in the range of 10 to 100 mg kg^{-1} (Soon and Abboud, 1993), but in polluted soils especially near mines or by sewage sludge applications, its contents are even higher than 1000 mg kg^{-1} (Peters and Shem, 1992; Pichtel *et al*., 2000). Higher levels of Pb in soil may adversely affect the activities of soil

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enzymes, which in turn may result in adverse effects on various plant parameters influencing crop quality, yield and possibly human health through food chain. Accordingly, we conducted this study and selected wide levels of Pb to determine their impact on dehydrogenase, urease and phosphatase activities in soil.

Materials and Methods

Soil sampling, preparation and Pb contamination

A bulk soil sample was collected at 0-15 cm depth from the research field of Hua Jia Chi campus of Zhejiang University, Hangzhou, China. The soil was brought to the laboratory, hand-picked to remove stones, discrete plant residues and large soil animals (earthworms etc.), passed through 2 mm sieve and mixed thoroughly. A sub sample of the soil was taken, air-dried, mixed and analyzed for selected physico-chemical properties (Table 1). The moist soil (equivalent to 100 g oven-dry weight) was transferred to 250 mL capacity glass beakers. The soil samples were first adjusted to 40% of the soil water holding capacity (WHC) by adding distilled water and then pre-incubated at 25 °C for seven days (conditioning period). After conditioning, Pb was applied as $Pb(NO_3)_2$ solution to get the concentrations of 0 (Control), 200, 400, 600, 800 and 1000 mg Pb kg^{-1} soil. The moisture contents in the soil were adjusted to 50% of WHC and soil samples were incubated for 60 days at 25 °C. The soil moisture was kept at the same level throughout the incubation period.

Table 1. Basic physical and chemical properties of soil used in the study

Property	Value	Property	Value
Sand	700 g kg^{-1}	CEC	11.3 cmol kg^{-1}
Silt	190 g kg^{-1}	Total organic carbon	19.3 g kg^{-1}
Clay	110 g kg^{-1}	Total nitrogen	1.6 g kg^{-1}
$pH_{(1:2.5)}$	5.51	Total lead	28.5 mg kg^{-1}

Analysis of soil enzymes activity

Soil samples for various treatments were taken at 0, 15, 30, 45 and 60 days after Pb contamination (DAC) and analyzed for dehydrogenase, urease, and phosphatase activities. Soil dehydrogenase activity was measured by the reduction of triphenyl tetrazolium chloride (TTC) to triphenyl formazan (TPF). Briefly, 5 g soil sample was incubated for 24 h at 37 °C in 5 mL of TTC solution (5 g L^{-1} in 0.2 M Tris–HCl buffer, pH 7.4). Two drops of conc. H_2SO_4 were immediately added after the incubation to stop the reaction. The sample was then blended with 5 mL of toluene to extract TPF and shaken for 30 min at 250 rpm,

followed by centrifugation for 5 min, and the color absorbance of the extract was measured at 492 nm. For analysis of soil urease activity, 5 g soil was taken into 250 mL conical flask, and 10 mL of 100 g L^{-1} urea solution and 20 mL citric acid buffer (pH 6.7) were added into flask. Soil sample was incubated at 37 °C for 24 hours. After 24 hours, the solution was filtered and 3 mL of filtrate was taken into 50 mL vol. flask, and 20 mL distilled water and 4 mL of mixed reagent (Phenol + NaOH) were added. Then, 4 mL of sodium hypo chlorite solution was added, mixed and made the volume to 50 mL with distilled water, and absorbance of color was checked at 578 nm. Soil phosphatase activity was measured by disodium phenyl phosphate method. Briefly, 5 g of soil sample was carefully transferred into 250 mL flask and 2 mL of toluene was added to inhibit the growth of microorganisms. After standing for 15 min, added 20 mL of 0.5% (w/v) disodium phenyl phosphate prepared in acetic acid buffer (pH 5), and sample was incubated at 37 °C for 24 h. After incubation, 100 mL of 0.3% $Al_2(SO_4)_3$ solution was added to the sample, filtered, and 3 mL of filtrate was taken into 50 mL vol. flask. Then 5 mL of borate buffer (pH 9.4) and 4 drops of indicator were added, and made up the volume. The absorbance of color in the solution was measured at 660 nm. Soil dehydrogenase, urease and phosphatase activities are expressed as mg TPF g^{-1} dry soil 24 h^{-1}, mg NH_3-N g^{-1} dry soil 24 h^{-1} and mg phenol produced g^{-1} dry soil 24 h^{-1}, respectively (Li, 1996; Min *et al*., 2001). Soil enzymes activity data for different treatments were analyzed by ANOVA and means (n = 3) were compared at 5% level of significance using Duncan's multiple range test. The effects of different treatments were compared at specific as well as over different incubation periods (Gomez and Gomez, 1984).

Results

The activity of dehydrogenase affected by lead (Pb) contamination in soil (Figure 1) showed a significant (P<0.05) decline in activity for various Pb treatments after 15 days of heavy metal contamination (DAC). The declining rate of enzyme activity was comparatively higher from start to 30 DAC, than from 30 to 60 DAC, and until 60 DAC, 800 and 1000 mg kg^{-1} Pb treatments resulted in 47.3 and 53.8% reduction in activity compared to their initial values, respectively. However, there was only slight decrease (13.4%) in dehydrogenase activity for control from start to 60 DAC. Soil urease activity affected by Pb contamination (Figure 2) showed significant declines in activity for all Pb treatments from start to 60 DAC, except for 200 mg kg^{-1} Pb which was not significant with that of control from 30 to 45 DAC. A relatively higher decline in urease activity was shown for 400, 600, 800 and 1000 mg kg^{-1} Pb from start to 30 DAC, than from 30 to 60 DAC.

From 15 to 60 DAC, 1000 mg kg^{-1} Pb had significantly lower urease activities compared with other Pb treatments and until 60 DAC, it showed a 55.6% decrease in activity compared to its initial value. However, in case of control soil, urease activity showed 20.3% decrease from start up to 60 DAC. Soil phosphatase activity also declined significantly ($P<0.05$) from start to the end for all Pb treatments except for 200 mg kg^{-1} Pb, which did not show significant difference with control throughout the incubation period (Figure 3). Soil contaminated with 800 and 1000 mg kg^{-1} Pb showed comparatively more decreases for phosphatase than urease and dehydrogenase activities, and up to 60 DAC, they had 52.6% and 60.4% decrease in phosphatase activity compared to the initial values, respectively. A considerable decline (24.1%) in phosphatase activity was also observed for control from start to the end of incubation.

Discussion

Pb is one of the major pollutants among heavy metals in the soil which results in adverse effects on crop yield and food quality. The results of present study demonstrated a significant ($P<0.05$) decline in dehydrogenase, urease and phosphatase activities for soil amended with more than 200 mg kg^{-1} Pb from 15 to 60 days after heavy metal contamination (DAC). Dehydrogenase is an intracellular enzyme that is involved in microbial oxidoreductase metabolism and its activity basically depends on the metabolic state of the soil biota, while urease and phosphatase are hydrolytic enzymes, involved in N and P cycling in the soil. Activities of these enzymes are considered sensitive to heavy metal pollution and have been proposed as indicators for measuring the degree of soil sustainability (Brookes, 1995; Sparling, 1997). In general enzymes activities in soils have been shown to decrease in response to increasing metal pollution (Serra- Wittling *et al*,. 1995). Belitsyna *et al*. (1989) found, the greatest suppre -ssion of invertase and dehydrogenase activity in soil at 500 mg kg^{-1} Pb. In our experiment we observed a greater declining rate for dehydrogenase and urease activities from start to 30 DAC than from 30 to 60 DAC, suggesting a greater Pb-toxicity in the first 30 days after which the microbial activities reached at equilibrium. However, phosphatase activity for various Pb amended soils decreased gradually from start to the end of incubation. The dynamics of dehydrogenase, urease and phosphatase at various times during incubation after the heavy metal contamination might be related to the dynamics of microbial populations in the soil (Giller *et al*., 1998; Renella *et al*., 2003).
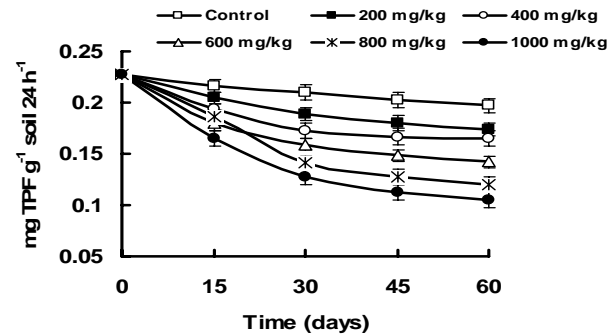

Figure 1. Effect of Pb on dehydrogenase activity in soil. Dehydrogenase activity is expressed as mg TPF g^{-1} dry soil 24 h^{-1}. The error bar is standard error of means (n = 3)
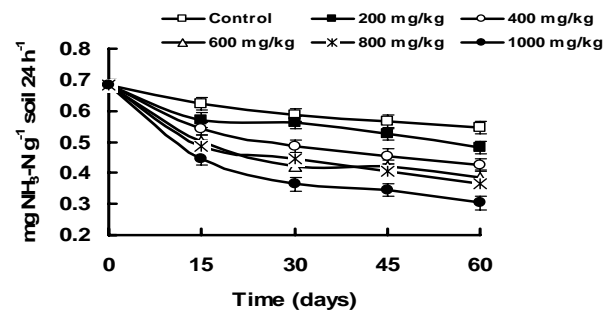

Figure 2. Effect of Pb on urease activity in soil. Urease activity is expressed mg NH$_3$-N g^{-1} dry soil 24 h^{-1}. The error bar is standard error of means (n = 3)
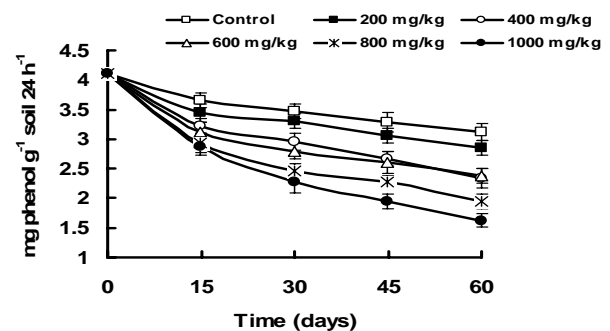

Figure 3. Effect of Pb on phosphatase activity in soil. Phosphatase activity is expressed as mg phenol produced g^{-1} dry soil 24 h^{-1}. The error bar is standard error of means (n = 3)

The ecological dose concept represents a promising attempt to quantify the effect of heavy metal on soil microbiological activity. Doelman and Haanstra (1986) defined the ecological dose 50% (ED$_{50}$) as the concentration of a toxicant that reduces the microbial activity to 50% of its initial value. As soil enzymes are

essential factors of soil fertility, and are sensitive to pollutants such as heavy metals, their activity can be used to asses the ecological status in soil monitoring programs (Brookes, 1995; Dick, 1997). In the present study, we found that the ecological dose 50% (ED_{50}) value of Pb for dehydrogenase and urease activities was 1000 mg kg^{-1}, however for phosphatase activity it was 800 mg kg^{-1} as at these concentrations, activities were reduced to 50% up to 60 DAC. The observed decreases of soil enzymes activities under Pb pollution in the present study can be interpreted as the binding of Pb with the functional group of enzyme and reduction in the microbial activity as a result of its direct toxicity to microbial populations in soil. As Wittekind *et al.* (1996) found that the inhibition of urease in the presence of heavy metals is due to changes in the molecular structure of the enzyme. For heavy metals like Pb, it is assumed that they bind to the sulfhydral groups of the active site, forming metal sulphide equivalents and consequently inhibit enzyme's activity. The present study depicted the dynamics of soil enzymes only up to 60 days after Pb contamination. Further extensive studies involving more soil enzymes and heavy metals are needed to make picture clearer.

Acknowledgement

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