

Effects of cadmium, zinc and lead on soil enzyme activities

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Abstract: Heavy metal (HM) is a major hazard to the soil-plant system. This study investigated the combined effects of cadmium (Cd), zinc (Zn) and lead (Pb) on activities of four enzymes in soil, including catalase, urease, invertase and alkaline phosphatase. HM content in tops of canola and four enzymes activities in soil were analyzed at two months after the metal additions to the soil. Pb was not significantly inhibitory than the other heavy metals for the four enzyme activities and was shown to have a protective role on catalase activity in the combined presence of Cd, Zn and Pb; whereas Cd significantly inhibited the four enzyme activities, and Zn only inhibited urease and catalase activities. The inhibiting effect of Cd and Zn on urease and catalase activities can be intensified significantly by the additions of Zn and Cd. There was a negative synergistic inhibitory effect of Cd and Zn on the two enzymes in the presence of Cd, Zn and Pb. The urease activity was inhibited more by the HM combinations than by the metals alone and reduced approximately 20%–40% of urease activity. The invertase and alkaline phosphatase activities significantly decreased only with the increase of Cd concentration in the soil. It was shown that urease was much more sensitive to HM than the other enzymes. There was a obvious negative correlation between the ionic impulsion of HM in soil, the ionic impulsion of HM in canola plants tops and urease activity. It is concluded that the soil urease activity may be a sensitive tool for assessing additive toxic combination effect on soil biochemical parameters.

Keywords: multiple heavy metal pollution; canola plants; soil enzyme; ionic impulsion

Introduction

The ability of the soil to serve as a habitat for plants, microorganisms and soil-living animals is the most important function of agricultural land (Riepert and Wilke, 1998). Soil enzyme activity is involved in nutrient cycling and availability to plants and can be used as an index of soil functioning (Nannipieri *et al.*, 2003). They are not only essential for plant growth but also equally important for soil fertility. Anthropogenic activities leading to the intentional or unintentional deposition of contaminants may be harmful to soil environment, affect the amount and activities of soil enzymes at different functional levels, reduce the growth and the yield of plants and cause excessive pollutant concentrations in plants.

Heavy metals (HM) are one of the major groups of pollutants in soil environment, arising from repeated applications of sewage sludge, municipal wastes and animal slurries, the activity of smelting industries, impurities in fertilizers and deposition of air pollutants from burning of fossil fuels and various industrial activities. A number of soil characteristics, including biological properties, are profoundly influenced by HM. The strong inhibition of the activities of a variety of enzymes has been reported in metal polluted soils over the past years (Tyler, 1974; Stott *et al.*, 1985; Fu and Tabatabai, 1989; Deng and Tabatabai, 1995; Marzadori *et al.*, 1996; Xia, 1988; LIU, 1996; Huang and Shindo, 2000; Ekenler and Tabatabai, 2002). Recent determinations of HM in the sewage irrigation soils in Baoding City of China

indicated that the content of these contaminants in the majority of the soils is very high (Liu, 1996) and the content has been increasing with increasing sewage irrigation in soil. At the same time, the sewage irrigated soil has been affected by a combination of multiple HM, and the vegetables planted on the soil have also been polluted. However, only single additions of HM have been studied in the past and there is insufficient information available on the additive effects of multiple HM pollution. Among the HM polluted soil, cadmium (Cd) is one of the most toxic, the other is lead (Pb), whereas zinc (Zn) may be less toxic but generally is present in higher concentrations. There is also a possibility of synergistic effect of groups of pollutants on soil enzymes. However, information on this subject in the literature is very scarce. Hence, it is useful to carry out the studies on the combined effects of multiple-HM contamination on soil enzyme activities and metal contents in plants. The aim of this work was to study the effects of Cd, Zn and Pb on activities of four soil enzymes.

1 Materials and methods

1.1 Soil sampling and preparation

Soil was sampled from the surface layer (0–40 cm) of the agricultural field of the experiment station at Agricultural University of Hebei, China. The soil is classified as meadow cinnamon soil according to international classification and its main physical and chemical properties are presented in Table 1. The sampled soil was air-dried and then sieved through a 2-mm mesh.

Table 1 Some physical and chemical properties of the soil used

Soil type	OM, g/kg	Available			pH	CaCO ₃ , g/kg	Particle size distribution, %						Cd	Zn	Pb
		N	P	K											
		(×10 ³ g/kg)						①	②	③	④	⑤	⑥		
Meadow cinnamon soil	10.9	22.7	14.0	98.0	7.43	165	9.85	7.24	44.07	9.79	8.57	20.48	0.83	75.9	30.54

Notes: Soils particle ①>0.25 mm; ②0.25--0.05 mm; ③0.05--0.01 mm; ④0.01--0.005 mm; ⑤0.005--0.001 mm; ⑥<0.001 mm; OM. organic material

1.2 Pot experiment

Five kilogram soil was put in a plastic bucket (20 cm diameter×20 cm height) after thoroughly mixing with 75 g dry poultry manure, 1 g urea and 2 g di-ammonium phosphate. Cadmium acetate, or zinc acetate or lead acetate was added at five rates in order to gain the regression orthogonal design method which is designed to have 3 factors (three heavy metal elements) and 5 levels (Table 2). In addition, for investigating the combined impact of the three heavy

metals, soil was added with the solution containing Cd, Zn and Pb. There were 5 levels for each heavy metal so the total number of treatment is 15 and single element treatments are also 15 (Table 3). Each treatment was replicated three times so the total number of pots in this study was 90. Then all pots were added with water to the full soil water holding capacity (WHC) and then equilibrated for 15 d before seeding.

Table 2 Concentrations of Cd, Zn and Pb supplied in soil in the 3-element combination treatment in the experiment

Heavy metal	Treatment number														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Cd, mg/kg	4.42	4.42	4.42	4.42	45.58	45.58	45.58	45.58	0	50	25	25	25	25	25
Zn, mg/kg	70.78	70.78	729.22	729.22	70.78	70.78	729.22	729.22	400	400	0	800	400	400	400
Pb, mg/kg	88.48	911.52	88.48	911.52	88.48	911.52	88.48	911.52	500	500	500	500	0	1000	500

Table 3 Concentrations of Cd, Zn and Pb supplied in soil in the single element treatment

Treatment number	Heavy metal, mg/kg		
	Cd	Zn	Pb
1	0	0	0
2	1	100	100
3	5	200	300
4	10	400	500
5	50	800	1000

Canola seeds were sown in each pot after germination, only eight plants with equal size were kept in each pot. Deionized water was added on the soil at 60% of the soil water holding capacity after the thinning and this level was kept during the whole period of the experiment. No symptoms of diseases and pest damage were observed in the period of growth. After growing for 60 d, all plants were harvested and concentrations of Cd, Zn and Pb in shoots were analysed separately. The activities of four soil enzymes calatase, urease, invertase and alkaline phosphatase were measured accordingly after the harvest.

1.3 Chemical analysis

The urease activity in the soil was determined by the buffered method of Zhou and Zhang (1980). In this procedure, a solution of urea (10%) and citrate buffer (pH 7) were added to soil in hermetically sealed flasks, and then incubated for 24 h at 37°C. The ammonium content of the centrifuged extracts was

determined by a modified indophenol-blue reaction. Controls were prepared without substrate to determine the ammonium produced in the absence of added urea. Soil calatase activity was measured by potassium permanganate (KMnO₄) titration method reported as Zhou and Zhang (1980). In the procedure, a solution of peroxid (0.3%), H₂O₂, was added to soil as substrate. The activity of soil alkaline phosphatase was measured by using the spectrophotometric method of Zhao and Jiang (1986). This method used *p*-nitrophenyl phosphate as a substrate. The invertase activity assay was a titration method by sodium thiosulfate (Na₂S₂O₃) as reported by Zhou and Zhang (1980).

In the study HM content in canola tops were extracted by nitric-perchloric acid digestion and quantified by using an atomic absorption spectroscope (WFX-120) (Bao, 2000). The other analytical items are done according to the general analysis method of soil and agriculture chemistry (He, 2000). The determination of available nitrogen was carried out by hydrolyzing the soil with 1 mol/L sodium hydroxide (NaOH) in a petri dish and then the amount of diffused N trapped in the boric acid-indicator solution was determined by titration with standard 0.01 mol/L sulfuric acid (H₂SO₄). The determination of available phosphorus was carried out by extracting the soil with 0.5 mol/L NaHCO₃ extraction solution and the filter suspension was added with ammonium molybdate and antimony potassium tartrate solution, then determine the absorbance by spectrophotometer. Soil was

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