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Effects of cadium, 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 cadium (Cd), zinc (Zn) and lead (Pb) on activities of four enzymes in soil, including calatase, urease, invertase and alkalin 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 calatase activity in the combined presence of Cd, Zn and Pb; whereas Cd significantly inhibited the four enzyme activities, and Zn only inhibited urease and calatase activities. The inhibiting effect of Cd and Zn on urease and calatase 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 intertase 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, cadium (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.

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Table 1 Some physical and chemical properties of the soil used

Soil type	OM, g/kg	Available N	Available P	Available K	pH	$CaCO_3$, g/kg	Particle size distribution, % ①	②	③	④	⑤	⑥	Cd	Zn	Pb
		($\times 10^3$ g/kg)											($\times 10^3$ g/kg)		
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 alkalin 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℃. 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 ($KMnO_4$) titration method reported as Zhou and Zhang (1980). In the procedure, a solution of peroxid (0.3%), H_2O_2, was added to soil as substrate. The activity of soil alkalin 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 ($Na_2S_2O_3$) 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 (H_2SO_4). The determination of available phosphorus was carried out by extracting the soil with 0.5 mol/L $NaHCO_3$ extraction solution and the filter suspension was added with ammonium molybdate and antimony potassium tartrate solution, then determine the absorbance by spectrophotometer. Soil was

analysed for available potassium from 1 mol/L NH_4OAc extract.

1.4 Statistical analysis of data

Multivariate regression equation was used to assess the impact of single and combination of heavy metals on soil enzyme activities, HM content of canola plant. The algebraic expression of the relationship is:

$$y = b_0+b_1x_1+b_2x_2+b_3x_3$$

where x is the co ntents of soil HM; y is the soil enzyme activities. The most significant element is analyzed by stepwise regression method.

Significance of differences of the relations was analysed by using the statistical computer package (SPSS11.5 statistics software).

2 Results and discussion

2.1 Effect of added HM in soil on four soil enzyme activities

Tables 4 and 5 show the effects of the combination of Cd, Zn, Pb and single metal treatments on the four enzyme activities in the soils. The changes of the activities of four enzymes with the added metal concentrations are shown in Figs. 1 to 4. The calatase activity was inhibited by higher Cd concentrations in the Cd treatment when the added Cd concentration reached 50 mg/kg, whereas the low Cd concentration from 0 to 10 mg/kg activated the enzyme activity (Fig. 1). However, the enzyme activity in the combined Cd, Zn and Pb treatments was decreased by the low Cd concentration whereas it evidently was increased by higher Cd concentrations, and similar trends were also observed with Zn mainly because the enzyme activity was also affected by the interaction of coexisting metals (Fig.1). In the Zn only treatment, the calatase activity decreased with increased Zn concentration from 0 to 800 mg/kg, whereas it increased with the increased Pb concentration in both the Pb alone and multiple-metal treatments. Thus, in all the treatments Pb, either alone or in combination with the other two metals significantly stimulated calatase activity (Fig.1). Liu *et al.* (2002) also reported an increase in the enzyme activity in the presence of Pb and showed that the concentration of the exchangeable Pb in soil was very low and the stimulating effect for the enzyme stemmed from Pb associated with Fe-Mn oxide. Consequently, the observed activation of calatase in the soil in the presence of Pb probably was a result of the reaction between the Pb ion in soil and the functional groups of calatase. At present, very little information is available on the enzyme activation mechanism in the presence of Pb. In the study, calatase activity in the soil was lower in the Cd and Zn alone treatments than in the Cd, Zn and Pb combined treatments, whereas it was higher in the Pb alone treatments than in the Cd, Zn and Pb combined treatments (Fig.1). That is to say, the calatase activity in the soil with Cd was stimulated when Zn and Pb were both added to the soil and the calatase activity in soil with Zn was stimulated when Cd and Pb were both added to the soil. Contrary to what was observed in soil with Cd or Zn, the enzyme activity in soil with Pb was inhibited when Cd and Zn were both added to the soil (Fig.1). Therefore, Cd, Zn and Pb combination, especially Pb, has a protective effect on

Table 4 Four soil enzyme activities in the presence of added Cd, Zn and Pb

Number	Catalase activity[a]		Urease activity[b]		Phosphatase activity[c]		Invertase activity[d]	
1	3.75	(0.09)	0.49	(0.03)	0.71	(0.09)	4.55	(0.40)
2	3.97	(0.05)	0.55	(0.08)	0.64	(0.09)	4.15	(0.12)
3	3.41	(0.08)	0.47	(0.00)	0.96	(0.04)	4.98	(0.34)
4	3.74	(0.02)	0.42	(0.06)	0.69	(0.07)	4.04	(0.30)
5	3.61	(0.03)	0.44	(0.02)	0.45	(0.06)	3.81	(0.35)
6	3.87	(0.09)	0.49	(0.03)	0.44	(0.01)	3.64	(0.21)
7	3.25	(0.01)	0.40	(0.009)	0.55	(0.09)	3.49	(0.40)
8	3.63	(0.09)	0.39	(0.01)	0.45	(0.06)	2.99	(0.38)
9	3.77	(0.07)	0.48	(0.03)	0.82	(0.07)	4.19	(0.50)
10	3.72	(0.04)	0.42	(0.003)	0.59	(0.09)	3.17	(0.03)
11	3.91	(0.04)	0.43	(0.01)	0.39	(0.03)	4.17	(0.19)
12	3.67	(0.001)	0.33	(0.013)	0.59	(0.05)	3.28	(0.17)
13	3.47	(0.03)	0.45	(0.014)	0.79	(0.09)	4.55	(0.19)
14	3.95	(0.09)	0.45	(0.01)	0.67	(0.09)	4.51	(0.28)
15	3.68	(0.06)	0.43	(0.077)	0.68	(0.01)	3.90	(0.14)
Blank test	3.71	(0.01)	0.67	(0.04)	0.55	(0.01)	4.68	(0.39)

Notes: Values are averages with standard error in parentheses; [a] ml(0.1 mol/L $KMnO_4$)/g dw soil (25℃, 20 min); [b] mg NH_3-N/g dw soil (37℃, 24 h); [c] mg phenol/g dw soil (37℃, 24 h); [d] ml(0.1 mol/L $Na_2S_2O_3$)/g dw soil (37℃, 24 h)

Table 5 Four soil enzyme activities in the presence of single added Cd, Zn and Pb metals

HM	Number	Catalase activity[a]	Urease activity[b]	Phosphatase activity[c]	Invertase activity[d]
Cd	1	3.570	0.7236	0.544	5.110
	2	3.566	0.7864	0.462	4.770
	3	3.600	0.6976	0.470	4.599
	4	3.697	0.6831	0.485	4.579
	5	3.508	0.6350	0.388	4.331
Zn	1	3.762	0.6412	0.570	4.162
	2	3.627	0.6363	0.735	4.373
	3	3.584	0.6271	0.821	4.614
	4	3.517	0.6066	0.985	4.416
	5	3.260	0.5346	0.787	3.207
Pb	1	3.803	0.6566	0.560	4.782
	2	3.880	0.7872	0.590	4.805
	3	3.975	0.7930	0.609	4.858
	4	4.063	0.7431	0.640	5.187
	5	4.127	0.7273	0.605	4.642

Notes: a, b, c, d are the same as Table 4

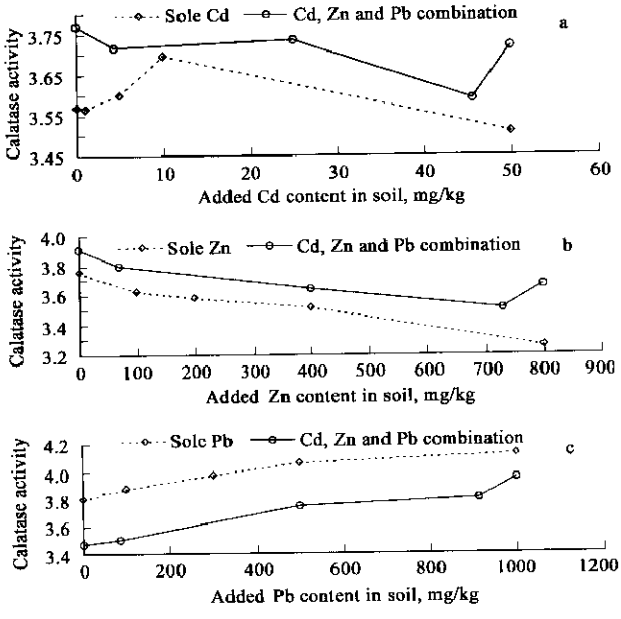

Fig.1 Comparison of effect of Cd (a), Zn (b), Pb (c) with the combination (Cd+Zn+Pb) in soil on calatase activity

calatase in this study.

Contamination of soil with HM had deleterious influence on soil urease activity. Cd and Pb activated urease activity at a low concentration but inhibited this enzyme with rising concentrations in soils when Cd and Pb were the only HM added (Fig.2). Moreover, urease activity was gradually reduced while the concentration of Zn increased in the Zn treatment. In the Cd, Zn and Pb combined treatment, the enzyme activity was inhibited with the increase of Cd and Zn concentration, but the effect of Pb on the enzyme was not evident with the increase of its concentration (Fig. 2). However, the effect was stronger in the case of soils contaminated with a combination of Cd, Zn and Pb than in soils amended with Cd, Zn and Pb separately (Fig.2). Urease activity was more inhibited by HM combination treatments than single Cd, Zn and Pb treatments, being reduced by 20%—40% (Fig.2). Therefore, multiple-metal treatments inhibited urease activity far more than the single HM treatments, with the synergistic inhibiting effect of Cd and Zn being particularly obvious.

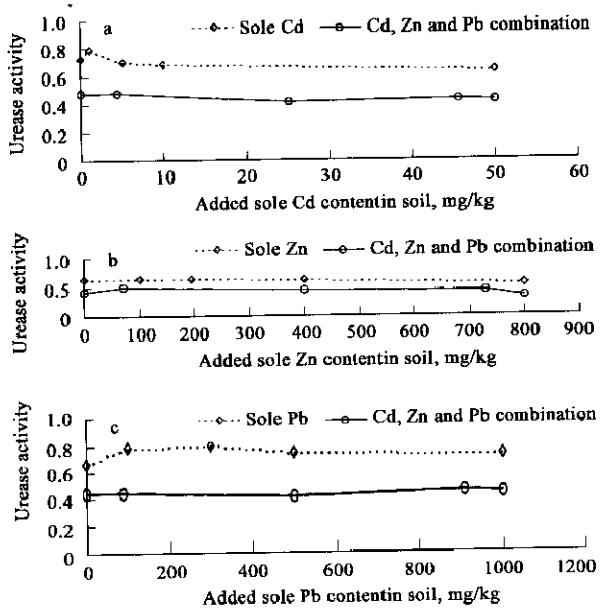

Fig.2 Comparison of effect of Cd (a), Zn (b), Pb (c) with the combination (Cd+Zn+Pb) in soil on urease activity

Multiple-metal treatments activated the alkaline phosphatase activity more than the single Cd treatment, but inhibited it more than the Zn only treatment, the difference of activation or inhibition was not evident for the Pb only treatment (Fig.3). The enzyme activity in the Cd, Zn and Pb combined treatment decreased markedly with the increase of Cd concentration but increased with that of Zn concentration (Fig.3). The invertase activities were more inhibited by the Cd, Zn and Pb combination treatments than the single metal treatments (Fig.4).

To sum up, each of the four soil enzyme activities studied here was affected differently by the added Cd, Zn and Pb combinations or single metal treatments. The degree of enzyme inhibition or activation varied with the concentration and different HM ion and the type of enzyme assayed. The inhibition or activation of these enzyme activities in soils in the presence of HM combination was a result of the interaction of HM and the reaction between the HM in solution and the functional groups of enzymes. The chemical and physical properties of the soil, such as organic matter content, type and amount of clay and soil pH, influence the toxic effect of trace elements (Bunzl *et al.*, 1976; Gadd and Griffiths, 1978; Sinha *et al.*, 1978). These processes are perhaps responsible for

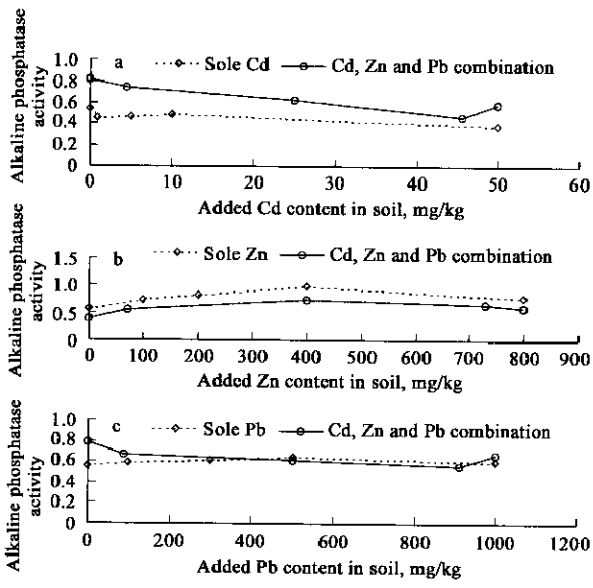

Fig.3 Comparison of effect of Cd (a), Zn (b), Pb (c) with the combination (Cd+Zn+Pb) in soil on alkaline phosphatase activity

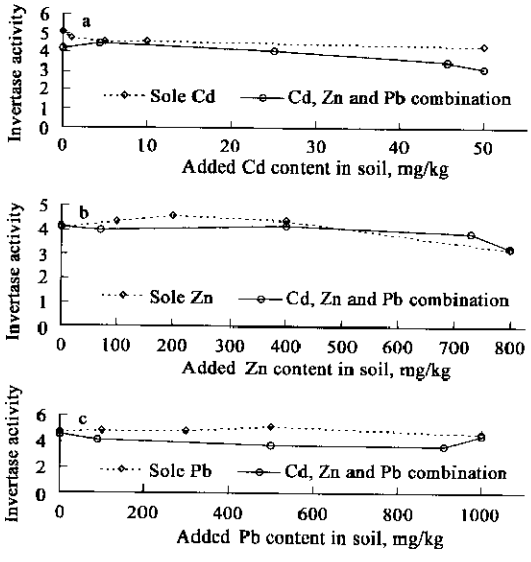

Fig.4 Comparison of effect of Cd (a), Zn (b), Pb (c) with the combination (Cd+Zn+Pb) in soil on invertase activity

the variation in the inhibition or activation observed in soil with different HM.

Results from a multivariate regression and stepwise regression analysis of the effects of combined HM pollution on the activity of four enzymes are presented in Tables 6 and 7. The stepwise regression analysis indicated that the concentration of Cd was negatively correlated with the four enzyme activities in the Cd, Zn and Pb combined treatments (Table 7). Urease and calatase activities were correlated negatively with the concentration of Zn and the calatase activities positively correlated with the concentration of Pb (Table 7). Pb in this study was significantly less inhibitory for the four enzyme activities than the other HM and was confirmed as a protective metal for calatase activity whereas Cd significantly inhibited the four enzyme activities, whereas Zn only inhibited urease and calatase activities (Table 7). The enhancement effect of Pb on calatase might also be explained by the fact that calatase maybe metallo-enzymes in which the metal is Pb. Furthermore, the inhibiting effect of Cd on urease and calatase activity can be intensified significantly by the addition of Zn. Similar to Cd, the inhibiting effect of Zn on urease and calatase activity can be intensified significantly by the addition of Cd (Tables 6 and 7). This shows a negative synergistic inhibitory effect of Cd and Zn on the two enzymes in the presence of Cd, Zn and Pb. A possible mechanism explaining Cd toxicity can be the substitution of Zn by Cd due to the similar ionic properties of these ions. The results for Cd and Zn are consisteat with other studies where enzyme activities were shown to reflect the extent of HM combination (Teng *et al.*, 2002). Cd was also found to have a significant inhibitory effect on the activities of alkaline phophatase and invertase, so the effect of combined Cd, Zn and Pb on invertase and alkalin phosphatase decreases mainly with increasing concentrations of Cd, and the effect of Pb and Zn is slight (Table 7). Therefore, Cd was the most effective inhibitor of the four enzymes in this study, followed by Zn. Thus the effect of Cd, Zn and Pb on four soil enzyme activities follows the order: Cd>Zn>Pb. The difference in the order of effectiveness of inhibition among HM may be related to the different HM ion-soil interactions and their different forms in soil. Metal oxides and hydroxides as well as amorphous aluminosilicates provide surface sites for chemisorption of HM. Usually Cd^{2+} has a higher affinity than Mg^{2+} but a lower affinity than Pb^{2+}, Cu^{2+}, Zn^{2+} and Ni^{2+} for these surfaces (Moreno *et al.*, 2001). Liu *et al.* (2003) reported that the contribution of the exchangeable Cd on uease activity inhibition is the greatest one in all the forms of Cd in alluvial meadow soils and meadow cinnamon soils, thus confirming the different role of different Cd forms on soil enzyme activity. On the other hand, urease activity was much more sensitive to HM combination pollution than the other enzymes in the study as it was inhibited by all metals and the enzyme activity markedly decreased comparing with the sole Cd, Zn and Pb treatment (Table 7, Fig.2). This behavior could be due to differences in the composition of soil enzyme with different sensitivities to metal toxicity. Enzyme reactions are inhibited by metals, which may complex the substrate, combine with the protein-active groups of the enzymes, or react with the enzyme-substrate complex. Sulfhydral groups of enzymes serve as catalytic sites or as groups involved in maintaining the correct conformation of the protein. Metals can react with sulfhydral groups causing inactivation or inhibition of enzyme activity (Shaw and Raval, 1961).

Table 6 Multivariate regression analysis between Cd, Zn, Pb pollution in soil and soil enzyme activity

Dependent variable	Multivariate regression equation	Correlation coefficient (r)	F value	Partial correlation coefficient and F
Calatase activity	y=3.7226−0.0024(Cd)−0.0004(Zn)+0.0004(Pb)	0.947**	32.1**	F_{Cd}=5.17**, F_{Zn}=36.2**, F_{Pb}=54.82**
Urease activity	y=0.5218−0.0013(Cd)−0.00012(Zn)+0.000009(Pb)	0.7257*	6.19*	F_{Cd}=5.87*, F_{Zn}=12.57**, F_{Pb}=0.13
Alkaline phosphatase activity	y=0.7782−0.0061(Cd)+0.00018(Zn)−0.00013(Pb)	0.7857**	8.53**	F_{Cd}=18.09**, F_{Zn}=4.19, F_{Pb}=3.30
Invertase activity	y=4.9359−0.02231(Cd)−0.00047(Zn)−0.00046(Pb)	0.7470**	6.84**	F_{Cd}=16.04**, F_{Zn}=1.79, F_{Pb}=2.68

Note: * and **. Significance at 0.05 and 0.01 levels of probability, respectively

Table 7 Stepwise regression results between Cd, Zn, Pb pollution in soil and soil enzyme activity

Dependent variable	Stepwise regression equation	Correlation coefficient (r)	F value
Calatase activity	y=3.723−0.002(Cd)−0.0004(Zn)+0.0004(Pb)	0.947**	32.1**
Urease activity	y=0.527−0.001(Cd)−0.0001(Zn)	0.790*	9.94*
Alkaline phosphatase activity	y=0.786−0.006(Cd)	0.703**	12.7**
Invertase activity	y=4.523−0.022(Cd)	0.713**	13.4**

Note: * and **. Significance at 0.05 and 0.01 levels of probability, respectively

The mode of action of metals varies with enzymes and little is known about the exact mechanisms by which metals interact with the multitude of enzymes that exist in soils.

Generally, different metal ions show different behaviors in their ability to act as inhibitors. The effect of combined pollution by Cd, Zn and Pb on soil enzyme activities mainly showed the inhibiting effect, especially Cd and Zn. In natural soils, under conditions of biovalibility, HM exhibit toxic activity towards soil biota (Kabata-Pendias and Pendias, 1999; Scott-Fordsmand and Pedersen, 1995; van Hesteren *et al.*, 1998) which may lead to the decrease of the number and the activity of soil microorganisms, thus the decrease on enzyme activities primarily by direct suppression of microbial growth by soil conditions in these contaminated sites. Alternatively, a portion of the reduction in enzyme activity may possibly have been caused by direct interactions between enzymes and HM (Gadd, 1993; Chen *et al.*, 2005). Landi *et al.* (2000) reported a decrease in the enzyme activity-to-microbial biomass ratio in the presence of available Cd. Effectiveness of the trace elements depends on the type and concentration added because a proportion of the SH groups of enzyme-active sites reacts with the trace element ions.

2.2 Effect of the ionic impulsion of combined Cd, Zn and Pb pollution on soil enzyme activities

Azpiazu and Romero (1986) reported that the ionic impulsion (I) could be the index of plant-soil pollution. It means that the total amount of metal pollution can be controlled by the ionic impulsion, which was expressed as:

$$I = \sum C_i^{1/n}$$

where C_i is metal c oncentration and n is metal oxidation amount. So the concept of "ionic impulsion" was introduced and ionic impulsion values of Cd, Zn and Pb supplied in soil and that of Cd, Zn and Pb accumulated in canola plant tops were calculated by the equation in this study (Table 8). Information about the relationship between the soil enzyme activities and ionic impulsion was presented (Tables 9 and 10). Table 9 shows that four soil enzyme activities have decreased with increasing ionic impulsion of soil HM, suggesting that soil enzyme activities have been inhibited with increasing total HM, and soil urease was sensitive to pollution of HM, with a significant negative correlation between urease activity and ionic impulsion of HM in soil. It was interesting that the enzyme activities also decreased with increasing ionic impulsion of accumulated HM in canola plant tops except for alkalin phosphatase, also with a significant negative correlation between urease activity and ionic impulsion of HM in canola plant tops, indicating that soil enzyme activities decreased with the increase of total heavy metals in soil and canola plant tops (Tables 9 and 10). It is concluded that soil urease activity may be a sensitive tool for assessing additive toxic HM combination effect to soil biochemical parameters and plant HM content. Very little information is available on the action mechanism between soil enzyme activity and ionic impulsion of HM accumulated in plants.

3 Conclusions

The present study demonstrated that the addition of Cd, Zn and Pb to soil, especially Cd and Zn, synergistically and negatively inhibited soil enzyme activities except for invertase activity. Cd significantly inhibited the four enzyme activities, and Zn only inhibited urease and calatase activities whereas Pb was significantly less inhibitory than the other HM for the four enzyme activies and was shown to have a protective role on calatase activity. With regard to the four enzymes, uease is the most sensitive to combined

Table 8 Ionic impulsion value of Cd, Zn, Pb pollution in soil and canola plant tops in the presence of every treatment level

Treatment number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
HM1	1.89	3.33	4.19	5.63	2.33	3.77	4.63	6.07	4.03	4.03	2.02	5.52	2.94	5.14	4.05
HM2	2.22	2.42	4.17	4.21	2.71	3.00	4.09	4.51	3.29	3.77	2.25	4.78	3.51	3.42	3.48

Notes: HM1. HM ionic impulsion in soil; HM2. HM ionic impulsion in canola plant tops

Table 9 Relationship between ionic impulsion of the supplied metals in soil and four soil enzyme activities

Dependent variable	Single variate regression equation	Correlation and significance test (r)
Catalase activity	y=3.7788–0.0197I	-0.13
Urease activity	y=0.5344–0.0212I	-0.54*
Alkaline phosphatase activity	y=0.5968–0.009I	-0.08
Invertase activity	y=4.7964–0.2053I	-0.048

Note: * Significance at 0.05 level of probability

Table 10 Relationship between ionic impulsion of the accumulated HM in canola plant tops and four soil enzyme activities

Dependent variable	Single variate regression equation	Correlation and significance test (r)
Catalase activity	y=4.144–0.129I	-0.52
Urease activity	y=0.606–0.046I	-0.720*
Alkaline phosphatase activity	y=0.499+0.039I	0.201
Invertase activity	y=4.936–0.281I	-0.402

Note: * Significance at 0.05 level of probability

pollution of Cd, Zn and Pb with a significant negative correlation between urease activity and ionic impulsion of HM. Therefore it is feasible to take soil urease activity as the main biochemical index to evaluate multiple metal pollution by soil Cd, Zn and Pb. However, only Cd was the most effective inhibitor on the four enzymes in this study. Further studies on the possibility of combined toxic effect mechanism for HM in soil are necessary, since in natural environment, contaminated soil often contain high amounts of multiple metal pollutants, suggesting that more further experiments should be performed to confirm the enzyme kinetics and mechanisms for the effects of HM interaction.

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