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Original Research Paper

Kinetic Model and Ecological Dose Values for the Inhibition of Effects of Cd^{2+} and Hg^{2+} on Soil Enzymes

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ABSTRACT

Effects of Hg^{2+} and Cd^{2+} on the activities of urease, invertase and amylase in different periods and concentration were studied by indoor soil cultivating method. Results showed that the activities of soil urease, invertase, and amylase by heavy metals were inhibited markedly, but this inhibitory effect differed among enzymes. During the treatment of 45 days, the activities of urease, invertase and amylase were decreased with the increase of the concentrations of Hg^{2+}. There were significant logarithmic correlations between the concentration of heavy metals and the inhibition ratios of the activities of these three enzymes ($r^2 > 0.902$). The inhibitory effect was also characterized in the term of ecological dose value (ED_{50}). It indicated that urease activity was more sensitive than the other two enzymes to heavy metals contamination. So, urease activity may be a suitable early warning index to be used in the characterization the soil pollution condition of heavy metals.

INTRODUCTION

In recent years, soil pollution is becoming more and more severe due to the increased using in mining, manufacturing and of synthetic products, such as pesticides, paints, batteries, industrial waste and land application of industrial or domestic sludge (Fang 2000). Polluted soil area has reached 20 million hm^2 in China, accounts for about 1/5 of the total cultivated area (Yang 2007, Liang 2009), threatening the soil ecological environmental quality, food security and sustainable social and economic development (Sun et al. 2003).

Soil microbes are important reflection for soil quality and enzyme activity involved in the biogeochemical cycling of carbon, nitrogen, phosphorus, sulphur and other nutrients (Caldwell 2005). The major parameters of soil quality are the soil biological properties. Among these, special emphasis is made of the enzyme activities. Soil enzyme activities have been generally accepted as one of the diagnostic indices of soil fertility quality and can quantify changes as a result of human disturbance (Bandick 1999, Dick 1994). Application of enzyme activity as a diagnostic index promotes high sensitivity to external effects, simplicity of definition and low errors (Bhattacharyya 2008).

Ecological dose 50 % (ED_{50}), which was first developed by Babich et al. (Babich 1983), the concentration of the heavy metal at which the enzyme, or other biological activities, is reduced to 50% of the uninhibited value. Michaelis "Menten kinetic approach (Speir 1999) and sigmoidal dose" response model (Haanstra 1985) have been used to calculate the ED_{50} values in relating to tested factors.

The effects of Hg^{2+} and Cd^{2+} on the activities of urease, invertase and amylase in different periods and concentration were studied by indoor soil cultivating method in this research, and the specific objectives are to: (1) modify the dose-response model; (2) compare the inhibition effect of Hg^{2+} and Cd^{2+} on soil enzymes; and (3) to establish which kind of soil enzyme is more sensitive to heavy metal contamination.

MATERIALS AND METHODS

We used surface soil (0-20 cm) from east campus of Yancheng Institute of Technology, Jaingsu Province, China (33°38'22"N and 120°13'58"E). According to the World Reference Base for Soil Resources (IUSS Working Group WRB 2006) and Chinese Soil Taxonomic Classification (Gong 1999), this soil is a silty clay (Stagnic Anthrosol). Stones and obvious pieces of organic materials were removed. After that, samples were stored in plastic bags in a refrigerator at 4°C for further experiments.

Some physico-chemical characteristics of the soil are given in Table 1. Soil organic C content was determined by the Walkley and Black dichromate oxidation method (Blakemore 1972). Soil pH was measured in a 1:2.5 soil-water slurry using a glass electrode. Saturation paste extracts were prepared, the electrical conductivity (EC) was measured and the K^+ and Na^+ concentrations were analysed by a PE-Zeemam-5100 atomic absorption spectrophotometry (AAS). Soil total N (TKN) was estimated using the semi-micro Kjeldahl method (SAS 1988). Available P was determined

Table 1: Physico-chemical properties of soils tested.

Soil characteristics	Values ± Standard errors
pH	7.84 ± 0.05
EC (ds m^{-1})	3.12 ± 0.16
CEC (meq 100 g^{-1})	19.40 ± 1.0
Clay (%)	25.20 ± 0.78
Silt (%)	32.30 ± 1.28
Sand (%)	42.50 ± 1.46
TOC (%)	1.06 ± 0.12
TKN (%)	0.073 ± 0.018
Available phosphorus (%)	0.042 ± 0.011
Available potassium (%)	2.43 ± 0.06
Available sodium (%)	2.34 ± 0.08
Cu (mg kg^{-1})	65.12 ± 0.66
Pb (mg kg^{-1})	25.26 ± 0.94
Cd (mg kg^{-1})	1.02 ± 0.22
Hg (mg kg^{-1})	0.18 ± 0.04

by the classical Olsen method (ISSCAS 1978). Exchangeable cations were extracted with 1.0 mol/L ammonium acetate (1:4 w:v for 2 h in a rotary shaker) and Ca^{2+}, Mg^{2+}, K^{+} and Na^{+} in extracts were analysed by AAS. The soil total concentration of heavy metals (Cu, Pb and Cd) was also detected by AAS in a solution prepared after digestion with HNO_3-$HClO_4$ (2:1, V:V). Clay mineral compositions were identified by X-ray diffraction analysis. Total mercury contents were determined in solid soil samples using mercury analyser F732-VJ (Hangzhou, China) with a detection limit of 0.01 μg/L.

The soils (100 g) were treated with 5 mL of $CdCl_2$ or $HgCl_2$ solutions to give the heavy metal concentration at six different levels ranging from 0.5 to 100 mg/kg soil. Untreated soils were served as controls. The soil moisture was adjusted to 60% of the field water holding capacity (WHC) with distilled water and then the soils were incubated at 25°C and kept in darkness for 45 days.

Amylase (EC 3.2.1.1) and invertase (EC 3.2.1.26) were assayed by the methods of Guan (Guan 1983). For measuring amylase activity, excessive starch was added with phosphoric acid buffer (PAB, pH5.5), and then the released reducing sugar was determined using Nelson reagent after 96h incubation (Nelson 1944). Amylase activity was expressed as mL 0.05 mol/L $Na_2S_2O_3$ h^{-1} g^{-1}. The method for invertase measurement was similar to that for amylase excepting substrate and the investigation for reducing sugar; here we take saccharose as the substrate for invertase, take acetic acid buffer instead of PAB, and measured released reducing sugar using absorbance at 551 nm for sugar and anthrone with the absorbance was calibrated against standard solutions of glucose. Invertase activity was expressed as mg glucose per g soil per hour. Urease (EC 3.5.1.5) activity was measured with the methods described by Tabatabai & Bremner (1972), with urea as the substrate, expressed as mg NH_4-N g^{-1} h^{-1}.

All determinations were performed in triplicate; all values reported are means with their standard error on the basis of the oven dry (105 °C) weight of soil. Excel 2007 and Origin 7.0 analysis software for windows were used for statistical tests.

RESULTS AND DISCUSSION

Effects of Cd^{2+} and Hg^{2+} on soil amylase, invertase and urease activities were given in Tables 2 and 3. Compared with the controls, the enzymes activities decreased with the increasing concentration of heavy metals and the incubation periods except for Cd^{2+} treatment at 0.5 mg/kg.

Urease activities were found to be more sensitive to the inhibition effect of heavy metals than amylase and invertase. After 45 days incubation under the concentration of Cd^{2+} at 100 mg/kg, the inhibition rates of soil amylase, invertase and urease activities were determined at 93.09, 53.99 and 58.62 % compared to the control, respectively. As for Hg^{2+} treatment, the inhibition rates were 94.74, 63.21 and 65.52 %, higher than the Cd^{2+} treatment, which indicated that the inhibition effect was different from the kinds of heavy metals. Disagree with the results of Zhou et al. (1985), soil enzyme activity was found to have a different degree of recovery after a long period of heavy metals stress, which would be contributed to the different concentrations of heavy metals.

The inhibition effect of heavy metals on soil enzyme activities was the result of the changes of chemical conformation mainly due to the coordination reaction. Based on Lewis's hard and soft acids and base theory, the active sites in enzyme protein molecular, such as thiol or imidazolyl groups, were preferred coordinated with soft heavy metals.

Different kinetic mathematical models have been used to assess the inhibition of soil biological and biochemical properties by heavy metals, such as dose-response models proposed by Speir et al. (1999) and Haanstra et al. (1985). The concept of ecological dose 50% (ED_{50}), was useful in determining the toxicity of heavy metals to soil enzymatic catalytic reactions.

The changes of inhibition ratio Y (%) of inhibitor on soil enzyme activities with different concentrations C (mg/ kg) of heavy metals are shown in Fig. 1 and 2, where the inhibition ratio (%) was calculated from the enzyme activities stressed by Cd^{2+} and Hg^{2+} with the control. The ED quantitative evaluation was also based on the inhibition ratio Y (%) on heavy metals concentrations. Natural logarithm calibration plots were obtained and the algebraic regression equation was determined as follows:

Table 2: Effects of Cd^{2+} on soil enzymes (n = 3).

Concentration (mg/kg)	Incubation period (days) 1	3	5	7	10	25	45
Urease activities (μg NH_4-N g^{-1} soil h^{-1})							
0	4.20 ± 0.36	4.22 ± 0.32	4.07 ± 0.20	4.26 ± 0.14	4.29 ± 0.25	4.31 ± 0.27	4.14 ± 0.23
0.5	3.78 ± 0.28	3.79 ± 0.25	3.84 ± 0.27	3.86 ± 0.20	3.94 ± 0.24	3.98 ± 0.24	4.02 ± 0.27
1	3.52± 0.30	3.21 ± 0.24	3.14 ± 0.31	2.86 ± 0.33	2.71 ± 0.18	2.50 ± 0.18	2.36 ± 0.26
5	3.03 ± 0.28	2.71 ± 0.30	2.43 ± 0.26	2.36 ± 0.35	2.14 ± 0.16	1.86 ± 0.22	1.71 ± 0.18
10	2.71 ± 0.31	2.57 ± 0.18	2.14 ± 0.14	2.04 ± 0.28	1.86 ± 0.20	1.71 ± 0.35	1.57 ± 0.16
50	2.24 ± 0.20	1.79 ± 0.24	1.57 ± 0.18	1.43 ± 0.31	1.29 ± 0.26	0.86 ± 0.20	0.71 ± 0.24
100	1.43 ± 0.24	1.28 ± 0.38	1.00 ± 0.24	0.86 ± 0.26	0.79 ± 0.12	0.57 ± 0.26	0.29 ± 0.08
Invertase activities at different periods (mg glucose kg^{-1} soil h^{-1})							
0	5.98 ± 0.33	6.17 ± 0.28	6.38 ± 0.35	6.01 ± 0.32	6.01 ± 0.23	6.01 ± 0.32	5.86 ± 0.34
0.5	5.98 ± 0.28	5.93 ± 0.32	6.17 ± 0.27	6.17 ± 0.36	6.17 ± 0.27	6.29 ± 0.26	6.32 ± 0.31
1	5.67 ± 0.29	5.55 ± 0.24	5.35 ± 0.26	5.09 ± 0.26	4.78 ± 0.24	4.62 ± 0.24	4.62 ± 0.26
5	5.35 ± 0.30	5.09 ± 0.26	4.80 ± 0.18	4.32 ± 0.20	4.01 ± 0.32	3.85 ± 0.28	3.70 ± 0.18
10	5.04 ± 0.32	4.47 ± 0.31	4.25 ± 0.30	3.70 ± 0.14	3.54 ± 0.30	3.24 ± 0.22	3.24 ± 0.24
50	4.88 ± 0.26	4.32 ± 0.27	3.93 ± 0.26	3.54 ± 0.32	3.24 ± 0.28	3.00 ± 0.25	2.93 ± 0.30
100	4.41 ± 0.22	3.70 ± 0.26	3.30 ± 0.29	3.00 ± 0.25	2.93 ± 0.31	2.77 ± 0.31	2.70 ± 0.22
Amylase activities at different periods (mL 0.05 mol/L $Na_2S_2O_3$ g^{-1} h^{-1})							
0	1.91 ± 0.18	1.91 ± 0.24	1.81 ± 0.20	1.84 ± 0.22	1.87 ± 0.24	1.84 ± 0.25	1.81 ± 0.25
0.5	1.88 ± 0.12	1.88 ± 0.20	1.90 ± 0.14	1.93 ± 0.16	1.93 ± 0.18	1.96 ± 0.14	2.01 ± 0.15
1	1.81 ± 0.14	1.75 ± 0.14	1.62 ± 0.14	1.56 ± 0.16	1.56 ± 0.12	1.51 ± 0.13	1.50 ± 0.10
5	1.69 ± 0.17	1.56 ± 0.18	1.50 ± 0.22	1.43 ± 0.12	1.37 ± 0.14	1.34 ± 0.18	1.31 ± 0.21
10	1.63 ± 0.12	1.47 ± 0.16	1.31 ± 0.12	1.25 ± 0.14	1.18 ± 0.12	1.12 ± 0.16	1.06 ± 0.14
50	1.51 ± 0.15	1.31 ± 0.08	1.18 ± 0.10	1.12 ± 0.20	1.03 ± 0.15	0.93 ± 0.19	0.87 ± 0.16
100	1.19 ± 0.16	1.12 ± 0.12	1.02 ± 0.26	0.87 ± 0.10	0.86 ± 0.19	0.81 ± 0.10	0.75 ± 0.22

Table 3: Effects of Hg^{2+} on soil enzymes (n = 3).

Concentration (mg/kg)	Incubation period (days) 1	3	5	7	10	25	45
Urease activities (μg NH_4-N g^{-1} soil h^{-1})							
0	4.20 ± 0.36	4.22 ± 0.32	4.07 ± 0.20	4.26 ± 0.14	4.29 ± 0.25	4.31 ± 0.27	4.14 ± 0.23
0.5	4.00 ± 0.32	3.78 ± 0.26	3.64 ± 0.22	3.50 ± 0.27	3.28 ± 0.29	3.14 ± 0.34	3.07 ± 0.21
1	3.71 ± 0.34	3.50 ± 0.28	3.28 ± 0.32	3.00 ± 0.21	2.93 ± 0.20	2.85 ± 0.23	2.78 ± 0.24
5	3.28 ± 0.30	2.92 ± 0.33	2.64 ± 0.23	2.36 ± 0.25	2.21 ± 0.17	2.16 ± 0.22	2.01 ± 0.25
10	2.78 ± 0.28	2.35 ± 0.25	2.93 ± 0.34	1.78 ± 0.28	1.71 ± 0.30	1.51 ± 0.26	1.35 ± 0.19
50	2.07 ± 0.28	1.50 ± 0.14	1.21 ± 0.28	0.93 ± 0.23	0.85 ± 0.21	0.71 ± 0.33	0.57 ± 0.30
100	1.35 ± 0.27	0.93 ± 0.20	0.64 ± 0.21	0.50 ± 0.26	0.50 ± 0.22	0.36 ± 0.23	0.21 ± 0.10
Invertase activities (mg glucose kg^{-1} soil h^{-1})							
0	5.98 ± 0.33	6.17 ± 0.28	6.38 ± 0.35	6.01 ± 0.32	6.01 ± 0.23	6.01 ± 0.32	5.86 ± 0.34
0.5	5.82 ± 0.24	6.02 ± 0.28	5.98 ± 0.22	5.70 ± 0.26	5.55 ± 0.30	5.40 ± 0.22	5.24 ± 0.21
1	5.50 ± 0.29	5.55 ± 0.29	5.51 ± 0.16	5.24 ± 0.29	4.93 ± 0.26	4.68 ± 0.38	4.47 ± 0.28
5	5.35 ± 0.33	5.09 ± 0.26	4.88 ± 0.19	4.39 ± 0.25	4.16 ± 0.22	3.93 ± 0.30	3.75 ± 0.12
10	4.87 ± 0.24	4.47 ± 0.33	4.25 ± 0.32	3.75 ± 0.21	3.51 ± 0.25	3.39 ± 0.26	3.34 ± 0.26
50	4.41 ± 0.25	3.85 ± 0.22	3.62 ± 0.25	3.24 ± 0.30	3.08 ± 0.18	2.92 ± 0.19	2.61 ± 0.37
100	3.77 ± 0.27	3.24 ± 0.21	2.99 ± 0.24	2.62 ± 0.26	2.63 ± 0.21	2.31 ± 0.21	2.16 ± 0.23
Amylase activities (mL 0.05 mol/L $Na_2S_2O_3$ g^{-1} h^{-1})							
0	1.91 ± 0.18	1.91 ± 0.24	1.81 ± 0.20	1.84 ± 0.22	1.87 ± 0.24	1.84 ± 0.25	1.81 ± 0.25
0.5	1.88 ± 0.13	1.82 ± 0.25	1.75 ± 0.14	1.68 ± 0.26	1.69 ± 0.18	1.62 ± 0.21	1.56 ± 0.17
1	1.82 ± 0.19	1.75 ± 0.16	1.66 ± 0.16	1.54 ± 0.13	1.50 ± 0.18	1.50 ± 0.23	1.43 ± 0.16
5	1.68 ± 0.17	1.52 ± 0.12	1.46 ± 0.23	1.37 ± 0.12	1.32 ± 0.16	1.30 ± 0.19	1.13 ± 0.27
10	1.50 ± 0.14	1.45 ± 0.19	1.32 ± 0.18	1.25 ± 0.18	1.10 ± 0.10	1.00 ± 0.26	0.94 ± 0.18
50	1.32 ± 0.17	1.31 ± 0.16	1.18 ± 0.19	1.22 ± 0.24	0.88 ± 0.19	0.88 ± 0.16	0.75 ± 0.26
100	1.19 ± 0.19	1.13 ± 0.16	1.00 ± 0.16	0.88 ± 0.15	0.81 ± 0.13	0.81 ± 0.15	0.63 ± 0.17

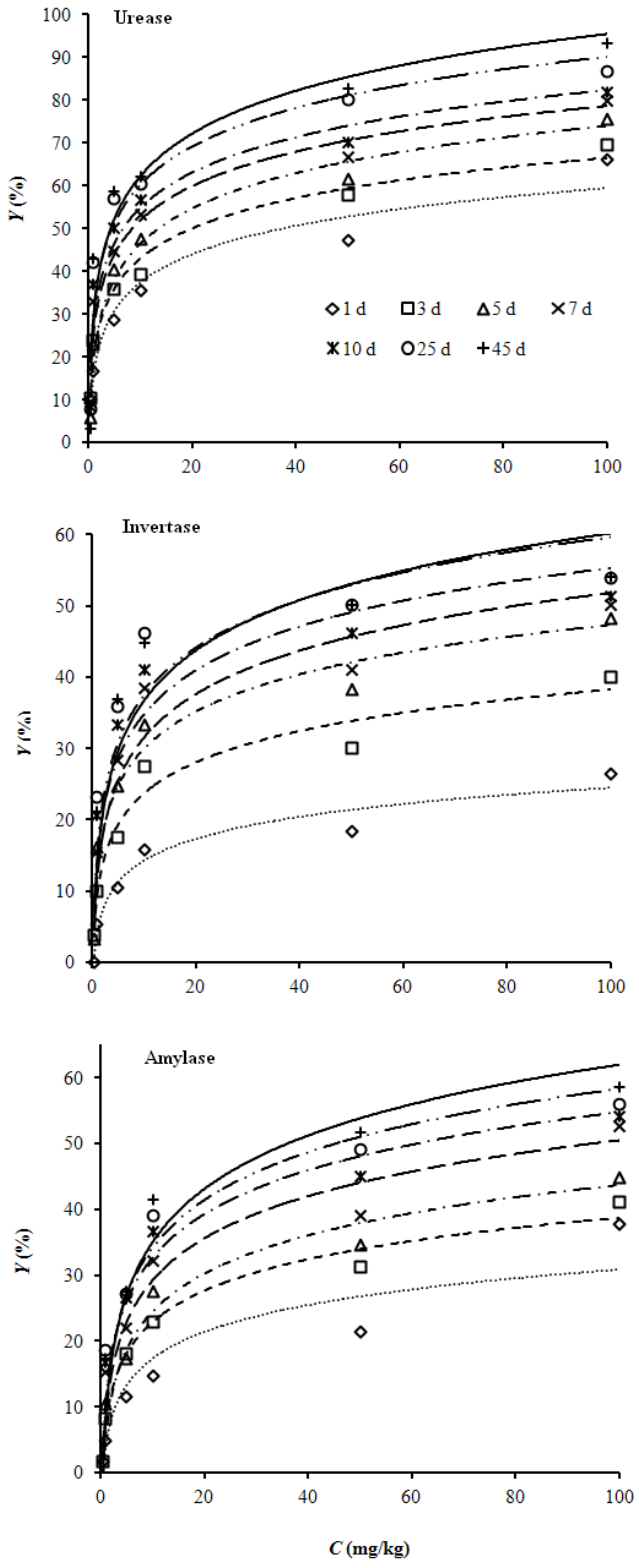

Fig. 1: Plots of the inhibition ratio of Cd^{2+} on soil enzyme activities.

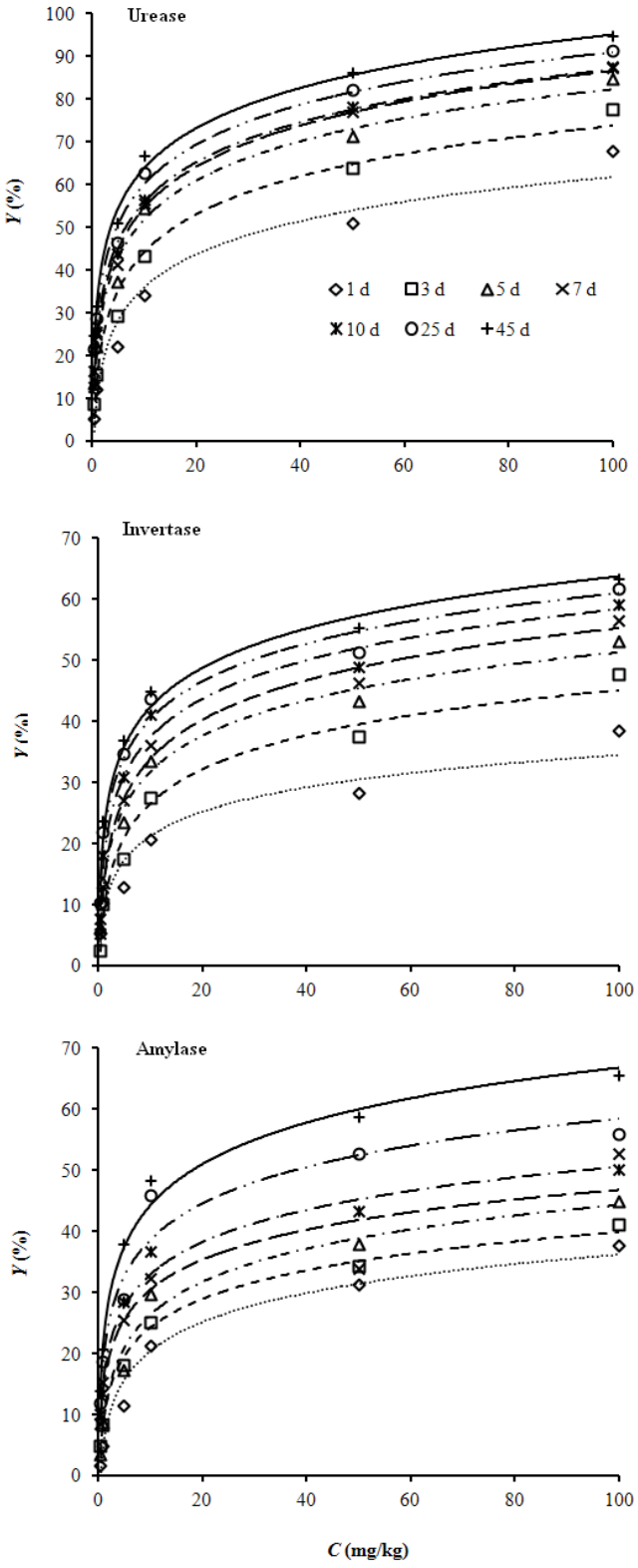

Fig. 2: Plots of the inhibition ratio of Hg^{2+} on soil enzyme activities.

Table 4: Correlations between the inhibition rates of soil enzymes activities and the concentration of heavy metals.

Incubation period (d)	Cd^{2+}				Hg^{2+}			
	$Y = A \ln C + B$		r^2	ED_{50} (mg/kg)	$Y = A \ln C + B$		r^2	ED_{50} (mg/kg)
	A	*B*			*A*	*B*		
Urease activities								
1	9.598	15.21	0.959	37.51	11.22	10.08	0.965	35.09
3	10.22	19.36	0.978	20.05	12.85	14.5	0.983	15.84
5	11.96	18.78	0.979	13.60	13.25	21.25	0.988	8.76
7	11.62	25.06	0.955	8.55	13.9	22.5	0.994	7.23
10	11.93	27.25	0.929	6.73	13.47	24.86	0.997	6.46
25	13.01	30.13	0.910	4.61	13.44	29.05	0.993	4.75
45	14.44	28.85	0.902	4.32	13.57	32.5	0.994	3.63
Invertase activities								
1	4.212	4.804	0.938	45720.15	5.754	7.988	0.938	1482.3
3	6.326	9.099	0.958	642.61	8.054	8.014	0.981	183.66
5	7.553	12.52	0.957	142.92	8.473	12.25	0.988	86.08
7	6.999	17.24	0.939	107.84	9.317	12.35	0.992	56.88
10	6.429	22.50	0.963	72.06	9.109	16.39	0.982	40.03
25	6.531	25.59	0.932	42.00	8.975	19.65	0.978	29.41
45	6.877	24.25	0.941	42.28	9.308	20.85	0.982	22.91
Amylase activities								
1	5.871	3.816	0.892	2608.32	6.851	4.631	0.977	751.63
3	6.878	7.038	0.986	516.10	6.481	8.36	0.982	617.04
5	7.278	8.804	0.958	287.25	7.777	8.064	0.988	219.71
7	7.686	13.09	0.938	121.78	7.094	14.08	0.915	158.13
10	7.927	16.08	0.980	72.09	7.640	15.33	0.982	93.50
25	8.246	17.46	0.977	51.74	8.615	18.73	0.957	37.70
45	9.135	16.60	0.975	38.72	9.796	21.64	0.989	18.08

$$Y = A \ln C + B \quad ...(1)$$

Where Y is the inhibition rate (%), C is the concentrations of heavy metals (mg/kg), and A and B are simulation constants. The characteristics of the calibration plots are listed in Table 4; better correlation was found in the simulation plots with the correlation coefficient $r^2 > 0.902$.

ED_{50} was also calculated based on the proposed regression equation and given in Table 4. Smaller the value of ED_{50}, stronger the inhibition effect of the heavy metals on soil urease, invertase and amylase. Results have shown that the ED_{50} values were decreased gradually with the incubation period from 1 d to 45 d, indicating that the toxicity of heavy metals Cd^{2+} and Hg^{2+} on soil enzyme activities was gradually strengthened along with the training time increased. ED_{50} values of Hg^{2+} stress on soil urease were calculated to be 35.09, 15.84, 8.76, 7.23, 6.46, 4.75 and 3.63 mg/kg at 1, 3, 5, 7, 10, 25 and 45 d, respectively.

ED_{50} values of heavy metals on urease activities were found to be smaller than soil invertase and amylase at every incubation period (Table 4), which indicated that soil urease was most sensitive in evaluating the toxicity of heavy metals on soil enzyme activities and could be use as a potential indictor on assessing the inhibition of soil biological and biochemical properties by heavy metals. As for the different kinds of heavy metals in this research, the toxicity of Hg^{2+} on soil enzyme activities was also found to be higher than Cd^{2+} with the lower values of ED_{50}. For example, ED_{50} values of urease, invertase and amylase under Hg^{2+} stress at the incubation time of 45 d were calculated to be 3.63, 22.91 and 18.08 mg/kg, respectively; meanwhile, these ED_{50} values under Cd^{2+} stress were 4.32, 42.28 and 38.72 mg/kg.

CONCLUSIONS

Soil enzyme activities were inhibited by heavy metals, but the inhibition effects were differed among different kinds of enzymes. Significant logarithmic correlations were determined between the concentration of heavy metals and the inhibition ratios of the activities of these three enzymes ($r^2 > 0.902$). Ecological dose value (ED_{50}), which was used to characterize the inhibitory effect, indicated that urease activity was more sensitive than amylase and invertase to heavy metal contamination.

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