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Effect of combined pollution by heavy metals on soil enzymatic activities in areas polluted by tailings from Pb-Zn-Ag mine

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Abstract: Some enzymatic activities were determined in the areas polluted by tailings from Tiantai Pb-Zn-Ag Mine in Zhejiang Province of China. The results indicated the soil enzymatic activities decreased significantly with increase of concentrations of heavy metals or the distance away from mining tailing center, especially dehydrogenase and urease activities. Multivariate regression analysis between heavy metal contents and soil enzymatic activities indicated that single dehydrogenase activity was very significantly correlated to combined effect of soil heavy metals in mine area. Moreover, single urease, protease and acid phosphatase activities were significantly related to the combined effect of heavy metals. The results suggest it is feasible to use soil enzymatic activities to indicate the pollution situation by combined heavy metals in the soil of mine area.

Keywords: heavy metal; Pb-Zn-Ag mine tailing; soil enzyme

Introduction

Heavy metals are of great concern because they can enter the bodies of human beings and animals by food chain and then lead to health impacts. Mining exploitation produces a great deal of wastes, which is a main source of heavy metals. Anthropogenic activities such as mining, smelting, electroplating, ore-refining and land disposal of wastes have led to an increase of heavy metal contents in the soil(Chen, 1996). The exploitation of Pb-Zn-Ag mine can cause grievous destruction, becoming a serious problem not only in China, but also in the world. Recently, more and more attention has been paid to the situation of heavy metal contamination in tailings. Some results on contents and characteristics of heavy metal were reported in this field, but only little work has been done on the effect of heavy metal contamination on soil enzymatic activities in areas of tailings.

Enzyme exists extensively in soil. It not only can activate complex organic compound change into simple inorganic compound to supply the plants(Srivastava, 1991; Hojeong, 1999), but is also one of the soil capacity indexes as well(Yu, 1998; Irvine, 1993; Zeng, 2001). The activity of soil enzyme is easily influenced by physical, chemical and biogenic factors in environment and enzymatic activities which may reflect the overall microbial activity of soil; it is sensitive to variations induced by natural and anthropogenic factors (Gianfreda, 1996). So it is of great importance to study the effect of heavy metal contamination on soil enzymatic activities for further understanding the influence of heavy metal pollution on soil fertility and soil quality. The use of enzymatic activities as bioindicators to evaluate the degree of soil contamination by heavy metals have been proposed(Dick, 1992; Nannipieri, 1995). Zhou *et al*.(Zhou, 1985) have succeeded in using urease, invertin and acid phosphatase as indices of soil contamination by heavy metals. These studies are mostly concentrated on the effect of a single metal. In the real environment, however, soil pollution by several metals is more common than by a single element. Moreover, the combinative effects of multiple metals are greatly different from the individual effect (Chen, 1996). So combined pollution comes to arouse attention and turn to one of the important research directions in environmental science (Zheng, 2001).

The objectives of this work were: (1) to study the effect of heavy metal contamination on soil enzymatic activities in areas polluted by tailings from Tiantai Pb-Zn-Ag Mine in Zhejiang Province of China; (2) to probe into internal relationship between combined heavy metal pollution and soil enzymatic activities for the further understanding the use of soil enzymatic activities as bioindicators to evaluate the situation of soil contamination by combined heavy metals in the soil of mine area.

1 Materials and methods

1.1 Sampling sites

The present study was in the areas polluted by tailings from Tiantai Pb-Zn-Ag Mine, located at 28°57′02″N and 120°41′24″ E longitude, of Tiantai County, Zhejiang Province, southeast China. The topography is hill with elevations ranging from 50 to 150 m above sea level. There is a humid subtropical climate with an average annual temperature of 16.8℃ and a mean annual rainfall of 1332 mm, mostly concentrated from March to August.

1.2 Soil sampling and processing

The soil samples is collected at eight sample belts (S1, S2, S3, S4, S5, S6, S7 and S8), including one comparison sample(S8), which was not polluted by heavy metals. The sketch-map showing the locations of soil samples is listed in Fig.1. The old tailing(S2) was one site to deposit mining tail before mining tailing bank(S1) being built, and most mining tail was wheathering. The eight samples from eight sampling locations of each sampling belt were mixed to give one sample and transferred, kept in sealed plastic bags to the laboratory. The composite soil samples were sieved through a 2 mm sieve, homogenized, a portion of which were air-dried and ground to pass through an 1 mm sieve, adjusting to 45% of field moisture capacity, stored in polythene bags at 4℃ prior to soil enzymatic activities analysis, and a portion of this was air-dried for physical and chemical analysis. Some physical and chemical properties of the soil were measured with the routine analytical methods(ACSSSC, 1983), listed in Table 1.

1.3 Analytical methods

The total metals (Pb, Zn, Ag, Cu and Cd) were determined by the atomic adsorption spectrophotometry(AAS) after digestion with a mixture of HNO_3-HCl(Soon, 1993). The available metals (Pb, Zn, Ag, Cu and Cd) were extracted with EDTA solution and analyzed by AAS(Soon,

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1993). Urease activity was estimated by colorimetric determination of the ammonium released during the soil sample incubation with urea at 37℃ for 24 h, expressed as $mgNH_3$-N/(kg · 24h) (Guan, 1986). Acid phosphatase activity was colorimetrically determined by using phenylphosphate as substrate and measuring the phenol produced, shown as mgC_6H_5OH/(kg · 24h) (MLISSCAS, 1985). Dehydrogenase activity(DHA) assay was based on the use of 2, 3, 5-triphenyltetrazolium chloride(TTC), a water-soluble compound, which was reduced to triphenyl formazan(TPF), a red-colored compound (Ross, 1971; Casida, 1964; Frankenberger, 1983; Gong, 1997). Moreover, activities of sucrase and protinase were measured by colorimetric method (Institute of Soil Science, 1985; Guan, 1986). While peroxidase activity was expressed by n ml 0.1 mol/L $KMnO_4$ for titration(Guan, 1986).

Table 1 Some physical and chemical properties of soil samples tested

Soil No.	pH (H_2O)	Organic C, g/kg	Total N, g/kg	Available N, mg/kg	CEC, cmol/kg	Size composition, %		
						2—0.02 mm	0.02—0.002 mm	< 0.002 mm
S1	7.84	4.52	0.87	8.25	1.51	58.35	32.53	9.12
S2	6.48	0.22	0.08	1.31	1.26	65.87	25.59	8.54
S3	7.61	2.05	0.96	9.71	2.75	67.23	23.81	8.96
S4	7.17	1.58	0.75	6.46	2.51	63.08	27.47	9.45
S5	5.38	5.79	0.95	67.87	6.01	54.45	33.20	12.35
S6	6.14	4.48	0.73	48.48	4.87	48.71	40.08	11.21
S7	5.18	6.24	1.97	105.44	9.75	31.06	58.30	10.64
S8	5.02	11.3	3.22	86.21	10.51	35.12	36.85	28.03

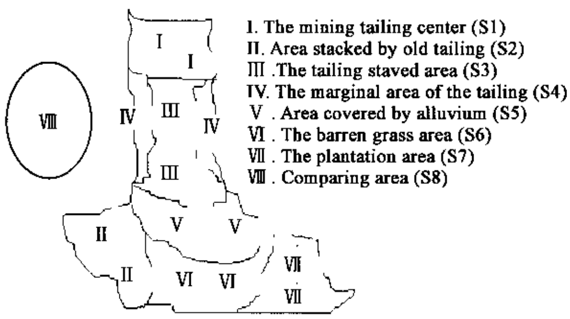

Fig.1 Sketch map showing the locations of soil samples

1.4 Data analysis

All experimental data were processed by Microsoft Excel 2000. The multivariate regression and the stepwise linear regression were conducted by using the statistical programs of SPSS (V10.1) and SAS (V6.12) Systems. The least significant difference(LSD) was used to test the significance between the means.

2 Results and discussion

2.1 Contents of heavy metals in the Pb-Zn-Ag mine

Heavy metal contents of soil samples collected in the Pb-Zn-Ag mine area are listed in Table 2. Results clearly showed that with the distance away from mining tailing center increasing and deposited time prolonging, the contents of heavy metals in the soil decreased diminishingly, especially total Pb and Zn markedly decreased, followed by Cu, Cd and Ag. This may be explained by leaching, translocation, accumulation of heavy metals released from the tailings into the soils. Therefore the concentration of total Pb, Zn, Ag, Cu or Cd near the center of the mine was higher than those of the relatively far away areas. On the other hand, the contents of total Pb, Zn, Ag, Cu and Cd from soil samples with heavy metals ranged from 433.94—1604.09 mg/kg, 597.80—1836.42 mg/kg, 9.44—41.92 mg/kg, 11.90—50.92 mg/kg, 4.62—15.21 mg/kg, with average contents of 996.61 mg/kg, 1138.64 mg/kg, 23.37 mg/kg, 28.15 mg/kg and 8.39 mg/kg, respectively, indicating that the concentration of heavy metals exceed soil environmental quality standards(National Standards for Soil Environmental Quality of China, GB15612-1995). In comparison with the soil sample without pollution by heavy metal(S8), the average content of total Pb, Zn, Ag, Cu and Cd increased by 33.99, 8.40, 9.97, 3.24, 37.14 fold, respectively. Moreover, the contents of available Pb, Zn, Ag, Cu and Cd from soil samples with heavy metals ranged from 16.68—53.55 mg/kg, 144.60—230.46 mg/kg, 0.46—1.40 mg/kg, 0.96—16.16 mg/kg and 0.35—1.96 mg/kg, with average contents of 33.80 mg/kg, 196.11 mg/kg, 0.99 mg/kg, 7.87 mg/kg and 1.01 mg/kg, respectively. Obviously, the average contents of available Pb, Zn, Ag, Cu and Cd increased by 31.82, 7.28, 3.11, 17.31, 49.57 fold, respectively, relative to S8, indicating that the soils in this region had been polluted severely, and might cause ecosystem problems and impacts on human health through food chain. The results of $LSD_{0.05}$ test indicated that there was significant variation among the contents of total and available Pb, Zn, Ag, Cu and Cd at $P < 0.05$ lever.

2.2 Soil enzymatic activities in the Pb-Zn-Ag mine

The enzymatic activities of soils samples tested are illustrated in Fig.2. The results revealed that there was a consistent increase in the enzymatic activities of soil with the distance away from mining tailing center increasing, especially all six enzymatic activities of S8 soil increased significantly, which were the highest of all samples. On the other hand, there was remarkable variation among soil enzymatic activities in different areas of the mine, particularly, the activities of urease, proteinase and dehydrogenase greatly changed, the variation coefficient ratio of which was 97.83%, 77.78% and 72.73%, respectively, followed by the activities of acid phosphatase, peroxidase and sucrase, which was 61.57%, 50.00% and 45.45%, respectively.

The results also showed that there was great variation among activities of different soil enzymes in the tailing. The average activities of urease, dehydrogenase, proteinase, acid phosphatase, sucrase and peroxidase in different soil samples with heavy metals were obviously lower than those from the sample without heavy metal(S8), accounting for 11.54%, 16.26%, 21.95%, 25.95%, 26.73% and 35.71% of S8, respectively. Therefore, it is obvious that the activities of urease and dehydrogenase in the tailing were less 20% of those of S8, indicating that urease and dehydrogenase could reflect special site conditions of the Pb-Zn-Ag mine, therefore, using urease and dehydrogenase as indices of soil contamination by heavy metals in the Pb-Zn-Ag mine may be taken as consideration.

Table 2 Heavy metal contents of soil samples collected in the Pb-Zn-Ag mine area, mg/kg

Soil No.	Total metal					Available metal				
	Pb	Zn	Ag	Cu	Cd	Pb	Zn	Ag	Cu	Cd
S1	1604.09	1836.42	41.92	50.92	15.21	53.55	230.46	1.40	16.16	1.96
S2	1399.09	1565.45	32.71	39.83	11.11	52.12	281.64	1.30	13.82	1.25
S3	1104.41	1272.54	25.15	31.75	9.77	40.21	198.41	1.31	10.48	1.21
S4	1061.37	1015.16	24.27	26.11	7.23	31.76	210.88	1.40	11.57	1.26
S5	714.54	923.14	17.03	19.81	5.10	22.66	163.00	0.53	1.09	0.59
S6	658.81	759.98	13.04	16.74	5.70	19.64	143.80	0.51	1.02	0.46
S7	433.94	597.80	9.44	11.90	4.62	16.68	144.60	0.46	0.96	0.35
Average	996.61	1138.64	23.37	28.15	8.39	33.80	196.11	0.99	7.87	1.01
S8	28.48	121.14	2.13	6.64	0.22	1.03	23.68	0.24	0.43	0.02
$LSD_{0.05}$	3.25	4.33	0.09	0.72	0.16	0.25	0.93	0.04	0.06	0.03

Note: data is means of three repeats in the table

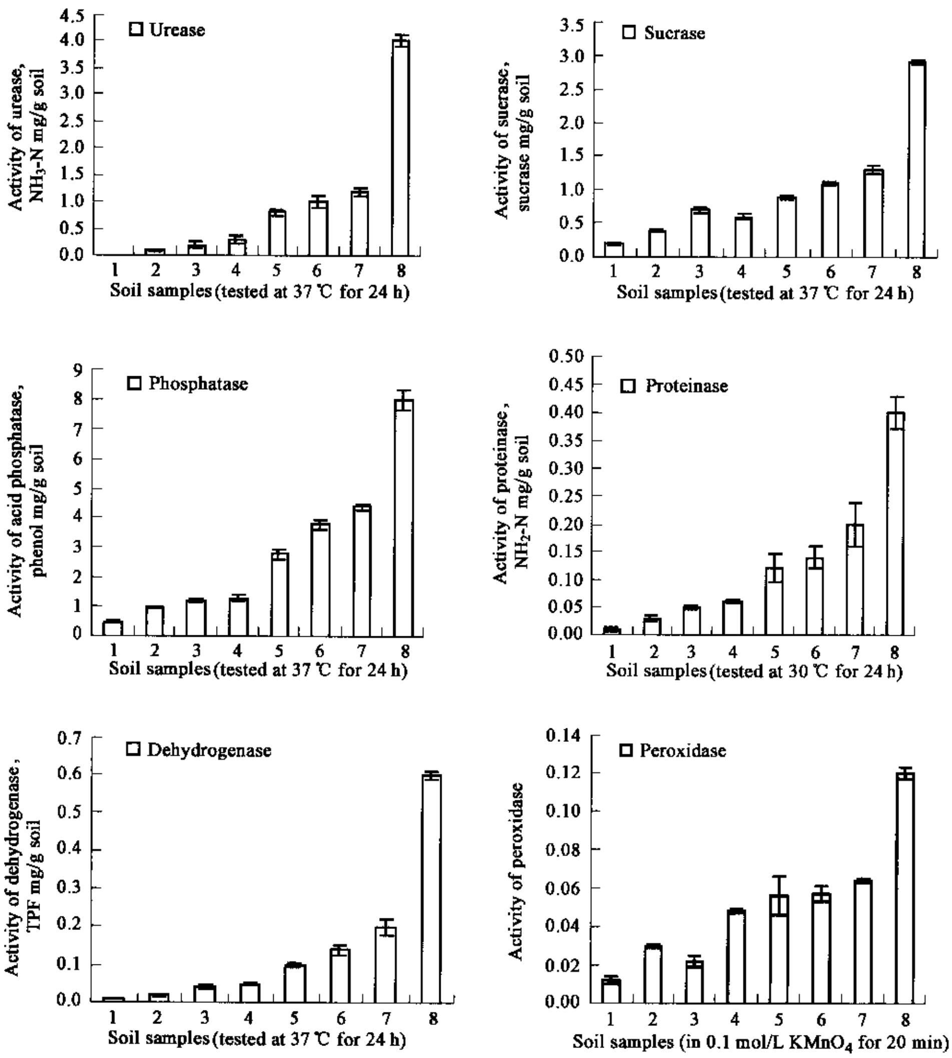

Fig.2 The enzymatic activities of soil samples tested

The factor analysis between the contents of heavy metal and basic physical and chemical properties of soil samples indicated that the variance contribution ratio of heavy metal contents as the first main factor was 83.03%, suggesting that the concentration of heavy metals was the main influencing factor caused decrease in soil enzymatic activities of the mine tailing. This may be explained that the heavy metals had inhibitory effects on the enzymatic activities. Similar influence of heavy metals was also reported by some other scientists (Yang, 2001; He, 2000). The mechanisms of inhibition of soil enzymes by heavy metals may be that the enzyme active site combines more or less loosely with the inhibitor, which is possibly structurally related to the substrate, thus preventing the attachment of the substrate to the enzyme-binding site. The inhibitor and substrate, therefore, compete for the same active site forming enzyme-substrate and enzyme-inhibitor complexes, respectively (Tabatabai, 1994).

The results also indicated that the combination of heavy metals and their interaction with nutrients made their effects on enzyme more complicated. Therefore, it is very difficult and complicated to study the interaction.

2.3 Relationships between soil enzymatic activities and combined pollution by heavy metals

Nowadays, in field of environment, soil combined pollution resulting from heavy metal is studied through multivariate regression analysis usually. Multivariate regression analysis between heavy metal contents and soil enzymatic activities are listed in Table 3. Multivariate regression model test indicated that Pb, Zn, Ag and Cd had inhibitory effects on the urease activities except that Cu could stimulate the activities of urease. The test suggested that under the circumstances of coexistance of Pb, Zn, Ag, Cu and Cd, the synergism of Pb, Zn, Ag and Cd chiefly exhibited the influence on the activity of urease, conversely the interaction of these metals and Cu exhibited antagonism on urease. Moreover, the effect of combined contamination by heavy metals on the activities of sucrase and proteinase were similar to that of urease. On the other hand, with the combined concentrations of Pb, Ag and Cd increasing, they had inhibitory influences on activities of dehydrogenase, acid phosphatase and peroxidase, while Zn and Cu had a slight stimulative effect, but the influence coefficient of heavy metals on activites of these three enzymes were discrepant. Inhibition of enzymatic activities might be due to the reduced microbial numbers and the activities in the soils, because the activity is derived from the intracellular enzymes (Skujins, 1967). The results described here on the suppression of soil enzymatic activities indicated the disruption of soil function by the heavy metal contamination in the mine soils.

The result of multivariate regression model test showed that with the confidence interval of 95%, the F value($F = 707.15$, $p = 0.001$) of the effect of combined heavy metals on single dehydrogenase activities was bigger than the critical value of $F_{0.001}$, indicating that single dehydrogenase activities was very significantly correlated to combined element content of soil heavy metals in mine area. Moreover, single urease, proteinase and acid phosphatase activities were significantly related to the combined content of heavy metals. Thus, it is speculated that the activities of dehydrogenase, urease, proteinase and acid phosphatase are important indices to reflect the pollution situation by combined heavy metals in the soil of mine area, which was consistent with the studies of He *et al*.(He, 2000).

Table 3 Multivariate regression analysis between heavy metal contents and soil enzymatic activities

Enzyme	Multivariate regression model	F value	P value
Urease	$Y = -1.390X_1 - 0.150X_2 - 1.067X_3 + 2.411X_4 - 0.934X_5$	24.69*	0.039
Sucrase	$Y = -0.703X_1 - 0.518X_2 - 1.440X_3 + 2.950X_4 - 1.500X_5$	8.09	0.114
Acid phosphatase	$Y = -1.193X_1 + 0.300X_2 - 0.494X_3 + 0.626X_4 - 0.349X_5$	78.24*	0.013
Proteinase	$Y = -1.458X_1 - 0.056X_2 - 0.861X_3 + 2.041X_4 - 0.788X_5$	22.00*	0.044
Dehydrogenase	$Y = -2.145X_1 + 0.409X_2 - 0.683X_3 + 1.866X_4 - 0.524X_5$	707.15**	0.001
Peroxidase	$Y = -4.099X_1 + 1.602X_2 - 0.725X_3 + 2.797X_4 - 0.417X_5$	4.78	0.182

Notes: X_1, X_2, X_3, X_4, X_5 indicate contents of Pb, Zn, Ag, Cu, Cd respectively; * factor which influenced significantly($p < 0.05$); ** factor which influenced particular significantly($p < 0.001$)

3 Conclusions

The soils were polluted by combined heavy metals, and Pb, Zn, Ag, Cu and Cd are the main pollutants in the mine tailing. Moreover, the contents of heavy metals in soils contaminated by tailings were higher than those in no-polluted soils. Soil pollution by heavy metals caused decreases in enzymatic activities. The pollution of combined metals alleviated their toxicity to enzymes. The soil enzymatic activities decreased significantly with increasing contamination of heavy metals, especially dehydrogenase and urease activities. The result of multivariate regression model test indicated that the activities of dehydrogenase, urease, proteinase and acid phosphatase are important indices to reflect the pollution situation by combined heavy metals in the soil of mine area. However, the combination of multiple metals and their interaction with nutrients made their effects on enzymes more complicated, indicating that further studies on the interaction are needed.

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