Front. Agric. China 2007, 1(1): 85–89
DOI 10.1007/s11703-007-0016-9

RESEARCH ARTICLE

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Effects of Cd and Pb pollution on soil enzymatic activities and soil microbiota

Abstract Based on a representative sampling method and pot experiment with different concentrations of Cd and Pd, the enzymatic activities (urease, phosphatase, catalase, invertase), population of bacteria, fungus and actinomycete in the soil, the Cd and Pd pollution status of soil samples (from the wastewater-irrigated area of Baoding suburb) were appraised. Unitary linear and nonlinear curve-fitting optimization models were applied in the research, and the relationship between Pb and Cd causing pollution and enzymatic activities of the tested soils were discussed. The research may provide a theoretical basis for protecting the environment in the region of Baiyangdian Lake, Hebei province, prevent soil pollution, and ascertain biochemical indexes, which reflect soil heavy metal pollution levels.

The research results indicated that: (1) there was obvious accumulation of Pb and Cd in the wastewater-irrigated area, also the accumulation in wastewater-irrigated soil is more than that in fresh water-irrigated soil, and accumulation on surface layer was more than that in the lower layer. Pb and Cd contents in the tested soils exceeded the standards of soil background values for some major cities at home and abroad and the world soil Cd and Pb contents range. This means that the tested soil had reached a lightly polluted level; (2) there existed an obvious negative correlation between soil enzymatic activities and Pb and Cd contents in wastewater-irrigated soil, where the soil urease and catalase activities decreased obviously with the increase of Pb and Cd contents in soil. Therefore, the urease and catalase can be considered as biochemical indexes that reflect the degree of soil Pb and Cd pollution; (3) the pot experiments indicated that the influence of Cd on soil enzymatic activities was greater than that of Pb. Generally, the effect of Cd on soil phosphatase, urease, catalase is more obvious than that on invertase, while Pb has a more obvious effect on invertase than Cd; (4) pot experiments of triple cropping showed that, Cd and Pb had an obvious inhibiting effect on microbes. The influence sensitivity of Cd and Pb on different microbes was ordered as bacteria $>$ actinomycete $>$ fungus. Cd had a greater inhibiting effect on microbes than Pb, the influence of Cd and Pb on actinomycete and fungus was relatively smaller, therefore, bacteria can be chosen as an important index that reflects the effect of Cd and Pb on microbe sensitivity.

Keywords soil enzymatic activity, wastewater-irrigated soil, heavy metal pollution, microbial effect

Received August 12, 2006; accepted October 5, 2006

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1 Introduction

Because of the increasingly more serious "Three Wastes" pollution, which has worsened the natural environment, it is of great significance to protect the soil environment from pollution to deepen our understanding of the environmental quality of regional soils, and to study heavy metal pollution, enzymatic activities and microbial effects in wastewater-irrigated soils.

The research and application of soil enzymology in environmental science has been looked at recently. However, related reports are still rather limited on a world scale (Gao, 1986; Guan, 1986; He et al., 2000; Kilaglish, 1981; Liu, 1996; Liu and Zhang, 1995; Liu et al., 2003; Tyley, 1974; Xu et al., 2004; Zhou, 1985). Researchers from the former U.S.S.R contributed to the researches on phenol organic wastewater purging soils. More attention was paid to the heavy metal pollution in Western Europe and Japan. In China, the research of soil enzymology was started in 1949 with the introduction of research methods, and was mainly concentrated on the relationship between soil enzymatic activities and soil fertility (Liu and Zhang, 1995; Liu, 1996), with fewer researches on the application of soil enzymatic activity in agricultural environmental protection. Even fewer researches have been done on the relationship between enzymatic activity and heavy metal pollution, the main biochemical indexes, the mechanism of soil purification of biochemistry and the protecting and harnessing measures in wastewater-irrigated areas upstream of the Baiyangdian Lake in China (Liu, 1996; Zhang et al., 2006).

This paper focuses on the researches ranging from soil-plant ecosystem to the relationship between heavy metal pollution and enzymatic activities and its varied situations, and influence of heavy metal pollution on microbes in the wastewater-irrigated area upstream of the Baiyangdian Lake. It provides a scientific basis for protecting the ecological environment, harnessing soil pollution and ascertaining its main biochemical indexes.

2 Materials and methods

2.1 Experimental soils

The soils were sampled from the pollutant sources near Baoding City, Hebei province and the area that has been irrigated with wastewater for 20–30 years at the suburban district of Baoding City, compared with the adjacent soils irrigated with fresh water (no wastewater irrigation by 2005). Twelve representative typical profiles divided into two layers (0–20 cm and 20–40 cm), and sixteen mixed soil samples were collected from each meadow cinnamon soil layer. The pH of the surface soils was from 7.91 to 8.03 and the content of $CaCO_3$ (calcium carbonate) was from 30 g/kg to 60 g/kg. The types of the soils were all medium loam meadow cinnamon soil. The basic characteristics of the soils are listed in Table 1. The potted soil was of medium loam meadow cinnamon soil, and collected from the experimental field of Agricultural University of Hebei. Each pot was loaded with 15 kg of soil. Seven different concentrations both for Cd and Pb were designed, with three replicates. The soils for analysis were sampled after a cropping cycle of wheat-Chinese cabbage-wheat (Table 4).

2.2 Methods

The contents of soil Pb and Cd were measured by using the AAS after extraction of HCl-HNO_3-$HClO_4$ (Li, 1984). Soil enzymatic activities were investigated by using the method developed by Guan Songmeng (1986), Zhou Likai (1980) and Zhao Lanbo et al. (1986; Liu, 1990). The soil microbial communities were determined with dilution-plate-count method.

3 Results

3.1 The heavy metal content of the soil and the situations of soil enzymatic activity in the wastewater irrigated area

The data in Tables 1 and 2 show that the total and rapidly available nutrients were notably increased in the wastewater-irrigated soil. However, the content of soil heavy metal accumulated obviously. The content of Pb was 31.6 mg/kg in the soil irrigated with wastewater compared with 25.7 mg/kg in the freshwater irrigated soil; The Cd content in the wastewater irrigated soil was 0.956 mg/kg compared with 0.8181 mg/kg in the freshwater irrigated soil. They all showed that the contents of Pb and Cd in the soil irrigated with wastewater were higher than those with freshwater, and the

Table 1 Physical and chemical properties of tested soil

Soil No.*	Irrigation type**	Depth / cm	O.M. / $g \cdot kg^{-1}$	Total N / $g \cdot kg^{-1}$	Total P / $g \cdot kg^{-1}$	Available N / $mg \cdot kg^{-1}$	Available P / $mg \cdot kg^{-1}$	Physical clay / $g \cdot kg^{-1}$
1	Wa	0–20	29.3	1.150	1.432	99.7	28.9	322
2	Wa	20–40	18.7	0.839	1.295	68.0	15.5	364
3	Fa	0–20	17.1	0.581	1.469	63.9	24.7	319
4	Fa	20–40	10.0	0.424	1.188	42.3	12.6	316
5	Wb	0–20	29.2	1.166	1.429	100.8	29.3	310
6	Wb	0–20	21.9	0.899	1.174	110.8	19.6	327
7	Wb	20–40	14.3	0.509	0.840	53.1	4.1	328
8	Fb	0–20	17.7	0.789	1.162	45.6	11.5	364
9	Fb	20–40	12.4	0.715	1.027	58.0	4.3	443
10	Wc	0–20	22.1	0.892	1.181	110.0	19.4	321
11	Fc	0–20	17.8	0.830	1.169	79.6	11.6	365
12	Wd	0–20	35.0	1.028	1.705	72.6	31.0	347
13	Fd	0–20	15.6	0.617	1.388	42.7	10.5	335
14	We	0–20	13.9	0.706	1.813	86.8	19.5	330
15	Fe	0–20	15.8	0.624	1.393	43.0	10.5	334
16	We	0–20	21.5	0.648	1.817	2.0	3.1	344

*: The soil samples from No. 1 to No. 11 were collected from the western outskirts of Baoding City, and those from No.12 to 16 were collected from the eastern outskirts of the city. All the soil types are meadow cinnamon soils and the soil texture was medium loam. P appeared in P_2O_5.

**: W = Irrigated with waster water; F = Irrigated with fresh water

Note: The locations of the sampled soils are as follows:

1, 2: wheat fields to the north of Chemical Fiber Factory; 3, 4: wheat fields to the south of Phosphate Fertilizer Factory; 5: wheat fields to the west of canal for Chemical Fiber Factory; 6, 7: paddy fields to the north of 604 Paper Mill; 8, 9: wheat fields to the west of road to Petroleum Works; 10: wheat fields to the north of Petroleum Works; 11: north wheat fields of Jiaozhuang Village to the north of Fuhe River; 12, 13, 14, 15: wheat fields of Fanzhuang Village to the south of Fuhe River; 16: wheat fields of Jiaozhuang Village to the south of Fuhe River

Table 2 Soil enzyme activities Pb and Cd contents in the wastewater-irrigated area of Baoding City

Soil No.*	Irrigation type*	Depth / cm	Catalase** / mL·g^{-1}	Invertase** / mg·g^{-1}	Urease / mg·g^{-1}	Phosphatase / mg·g^{-1}	Lead / mg·kg^{-1}	Cadmium / mg·kg^{-1}
1	W	0–20	2.12	1.60	0.24	0.70	34.20	1.05
2	W	20–40	2.30	1.78	0.43	0.43	16.45	1.06
3	F	0–20	2.28	1.66	2.23	0.55	16.40	0.66
4	F	20–40	2.12	0.73	0.82	0.22	12.00	0.86
5	W	0–20	1.16	1.64	0.82	0.76	34.10	1.15
6	W	0–20	2.40	3.04	0.71	0.81	34.30	1.06
7	W	20–40	2.68	0.56	0.23	0.30	16.50	0.86
8	F	0–20	2.70	3.90	1.50	0.90	29.70	1.05
9	F	20–40	3.71	1.74	0.78	0.45	—	0.86
10	W	0–20	2.43	3.06	0.73	0.83	34.00	1.05
11	F	0–20	2.69	3.88	1.45	0.89	31.90	1.05
12	W	0–20	2.29	3.24	1.67	1.01	29.80	0.86
13	F	0–20	2.85	4.53	4.60	1.00	25.20	0.66
14	W	0–20	2.74	6.17	2.30	1.21	29.70	0.86
15	F	0–20	2.83	4.51	4.58	0.98	25.30	0.66
16	W	0–20	2.45	3.63	3.40	0.74	25.40	0.67
Average	W	0–20	2.23	3.20	1.33	0.87	31.64	0.96
	F	0–20	2.67	3.70	2.87	0.86	25.70	0.82
Total average		0–20	2.41	3.41	1.97	0.86	29.17	0.90

*: same as in Table 1

content of metal in the surface layer was higher than in the lower layer, and the contents of Pb and Cd in the soils of wastewater irrigated area exceeded not only the standards of soil background values for some major cities at home and abroad, but also the content range of soil background values for the world's soils. The level of soil Pb and Cd pollution in the wastewater-irrigated upstream area of the Baiyangdian Lake showed a slight pollution. Therefore, integrated control measures such as rational wastewater irrigation and prudent wastewater supply should be taken to prevent further soil pollution.

3.2 Relationship between heavy metal pollution and enzymatic activities of soils in wastewater-irrigated areas

The data in Table 3 show that there existed a very significant negative correlation between soil enzymatic activity and contents of Pb and Cd in wastewater-irrigated areas. It also shows that the soil urease and catalase activities decreased evidently with the increasing contents of Pb and Cd. For the activities of the four soil enzymes, the most remarkable negative correlation exists between the contents of Pb and Cd and the urease and catalase. The regression fitting model equations of the correlation coefficient (r) and the maximum r^2 between Pb and the urease and catalase are

$$y_{\text{urease}} = 7.03xe^{-0.171x}\ (r = -0.8084^{**}),$$
$$y_{\text{catalase}} = 0.393xe^{-0.954x}\ (r = -0.795^{**}),$$

and the equations between Cd and the urease and catalase are

$$y_{\text{urease}} = 225.35xe^{-5.53x}\ (r = -0.899),$$
$$y_{\text{catalase}} = 13.01xe^{-1.757x}\ (r = -0.852^{**}).$$

Their negative correlation also reached a very significant level. Hence, it was feasible to use soil urease and catalase activities as the primary biochemical indexes to evaluate soil heavy metal pollution levels by Pb and Cd in the wastewater-irrigated area.

3.3 The influence of heavy metals on soil enzymatic activities and microbial effect

The data in Table 4 show the obvious influence of heavy metals of Cd and Pb on soil enzymatic activities and microbial effect. The pot experiments with different concentrations of heavy metals indicated that the influence of Cd on soil enzymatic activities was greater than that of Pb. In general, the influence of Cd on the soil alkaline phosphatase, urease and catalase activities was greater than the invertase activity. The activities of phosphatase, urease, and catalase decreased by 74%, 22% and 23%, respectively, with an increasing in the concentration of Cd. However, the invertase activity changed less than other enzymes under the influence of Cd. The effect of Pb on the invertase activity was greater than that of Cd, and mainly showed a promoting effect on other enzymes. Therefore, the influence of each heavy metal on soil enzymatic activities was greatly different.

4 Discussion

The pot experiments, in which the cinnamon soil was mixed with the heavy metals of Cd and Pb to grow continuously triple crops (wheat-cabbage, wheat-paddy), showed an obvious inhibiting effect from Cd and Pb on bacteria. The sensitive effect of Cd and Pb on different microbes was less

Table 3 Correlation between Pb and Cd contents and soil enzymatic activities and their regressive fitting results

Relation	Mod##	Lead and urease				Mod##	Lead and catalase			
		$r^{\#}$	r^2	a	b		$r^{\#}$	r^2	a	b
Regression fitting	*S*	−0.804**	0.654	7.03	−0.17	*S*	−0.795**	0.633	0.093	−0.05
	F	−0.715**	0.511	63.86	−0.13	*D*	−0.645*	0.415	−7.24	0.69
	H	0.673*	0.453	7.56	−0.19	*B*	0.497	0.240	25.99	1.49
Relation	Mod	Lead and invertase				Mod	Lead and alkaline phosphatase			
		r	r^2	a	b		r	r^2	a	b
Regression fitting	*D*	0.495	0.245	−4.62	0.51	*B*	0.705*	0.497	0.30	0.86
	S	−0.481	0.231	0.38	−0.43	*D*	0.637*	0.453	4.45	1.04
	B	0.238	0.057	47.14	1.77	*S*	−0.655*	0.429	0.07	0.03
Relation	Mod	Cadmium and urease				Mod	Cadmium and catalase			
		r	r^2	a	b		r	r^2	a	b
Regression fitting	*S*	−0.899**	0.808	225.35	−5.53	*S*	−0.852**	0.726	13.01	−1.76
	D	−0.892**	0.796	1.19	−1.39	*D*	−0.724*	0.524	−0.28	0.77
	B	−0.889**	0.791	8.32	−7.06	*B*	0.500	0.250	0.95	1.32
Relation	Mod	Cadmium and invertase				Mod	Cadmium and alkaline phosphatase			
		r	r^2	a	b		r	r^2	a	b
Regression fitting	*S*	−0.662*	0.438	19.49	−1.89	*D*	0.762**	0.581	−0.02	1.22
	D	0.617*	0.381	−0.22	0.60	*S*	−0.754**	0.568	2.82	−1.19
	H	−0.332	0.110	5.52	−2.36	*B*	0.716**	0.512	0.11	0.74

#: $P<0.05$, $r=0.567$; $P<0.01$, $r=0.708$; $n=12$; r = coefficient of correlation; r^2 = coefficient of determination
##: Mod = Model of function; x = Content of heavy metal; y = Soil enzymatic activity
$F=y=ae^{bx}$ and $S=y=axe^{bx}$ are of exponential of function; $D=y=x/(a+bx)$ and $B=y=(a+bx)/x$ are of hyperbola; $H=y=a+bx$ is linear equation.

Table 4 Influence of heavy metals on soil enzymatic activities and microbial effect

Heavy metal	The first treating concentration / $mg \cdot kg^{-1}$	Soil enzymatic activities*				Soil microbes/ $CFU \cdot g^{-1}$ $dw \cdot s^{-1}$		
		Urease	Phosphatase	Catalase	Invertase	Bacteria	Actinomycete	Fungus
Cd	0	2.26	1.09	2.97	4.55	6.345×10^{10}	1.904×10^{5}	1.19×10^{4}
	1	2.82	1.04	2.97	4.45	8.985×10^{10}	2.142×10^{5}	2.38×10^{4}
	5	2.04	1.03	3.09	4.75	4.762×10^{10}	2.140×10^{5}	2.97×10^{4}
	10	1.95	0.85	8.10	4.92	3.451×10^{10}	3.570×10^{5}	5.36×10^{4}
	50	1.74	0.60	2.97	4.88	2.737×10^{10}	2.380×10^{5}	3.57×10^{4}
	100	1.57	0.44	2.92	4.20	2.460×10^{10}	3.330×10^{5}	2.38×10^{4}
	200	0.57	0.17	2.44	2.08	1.428×10^{10}	2.570×10^{5}	2.38×10^{4}
Pb	0	0.90	0.66	8.61	4.15	6.347×10^{10}	2.499×10^{5}	8.33×10^{4}
	10	0.98	0.62	8.51	4.48	8.330×10^{10}	3.392×10^{5}	11.90×10^{4}
	50	1.90	0.72	9.59	3.90	6.185×10^{10}	2.620×10^{5}	6.54×10^{4}
	200	2.08	0.78	8.65	4.00	4.757×10^{10}	2.380×10^{5}	6.54×10^{4}
	500	2.19	0.66	8.85	4.08	3.094×10^{10}	2.261×10^{5}	5.95×10^{4}
	1000	1.89	0.69	9.89	4.25	2.495×10^{10}	1.964×10^{5}	1.19×10^{4}
	2000	0.80	0.68	4.02	4.00	1.666×10^{10}	1.488×10^{5}	1.19×10^{4}

*: The unit for soil enzymatic activities: (1) Urease, NH_3-N mg/g · s (37°C · 24h); (2) Alkaline, phosphatase-phenol mg/g · s (37°C · 24h); (3) Catalase, 0.1mol $KMnO_4$ mL/g · s (normal temperature 20 min); (4) Invertase, 0.1mol $Na_2S_2O_3$ mL/g · s (37°C · 24h)

on bacteria than on actinomycete and the greatest on fungus, especially on the influence of Cd and Pb on the amount of bacteria with a correlation of $r = -0.717$. There was an inhibiting effect on the amount of bacteria when the metals had high concentrations and there was an active effect when they were low. The inhibiting effect of Cd on bacteria was greater than that of Pb. The inhibiting concentration of Cd on bacteria started from 5 mg/kg and the inhibiting rate of Cd on bacteria was 25%. When the concentration of Cd was increased to 10, 50, 100 and 200 mg/kg, the inhibiting rate on bacteria was 45%, 57%, 61% and 77%, respectively. There was an inhibiting effect of Pb starting from 50 mg/kg and the rate of Cd inhibiting effect was only 2.55%. When the concentration of Pb increased to 200, 500, 1 000 and 2 000 mg/kg, the inhibiting rate of Pb was 25%, 51%, 61% and 74%, respectively. As for the influence of heavy metals on actinomycete and fungus, the inhibiting effect was low due to a small amount of the metals. However, the effect of Pb on those microbes was greater than Cd. In general, the inhibiting effect of Pb on actinomycete and fungus started from 200 or 500 mg/kg, and the inhibiting rates on those microbes were 4.8% and 21%, respectively. When the concentration of Pb increased to 1 000 and 2 000 mg/kg, the inhibiting rates were 21%–41% and 85%. Thus, it could be seen that the bacterium is an important index to reflect the influence of heavy metals of Cd and Pb in soil on microbial sensitivity.

References

Gao Z M (1986). Studies on Pollution Ecology of Soil-Plant-Systems. Beijing: China Science and Technology Press (in Chinese)

Guan S M (1986). Studies on Soil Enzyme and its Methods. Beijing: China Agriculture Press (in Chinese)

He W X, Zhu M E, Zhang Y P (2000). Recent advance in relationship between soil enzymes and heavy mental. Soil and Environmental Science, 9(2): 139–142 (in Chinese)

Kilaglish (1981). Heavy Metal Pollution in Soil of Japan. Tokyo: Japan Scientific Societies Press, 121, 89–90

Li X K (1984). Analytical Methods of Convention for Soil Agro-Chemistry. Beijing: Beijing Science Press, 67–99 (in Chinese)

Liu S Q (1990). Discuss relationship between soil enzymatic activities and soil pollution. Journal of Environmental Science in Hebei, 1(5): 52–57 (in Chinese)

Liu S Q (1996). Relationship between soil Pb and Cd pollution and enzyme activities in waste water irrigated area of Baoding city. Acta Pedologica Sinica, 33(2): 175–182 (in Chinese)

Liu S Q, Zhang L F (1995). Studies on Dryland Agriculture. China Science and Technology Press, 76–81 (in Chinese)

Liu X, Liu S Q, Tang Z H (2003). Relationship between heavy mental forms and soil environmental activities in alluvial meadow soils and meadow cinnamon soils. Acta Pedologica Sinica, 40(4): 581–587 (in Chinese)

Tyley G (1974). Heavy metal pollution and soil enzymatic activity. Plant and Soil, 41: 303

Xu D M, Liu G S, Wang L M, Liu W P (2004). Studies on the effects and corresponding mechanism of Hg^{2+} on the activity of soil acid phosphatase. Acta Scientiae Circumstiae, 24(5): 865–870 (in Chinese)

Zhang X G, Liu S Q, Dou T L, Ji Y Z, Xue B M (2006). Strategies for controlling water environmental pollution in the area of Baiyangdian Lake. Chinese Journal of Eco-Agriculture, 14(2): 27–31 (in Chinese)

Zhao L B, Jang H (1986). Analytical methods of soil phosphatase. Journal of Soil Science, 17(3): 137–141 (in Chinese)

Zhou L K (1980). Analytical methods of Soil enzyme. Journal of Soil Science, 11(5): 37–38 (in Chinese)

Zhou L K (1985). Heavy metal pollution of soil and soil enzymatic activity. Journal of Environmental Science, 5(2): 176–184 (in Chinese)