Journal of Environmental Sciences Vol.16, No.2, pp.177—180, 2004

ISSN 1001—0742
CN11—2629/X

Article ID: 1001-0742(2004)02-0177-04 **CLC number**: X131.3; X53 **Document code**: A

Effect of organic/inorganic compounds on the enzymes in soil under acid rain stress

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Abstract: The main effects of pollutions including acid rain, Cu^{2+}, atrazine and their combined products on the activities of urease, invertin, acid phosphatase and catalase were studied by means of orthogonal test. The results showed that H^+ and Cu^{2+} had significant influence on the activities of four enzymes and the ability of their inhibiting followed the order: $H^+ > Cu^{2+}$. Al^{3+} and atrazine only had litter effects on the activity of urease and phosphatase, respectively. Furthermore, interaction analysis revealed that Cu^{2+}-H^+ affected on the activity of acid phosphatase significantly and antagonism on invertin and urease, Cu^{2+}-atrazine only exhibited the synergism on the activity of acid phosphatase. But atrazine-H^+ had non-interaction within the investigated concentration range. Among four enzymes, acid phosphatase was the most sensitive one to the contaminations.

Keywords: combined pollution; orthogonal test; hydrolase; activity

Introduction

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. When the environment is polluted, enzyme activities change significantly. The use of enzyme activities as bioindicators to evaluate the degree of soil contamination by heavy metals have been proposed(Dick, 1992; Nannipieri, 1995). Zhou L-K (Zhou, 1985) has succeeded in using urease, invertin and acid phosphatase as indexes of soil contamination. Studies concerning the influences of pesticides on soil enzyme activity have been reviewed frequently(Sannino, 2001). The factor of pollution studied was simple, while with the rapid development of industry and agriculture in world many contaminations co-exist in soil and environmental problems can not be explained by simple contamination. So combined pollution comes to arouse attention and turn to one of the important research directions in environmental science(Zheng, 2001). Wu Y-Y (Wu, 1997) have made a systematic study on ecological effect of heavy metal combined pollution, which have been applied to the study of soil enzyme by He W-X(He, 2000). While the main effects of combined pollutions, for example acid rain, heavy metal and atrazine, on the activities of soil enzymes have not been reported. In order to provide scientific basis for environmental monitor the object of our work were to investigate the influences of the interaction of acid rain, Cu^{2+} and atrazine on the activities of soil enzymes, using urease, invertin, acid phosphatase and catalase as probes by means of orthogonal test.

1 Materials and methods

1.1 Reagent

Atrazine(99%) was purchased from Chemical Service Company of US. All the other reagents were A.R.

1.2 Soil

Soil tested was sampled from the farm of Zhejiang University Huajiachi Campus. Some physical and chemical properties of the soil are listed in Table 1.

Table 1 Physical and chemical properties of the soil tested

pH	Organic matter, g/kg	Total N, g/kg	Total P, g/kg	CEC, cmol/kg	Cu, mg/kg	Sand, %	Silt, %	Clay, %
6.5	25.0	16.2	10.9	14.7	15.3	31.3	46.3	22.4

1.3 Experimental design

Activities of urease, invertin, acid phosphatase and catalase were selected as indexes and acid rain, Cu^{2+}, atrazine and incubation time as factors. The factor of acid rain was decomposed as H^+ and Al^{3+} according to the theory of soil acidity in modern time. The factors and levels are shown in Table 2. Atrazine is a typical contamination and H^+, Cu^{2+} influence the activity of hydrolase significantly. After the analysis of each factor they were listed again for an orthogonal table in order to study the interactions of acid rain, heavy metal and pesticide.

The air-dry soil were sieved(1 mm) and mixed with all contaminations shown in Table 2 in flasks with enough shaking. The H^+ was mixed by sulfuric acid and nitric acid (5:1) and Al^{3+}, Cu^{2+} were added as aluminum sulfate and cupric sulfate. Water was added till 80% of the maximum water capacity of soil. The reaction mixture was incubated at 28℃ in dark, constant temperature and moisture place. Three duplicate samples were utilized contrasting with control tests.

Foundation item: The National Natural Science Foundation of China(No. 49973031); * Corresponding author

Table 2 Orthogonal experimental design

	Non-interactions					Interactions			
	[H^+], mmol/kg	[Al^{3+}], mg/kg	[Cu^{2+}], mg/kg	Time, d	Atrazine, mg/kg		[H^+], mmol/kg	[Cu^{2+}], mg/kg	Atrazine, mg/kg
Level 1	10	100	200	50	0	Level 1	44	80	0.5
Level 2	25	200	300	3	5				
Level 3	50	400	0	7	10				
Level 4	100	0	50	14	25	Level 2	110	240	1
Level 5	0	50	100	25	50				

1.4 Measurement method

Activities of urease and acid phophatase were measured by colorimetric method and interatase and catalase, titrimetric method(Institute of Soil Science, 1985; Guan, 1986).

2 Results

2.1 Activities of hydrolase in the presence of organic/inorganic compounds

Trend analyses of activities of enzymes are listed in Table 3 and 4. The trend analysis showed that H^+ had a slight stimulative effect on catalase and the activities of urease, invertin and acid phosphatase decreased significantly with increasing concentration of H^+ or Cu^{2+}; the maximal inhibition ratio of H^+ to urease, invertin, acid phosphatase and catalase was 43.1%, 61.4%, 73.1%, and 60.7%, respectively; the enzyme activities were inhibited to a maximum of 38.2%, 18.3%, 31.1%, and 21.4% by Cu^{2+}, respectively; the activities of four enzymes indicated a process of stimulation-inhibition with the increase of atrazine and incubation time; by contrast, Al^{3+} only had a slight effect on urease and its maximal inhibition ratio was 19.9%; and the maximal inhibition ratio of atrazine to four enzymes was 8.4%, 1.6%, 14.3% and 5.2%, respectively.

Table 3 Activity of hydrolase in the presence of non-interactions

No.	H^+	Al^{3+}	Cu^{2+}	Time	Atrazine
1	1	1	1	1	1
2	1	2	2	2	2
3	1	3	3	3	3
4	1	4	4	4	4
5	1	5	5	5	5
6	2	1	2	3	4
7	2	2	3	4	5
8	2	3	4	5	1
9	2	4	5	1	2
10	2	5	1	2	3
11	3	1	3	5	2
12	3	2	4	1	3
13	3	3	5	2	4
14	3	4	1	3	5
15	3	5	2	4	1
16	4	1	4	2	5
17	4	2	5	3	1
18	4	3	1	4	2
19	4	4	2	5	3
20	4	5	3	1	4
21	5	1	5	4	3
22	5	2	1	5	4
23	5	3	2	1	5
24	5	4	3	2	1
25	5	5	4	3	2
Trend analysis	55.2 8.3 222.4 64.6	45.0 6.7 177.6 52.5	55.5 7.1 206.5 60.6	40.2 6.2 148.6 56.1	45.3 6.4 163.9 53.5
	49.1 7.6 218.1 68.2	39.5 6.3 160.8 52.3	47.4 7.0 181.8 53.4	46.6 6.3 172.9 56.9	41.5 6.4 170.7 50.7
	45.3 7.3 203.9 59.2	39.8 6.4 171.0 55.0	42.1 6.0 160.3 55.8	46.2 7.1 191.3 53.5	46.6 6.3 186.0 55.9
	36.5 5.5 142.9 48.0	44.0 6.3 171.3 53.5	38.2 5.8 141.8 49.5	45.9 6.9 178.7 54.9	41.9 6.3 159.3 52.9
	31.4 3.2 59.8 26.8	49.3 6.1 166.0 53.6	34.3 6.0 156.6 47.6	40.9 5.4 155.5 45.5	42.2 6.4 167.1 53.4

Notes: Unit of enzyme activity: Urease: NH_3-N, mg/(100 g·8 h); invertin: $Na_2S_2O_3$, ml/(g·24 h); acid phosphatase, mg/(100 g·8 h); catalase: $KMnO_4$, ml/(g·8 h); the reported results of enzyme activities are averages of three duplicate samples

Table 4 Activity of hydrolase in the presence of interactions

No.	Atrazine	H^+	Atrazine-H^+	Cu^{2+}	Atrazine-Cu^{2+}	H^+-Cu^{2+}
1	1	1	1	1	1	1
2	1	1	1	2	2	2
3	1	2	2	1	1	2
4	1	2	2	2	2	1
5	2	1	2	1	2	1
6	2	1	2	2	1	2
7	2	2	1	1	2	2
8	2	2	1	2	1	1
Trend	30.2 4.5 84.2 50.4	33.4 4.8 90.6 53.5	31.6 4.3 87.2 49.2	32.7 4.9 86.7 52.8	30.2 4.6 90.1 48.8	30.7 4.7 91.1 48.1
analysis	31.4 4.3 87.5 47.5	28.1 4.0 81.2 44.3	30.0 4.6 84.5 48.7	28.8 3.9 85.0 45.0	31.3 4.3 81.6 49.1	30.8 4.2 80.6 49.8

The trend analysis of interaction test demonstrated that the interactions of atrazine and Cu^{2+}, H^+ and Cu^{2+} had significant inhibitory effects on the activity of acid phosphatase and the inhibition ratios were 9.4%, and 11.5%, respectively. Atrazine-H^+ had an inhibitory effect and atrazine-Cu^{2+} had a slight stimulative effect on the activity of urease while H^+-Cu^{2+} had no influence on it. The results could be explained by the antagonism of acid rain and Cu^{2+}. Atrazine-H^+ could slightly stimulate the activity of invertin, conversely atrazine-Cu^{2+} and H^+-Cu^{2+} inhibited its activity. But none of the interactions influenced the activity of catalase.

2.2 Activities of hydrolase in the presence of acid rain, Cu^{2+} and atrazine, respectively

The variance analyses of orthogonal test are listed in Table 5. With the confidence interval of 95%, the F value of the effect of H^+, Cu^{2+} and incubation time on the activities of four enzymes were all bigger than the critical value($F_{0.05}(4,4)$). The results indicated that H^+, Cu^{2+} and incubation time affected the enzyme activities significantly. Moreover the H^+ affected much more than the other two. The effect of H^+ on the enzyme activities had an order: acid phosphatase > invertin > catalase > urease; and Cu^{2+}: urease > acid phophstase > invertin > catalase; the effect of incubation time on them decreased in turns of urease, invertin, acid phosphatase and catalase. The results of variance analysis suggested that Al^{3+} had certain influence only on urease; atrazine had a slight effect on the activity of acid phosphatase. While no obvious effects on the activities of urease, invertin and catalase were observed.

Table 5 Analysis of variance of orthogonal test

Source of variation	Degree of freedom	Sum of squares Q_i				Mean square Q_i/f_i				F value				Critical value
		A	B	C	D	A	B	C	D	A	B	C	D	
H^+	4	74.1	3.49	3823.8	221	18.5	0.87	956.0	55.2	22.6**	104.8**	116.6**	25.6**	$F_{0.05}(4,4) = 6.39$
Al^{3+}	4	13.2	0.04	32	3.0	3.3				4.0△				$F_{0.10}(4,4) = 4.11$
Cu^{2+}	4	54.8	0.33	507.7	23.4	13.7	0.08	126.9	5.85	16.7**	9.6**	15.5**	2.71△	$F_{0.20}(4,4) = 2.50$
Time	4	44.2	0.42	240.8	19.0	11.1	0.10	60.2	4.75	13.5**	12.0**	7.3**	2.20	$F_{0.25}(4,4) = 2.06$
Atrazine	4	4.14	0.01	83.0	4.2			20.7				2.5△		
Error	4	2.41	0.05	33.3	18.7	0.82	0.008	8.2	2.16					
Total count	$f_T = 24$	163.2	4.33	4720.7	289.3									

Notes: A, B, C, D indicates urease, invertin, acid phophatase and catalase, respectively; **: factor which influenced in particular significantly; *: factor which influenced significantly; △: factor which had certain influence; Null: factor which had no influence

Relationships between enzyme activities and factors of combined pollution were established by calculating regression equations. All of tie extrinsic factors had inhibitory effects on the enzyme activities except that Al^{3+} could stimulate the activities of enzymes.

Urease:

$$Y = 12.02 - 0.0454(H^+) + 0.00339(Al^{3+}) - 0.0133(\text{time}) - 0.0107(Cu^{2+}) - 0.0124(\text{Atrazine}).$$

Invertin:

$$Y = 1.92 - 0.0102(H^+) - 0.000346(Al^{3+}) - 0.000950(\text{time}) - 0.00420(Cu^{2+}) - 0.000801(\text{Atrazine}).$$

Acid phosphatase:

$$Y = 55.225 - 0.341(H^+) - 0.00384(Al^{3+}) - 0.0350(\text{time}) - 0.0536(Cu^{2+}) - 0.0349(\text{Atrazine}).$$

Catalase:

$$Y = 15.72 - 0.0808(H^+) - 0.000579(Al^{3+}) - 0.00904(\text{time}) - 0.0383(Cu^{2+}) - 0.00518(\text{Atrazine}).$$

2.3 Activities of hydrolase in the presence of the interactions of acid rain, Cu^{2+} and atrazine

Analysis of variance of interactive orthogonal test of H^+, Cu^{2+} and atrazine indicated that no obvious effects of the interaction of H^+ and atrazine on the enzyme activities were observed; the synergism of atrazine and Cu^{2+} chiefly exhibited the influence on the activity of acid phosphatase; the interaction of Cu^{2+} and H^+ influenced the activity of acid phosphatase significantly and exhibited antagonism on invertin and urease (Table 6). The phenomenon that H^+ and Cu^{2+} had significant inhibitory effect on the activity of catalase but their interaction had no influence on it showed that H^+-Cu^{2+} had radical antagonism on catalase.

Table 6 Analysis of variance of interactive orthogonal test

Source of variation	Degree of freedom	Sum of squares Q_i				Mean square Q_i/f_i				F value				Critical value
		A	B	C	D	A	B	C	D	A	B	C	D	
Atrazine	1	0.2	0.02	1.4	1.01									$F_{0.05}(1,4) = 21.20$
H^+	1	3.5	0.31	15.3	10.63	3.5	0.31	15.3	10.63	3.07^{Δ}	10.3^*	8.5^*	8.86^*	$F_{0.10}(1,4) = 7.71$
Cu^{2+}	1	3.0	0.48	0.4	7.53	3.0	0.48		7.53	2.63^{Δ}	16.0^*		6.28^{Δ}	$F_{0.20}(1,4) = 4.54$
Atrazine-H^+	1	0.1	0.04	0.9	0.04									$F_{0.25}(1,4) = 1.81$
Atrazine-Cu^{2+}	1	1.6	0.03	13.1	0.02			13.1				7.3^*		
H^+-Cu^{2+}	1	1.8	0.12	13.8	0.35		0.12	13.8			4.0^{Δ}	7.7^*		
Error	1	2.0	0.03	4.7	4.59	1.14	0.03	1.8						
Total count	7	12.2	1.03	49.6	24.17									

3 Discussion

Without considering the interactions the effects of three contaminations on the hydrolases of soil had an order: $H^+ > Cu^{2+} >$ atrazine. The results of trend analyses which were consistent with that of variance analysis showed that the maximal inhibition ratio of H^+ to urease, interase, acid phosphatase and catalase was 43.1%, 61.4%, 73.1%, and 60.7%, respectively. As it is claimed that H^+ can influence the ionization radical of the enzyme and lead to one-way inactivation. Moreover H^+ can change the adsorption between enzymes and humics or clays thus influence the activities of enzymes(Srivastava, 1991). It have been proved that the optimal pH of catalytic reaction of free enzymes in soil solution and enzymes adsorbed are different(McLaren, 1957). The conclusion that Al^{3+} has a certain stimulative effect on urease was in consistent with the studies of Rao (Rao, 2000). The effects of Cu^{2+} on the activities of four enzymes are significant. The studies performed by Huang (Huang, 2000) indicated that the inhibition by Cu of enzymes immobilized on soil components are influenced by the properties of the adsorbent and the form of Cu, as well as pH. The conclusion proves the interaction of H^+ and Cu^{2+}.

The effect of the interaction of Cu^{2+} and acid rain on the activities of acid phosphatase was significant; the interaction of them exhibited antagonism on invertin; the activities of urase and catalase were hardly changed because of the antagonism of Cu^{2+} and acid rain. The synergism of Cu^{2+} and atrazine chiefly exhibited the influence on the activity of phosphatase. Within the range of concentration investigated, the effects of the interaction of atrazine and H^+ on enzymes were not been observed.

Among four enzymes, acid phophatase was most sensitive to the contaminations.

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(Received for review January 13, 2003. Accepted June 2, 2003)