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Effects of Copper Contamination on Enzyme Activities in Paddy Soils

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ABSTRACT

Soil contamination by heavy metals has rapidly increased in Southern China in the last decade due to rapid development of industries. One of the concerns regarding heavy metal pollution is the impact on soil functions. Enzyme activity, one of the important biochemical properties that is related to soil functions, has been reported to quickly respond to external heavy metal input. Laboratory and incubation studies were conducted to investigate the effects of anthropogenic loading of copper (Cu) on the activities of catalase, urease, invertase, and acid phosphatase in three paddy soils. The results indicate that external Cu^{2+} loading caused a significant reduction in the activities of all the four enzymes and the reduction (18-87%) was positively related to the increase in Cu^{2+} input from 0 to 1600 mg kg^{-1} soil. However, the pattern of response varied between soil types and among the different Cu^{2+} loading rates. The activities of invertase in purplish clayey soil (PCS) and catalase in PCS and yellowish red soil (YRS) were stimulated at low Cu^{2+} loadings (<200 mg.kg^{-1}) although external Cu^{2+} loading generally resulted in inhibition of activities for all the four enzymes, especially at higher loading rates (>200 mg kg^{-1}). Urease was the most sensitive whereas catalase was the most tolerant to Cu^{2+} contamination. The mean ED_{50} (ecological dose) of Cu^{2+} across the three soils was 652 mg.kg^{-1} for phophatase, 431 mg.kg^{-1} for invertase, and 269 mg.kg^{-1} for urease. These critical values varied with soil type, likely due to differences in soil properties and the nature of the enzyme. Based on the ED_{50} values of Cu for urease, the upper limits of Cu^{2+} contamination were 316 mg.kg^{-1}, 312 mg.kg^{-1}, and 180 mg.kg^{-1}, respectively, for the PCS, YRS, and silt loam soil (SLS).

Keywords: bioindicator, copper, ecological dose, enzyme activity, paddy soils
Abbreviations: CEC, cation exchange capacity; **DTPA**, diethylenetriaminepentaacetate; **ED**, ecological dose; **PCS**, purplish clayey soil; **SLS**, silt loam soil; **YRS**, yellowish red soil

INTRODUCTION

The preservation of soil quality is essential for sustainable land management. Increased attention has been directed to diagnosis and remediation of soils contaminated with heavy metals. According to Stratton *et al*. (1995), metals contained in sewage sludge and municipal solid-waste compost potentially contribute to the contamination of food chains. Soil pollution by heavy metals constitutes a persistent environmental problem. The input of heavy metals through application of municipal and industrial wastes or depositions may influence soil biochemical processes by inhibiting both microbial proliferation and enzyme activity (Abramyan 1993). Heavy metals can inhibit enzyme activity through the masking of catalytically active groups, denaturing effects on protein conformation, or competition with metal ions involved in the formation of enzyme-substrate complexes. In recent years, soil biochemical parameters have been used as indicators of soil stress and early changes of soil quality (Saviozzi *et al.* 2002). Enzyme activity has been suggested as a useful soil "fertility index" due to their functions in nutrient cycling (Stratton *et al.* 1995; Giller 1998; Roy *et al.* 2004). Soil enzyme activity may also serve as a measure of relative pollution of metals and other contaminants in soil. Of special interest is the potential role of soil enzymes in decontamination (Liliana and Bollag 1996). This goal may be fulfilled by a good understanding of soil enzyme behavior. Enzyme activities were found to be negatively correlated with the concentrations of metals. Eivazi and Tabatabai (1990) assumed that metal ions may inhibit enzyme reactions by: (1) complexing with the substrate, (2) binding with the protein-active group of the enzymes, and (3) reacting with the enzyme-substrate complex. Therefore, it is imperative to analyze soil properties along with the metals in order to examine the influence of heavy metals on soil enzyme activities.

Two groups of enzymes predominate in most soils: hydrolases and oxidoreductases. Therefore, three hydrolases (namely invertase, urease, and acid phosphatase) and one oxidoreductase (catalase) were selected in this study as a soil health indicator. Three paddy soils were sampled from the delta area of the Yangtze River, China, where rapid industrialization has been taking place in the last two decades. Among the enzymes measured in this study, phosphatase activity is related to organic P mineralization potential, as organic P compounds, the major pool of soil P, must first be hydrolyzed by phosphatase enzymes to inorganic forms before it is available to the plant (Dick *et al*. 1983). Hydrogen peroxide, a potential toxic intermediate product of respiration, is destroyed by catalase, which produces molecular oxygen and water. The decomposition of hydrogen peroxide makes catalase an important enzyme of soil biochemical activity (Perez *et al*. 1988). Hydrolysis of saccharose by invertase is a significant reaction of carbohydrate metabolism that occurs in soils. Urease, which catalyzes the hydrolysis of urea into ammonia and carbon dioxide, is one of the most studied enzymatic activities in soil. The origin, nature, properties, and inhibition of this enzyme have drawn the attention of numerous researchers (Abramyan 1993; Gianfreda and Bollag 1996; Geiger *et al*. 1998a, 1998b; Roy *et al*. 2004).

In the past, most studies on enzyme activity were conducted in upland soils. Minimal information is available regarding heavy metal effect on enzyme activity in paddy

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Table 1 The physical-chemical properties of the soils.

Soil code	Soil type	Sand	Silt	Clay	pH (H_2O)	Organic C	CEC	Total Cu	Available Cu
		g kg^{-1}				g kg^{-1}	cmol$_c$ kg^{-1}	mg kg^{-1}	
1	PCS	100.0 ± 5.6	556.0 ± 21.4	344.0 ± 11.3	5.09 ± 0.02	20.72 ± 0.32	13.45 ± 1.24	41.94 ± 1.52	5.83 ± 0.43
2	YRS	225.0 ± 18.6	570.0 ± 24.1	205.0 ± 12.7	6.36 ± 0.05	26.08 ± 1.36	12.47 ± 0.71	39.93 ± 0.78	0.98 ± 0.12
3	SLS	650.0 ± 33.6	288.0 ± 15.8	62.0 ± 3.2	7.80 ± 0.02	4.52 ± 0.22	8.42 ± 0.69	18.25 ± 1.11	1.20 ± 0.26

Data expressed as mean ± standard error.

soils. The objectives of the present study were to evaluate the response of enzyme activity to external Cu loading in paddy soils and to examine the possibility of using enzyme activity as a bioindicator for diagnosing and restoring Cu contaminated paddy soils.

MATERIALS AND METHODS

Three paddy soils were collected from Jiaxin County, Deqing County, and Xiasha District of Hangzhou City, Zhejiang Province, and locally referred to as a yellowish red soil (YRS), purplish clayey soil (PCS), and silty loam soil (SLS). They are comparable to fluvents under the US Soil Taxonomy. The physico-chemical properties of the soils are presented in **Table 1**. Soil pH was determined using a pH meter at 1:2.5 soil: water ratio, whereas soil particle distribution was measures by the pipette method (Miller and Miller 1987). Organic carbon, total and DTPA-extractable Cu and other soil properties were measured according to Page *et al*. (1982). For the incubation study, portions of moist soil (1 kg oven-dry basis) were each placed in a glass vessel and treated with a $CuNO_3 \cdot 3H_2O$ aqueous solution to reach the following treatment loadings: 0, 50, 100, 200, 400, 800, 1200, and 1600 mg Cu^{2+} kg^{-1} soil. After soil moisture was adjusted to 70% of water holding capacity, the treated soils were incubated at 25˚C in the dark. Each treatment was replicated three times. At the end of 4- and 8-week's incubation, subsamples of the soil were taken respectively from the vessels for analyses of enzyme activities in response to Cu treatments. Urease and acid phosphatase activities of the soils were estimated by the methods of Alef and Nannipieri (1995), and catalase and invertase activities by the methods of Perez *et al.* (1988) and Ross (1975a, 1975b).

Statistical analyses were performed on the regression between enzyme activity and external Cu loading in different soils using the SAS statistical package and Excel 2000 program.

RESULTS

Physico-chemical properties of the soils

The SLS contained a much higher percentage of sand, but a smaller percentage of silt and clay, than the YRS and PCS. The texture of three paddy soils varied from sandy clay loam to clay (**Table 1**). The pH of YRS was near neutral, PCS was acidic, but SLS was slightly alkaline. Organic carbon varied significantly among the soils, with approximately 6 times higher in the YRS than in the SLS. The YRS had higher CEC and total Cu than the SLS, whereas the PCS contained more DTPA-extractable Cu than the other two soils.

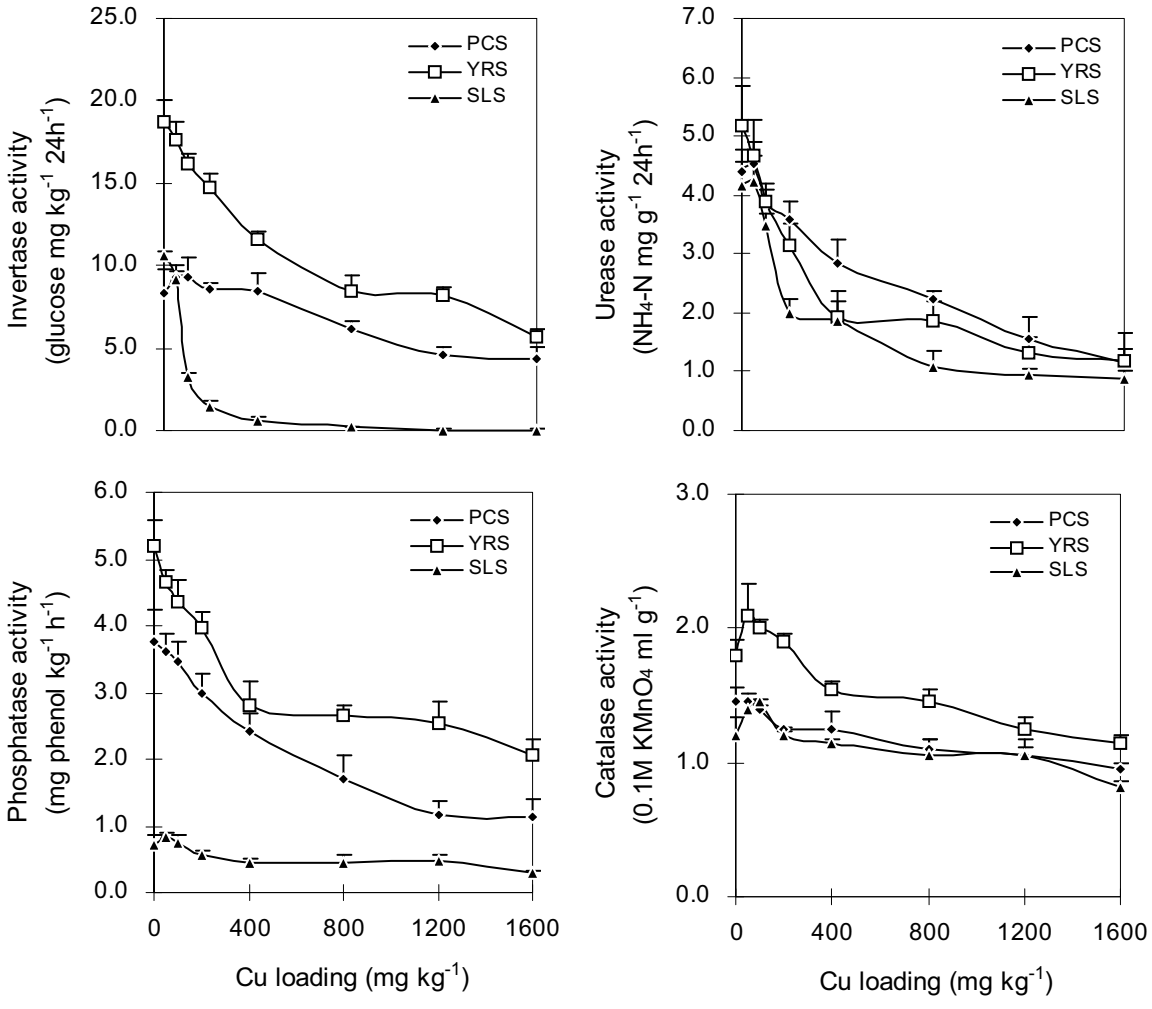

Fig. 1 Effects of Cu loading on the activities of urease, phosphatase, invertase and catalase in three paddy soils sampled at the 4th week of incubation. Error bar indicates standard error.

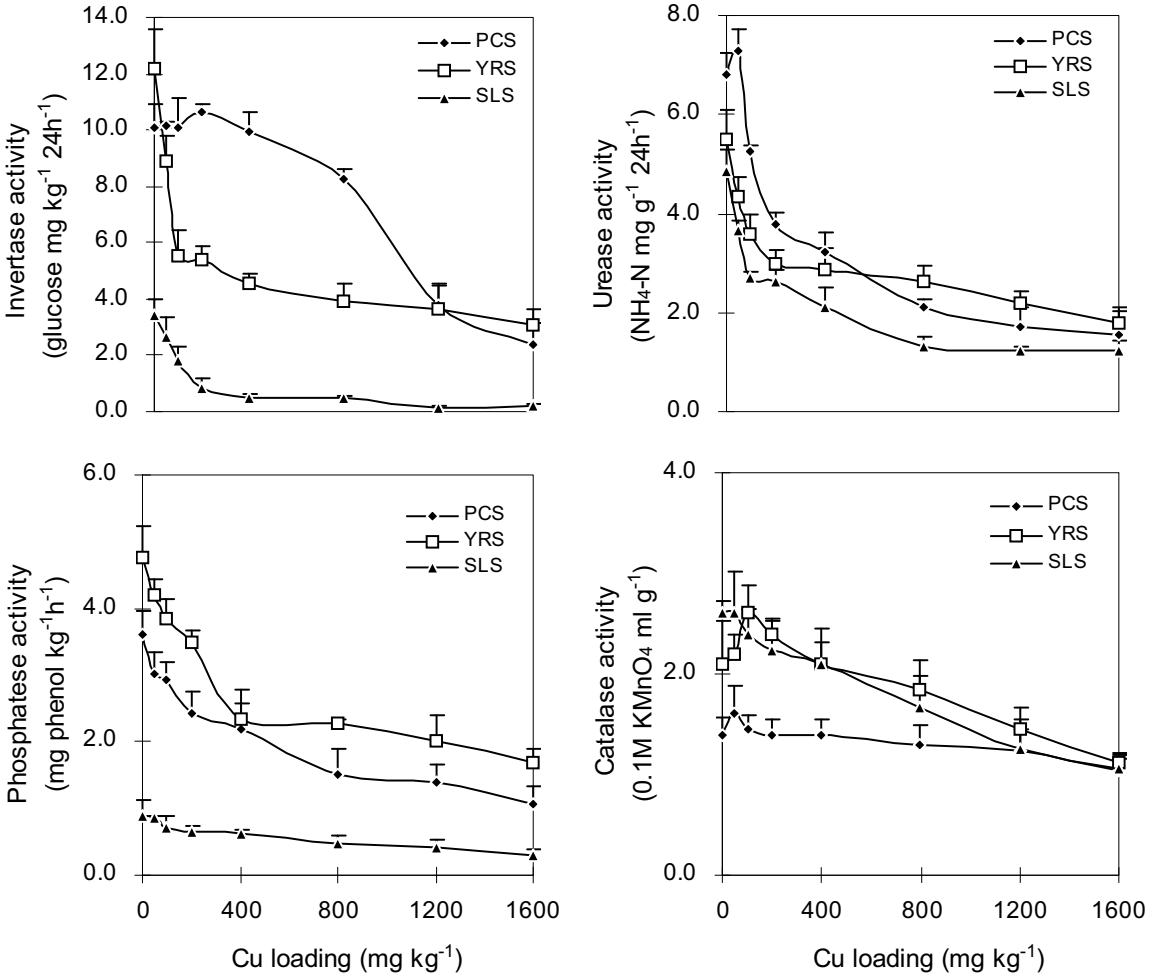

Fig. 2 Effects of Cu loading on the activities of urease, phosphatase, invertase and catalase in three paddy soils sampled at the 8th week of incubation. Error bar indicates standard error.

Enzyme activities

The measured activities of four enzymes were inhibited by external loading of Cu^{2+} in the tested soils, but the nature of effect varied in soils, individual enzymes, and loading rates of Cu^{2+} (**Figs. 1, 2**). Enhanced activities were observed at low Cu^{2+} loading rates (<100mg kg^{-1}) for catalase in all the paddy soils, invertase in PCS, and phosphatase and urease in SLS (**Fig. 1**). Their activities were raised by approximately 12%. Similar results were obtained from the samples analyzed at the end of 8th week's incubation. The differential response of enzymes to small Cu input could be related to soil properties and enzyme characteristics (**Table 1**). In most cases, the activities of enzymes were inhibited by external Cu^{2+} loading, especially at higher rates (>100 mg kg^{-1}; **Figs. 1, 2**). At the highest loading rate (1600 mg Cu^{2+} kg^{-1}), the activities of urease, phosphatase, invertase, and catalase in PCS were reduced by 74%, 70%, 47%, 28%, respectively, as compared to the control (without receiving Cu; **Fig. 1**). The corresponding values were 77%, 60%, 70%, and 36% for the YRS and 79%, 59%, 96%, and 21% for the SLS. The decreases in soil enzyme activities by Cu loading were similar in the samples analyzed by the end of the 8th week incubation (**Fig. 2**). These results indicate that soil contamination by Cu can significantly reduce enzyme activities, and urease is the most sensitive while catalase is relatively tolerant to external Cu loading. Urease can potentially serve as an indicator of Cu contamination to paddy soils.

Ecological dose

The concentration of a chemical that reduces the biological activity (or biomass) of a given organism to 50% of its initial value is defined as the 50% effective ecological dose, called ED_{50} (Doelman and Haanstra 1989). The ED_{50} value has been widely used to assess soil contamination including heavy metals. In this study, we found that the decreases in the activities of enzymes (urease, invertase, catalase, and phosphatase) in the paddy soils were closely related to external Cu^{2+} loading (**Table 2**), and this relationship can be described by a logistic equation [$Y = a/(1+EXP^{(b+cX)})$ where Y is enzyme activity, X is external Cu loading, and a, b, c are constants related to a specific enzyme], from which the ED_{50} can be obtained. The values of ED_{50} generally varied more among the different enzymes than between the soils (**Table 2**). The mean value of ED_{50} can be used as a threshold of Cu contamination for all the enzymes except for catalase, i.e., 652 mg kg^{-1} for phophatase, 431 mg kg^{-1} for invertase, and 269 mg kg^{-1} for urease. For catalase, its mean ED_{50} exceeded the highest level of Cu^{2+} loading and therefore, catalase activity may not be an adequate indicator of Cu^{2+} pollution. The variation in ED_{50} of a specific enzyme among different soils may be attributed to the differences in biogeochemical properties. SLS had the lowest ED_{50} likely because of its low organic C content and light texture. Urease appears to be the most sensitive bioindicator as it was significantly inhabited by increased Cu loading in all three paddy soils. Based on the ED_{50} of urease, the upper limits of Cu contamination were 316, 312, and 180 mg kg^{-1}, respectively for PCS, YRS, and SLS.

DISCUSSION

The response of enzyme activity to heavy metal contamination in upland soil has been extensively studied. A decrease of 70% in the activities of invertase and phosphatase in or-

Table 2 Relationships between enzyme activity and Cu loading rate at the 8th week of incubation and the ED_{50} values obtained from the regression equation for the three paddy soils.

Enzyme	Soil	Regression equation	R^2	ED_{50} (mg kg^{-1})	Mean ED_{10}
Phosphatase	PCS	$Y=3.68/(1+EXP^{(-4.76+1.74X)})$	0.9902	544.04	652.51
	YRS	$Y=4.88/(1+EXP^{(-4.39+1.58X)})$	0.9702	600.46	
	SLS	$Y=0.91/(1+EXP^{(-5.18+1.78X)})$	0.9793	813.04	
Catalase	PCS	$Y=1.45/(1+EXP^{(-23.53+6.97X)})$	0.7894	2376.28	1738.86
	YRS	$Y=2.32/(1+EXP^{(-14.91+4.67X)})$	0.8978	1558.55	
	SLS	$Y=2.59/(1+EXP^{(-8.36+2.69X)})$	0.9921	1281.76	
Invertase	PCS	$Y=10.24/(1+EXP^{(-27.54+9.04X)})$	0.9885	1112.91	431.01
	YRS	$Y=14.35/(1+EXP^{(-1.80+0.97X)})$	0.9518	71.72	
	SLS	$Y=3.47/(1+EXP^{(-6.39+3.14X)})$	0.9871	108.40	
Urease	PCS	$Y=7.40/(1+EXP^{(-5.50+2.20X)})$	0.9465	316.23	269.65
	YRS	$Y=5.98/(1+EXP^{(-2.52+1.01X)})$	0.9748	312.64	
	SLS	$Y=5.12/(1+EXP^{(-3.09=+1.37X)})$	0.9817	180.08	

ganic soils treated with Cu^{2+} was reported by Mathur *et al*. (1980). A strong inhibition of enzymes was also observed in Cu polluted soils (Doelman and Haanstra 1986, 1989; Fu and Tabatabai 1989; Deng and Tabatabai 1995). The results obtained from this study generally agree with the previous reports. Enzyme activities were significantly inhibited by external Cu loading, especially at high rates (>200 mg Cu kg^{-1} soil). However, we also found that the activity of some enzymes such as catalase was enhanced by external Cu loading at low rates (<100 mg Cu kg^{-1} soil; **Figs. 1, 2**), and this enhancement varied among the tested enzymes and between the soil types. In addition, in this study urease was found to be most sensitive to Cu contamination, and its activity drastically decreased with increasing Cu loading in all three paddy soils, whereas catalase was most tolerant to Cu input among the four tested enzymes. The response of invertase and phosphatase to Cu contamination was intermediate between urease and catalase. This finding is in agreement with the results reported by Krasnova (1983), who suggested that urease was most sensitive to heavy metal pollution and may be useful for diagnosing soil contamination by heavy metals. The enhancing effect of low Cu input on enzyme activity may be related to the differences in the intrinsic Cu requirement and tolerance of individual enzymes. For instance, catalase was most tolerant to Cu input, and the stimulating effect on catalase occurred in all the soils, whereas urease was most sensitive to Cu contamination and as a result, the stimulating effect of low Cu input on urease was minimal (**Figs. 1, 2**).

Developing a threshold for soil Cu^{2+} contamination was one of the research goals. By using ED_{50}, Doelman and Haanstra (1989) studied the short-and long-term effects of Cu^{2+} on phosphatase and urease activity of five soil types, and the results were presented graphically as logistic dose response curves. From this study we found that the relationships between Cu loading and enzyme activities agreed well with a logistic model. The ED_{50} values obtained from the regression equations (**Table 2**) generally varied more among the different enzymes than between the tested soils, indicating that these values are affected by both the nature of enzyme and the properties of the soils. However, the mean ED_{50} value across the tested soils is characteristic of each individual enzyme (**Table 2**), and decreased in the order of catalse > phosphatase > invertase > urease. Since the mean ED_{50} exceeded the highest level of Cu^{2+} addition in this experiment, the catalase activity may not be adequate for diagnosing Cu^{2+} pollution of the paddy soils. The mean values of ED_{50} of the rest three enzymes were 652 mg kg^{-1} for phophatase, 431 mg kg^{-1} for invertase, and 269 mg kg^{-1} for ureas. Obviously the urease is the most sensitive enzyme to Cu input followed by invertase and phosphatase. These mean ED_{50} values have the potential to be used as rough thresholds of Cu for the paddy soils. However, different type of paddy soils may have different thresholds of enzyme indices due to their differences in biogeochemical properties. For instance, the ED_{50} values of urease that is most sensitive to Cu contamination also varied among the three paddy soils: 316mg kg^{-1} for PCS, 312mg kg^{-1} for YRS, and 180 mg kg^{-1} for SLS. Therefore, the upper limits for Cu^{2+} contamination should also vary in different type of soils.

In conclusion, the responses of enzyme activity to Cu^{2+} contamination vary in enzyme, soil type, and external Cu loading rate. The activity of enzyme is generally inhibited by external Cu loading, especially at high rates (>200 mg kg^{-1} soil). Among the tested enzymes, urease is the most sensitive and catalase is the most tolerant to external Cu input. The ED_{50} of urease can be potentially applied for diagnosing soil Cu contamination, whereas catalase seems not adequate for this purpose.

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