

# Cadmium Phytoavailability and Enzyme Activity under Humic Acid Treatment in Fluvo-aquic Soil

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**Abstract.** A pot experiment was conducted to investigate the cadmium (Cd) availability to pakchois (*Brassica chinensis L.*) as well as the enzyme activities in fluvo-aquic soil under humic acid treatment. The results showed that the phytoavailability of Cd in soil decreased gradually as humic acid concentration rose (0 to 12 g·kg<sup>-1</sup>), while the activities of urease (UE), alkaline phosphatase (ALP) and catalase (CAT) kept increasing (P < 0.05). The correlation analysis indicated that humic acid was effective for reducing the devastation to soil enzymes due to the Cd pollution. In conclusion, humic acid is effective for the reduction of both Cd phytoavailability and the damage to enzyme activities due to Cd pollution in fluvo-aquic soil

**Key words:** soil pollution; cadmium; phytoavailability; humic acid; enzyme.

## 1. Introduction

In the last several decades, the increasing application of agrochemicals has brought a significant amount of heavy metals into farmland ecosystems [1, 2], which may lead to serious environmental risk because of the toxicity, stability and recalcitrance to biodegradation. The heavy metals can also accumulate in the crops [3, 4, 5], resulting in damage to human's health through the food chain. The humic acid has a molecular structure with abundant organic functional groups which can complex or chelate with the heavy metal ions in soil. Based on the properties, it can be speculated that humic acid has potential to inhibit the heavy metals uptake by crops and reduce the environmental risk. The aim of the present study is to find out the effect of humic acid on the cadmium (Cd) phytoavailability in fluvo-aquic soil. The urease (UE), alkaline phosphatase (ALP) and catalase (CAT) activities are employed to investigate the ecotoxicological effects in the process in order to assess the overall performance of humic acid.

## 2. Material and Methods

**Pot Experiment.** The fluvo-aquic soil was collected from Fangshan District, Beijing, China (in the depth of 0-20cm). The background Cd concentration was 0.072 mg·kg<sup>-1</sup>. The air-dried and sieved soil

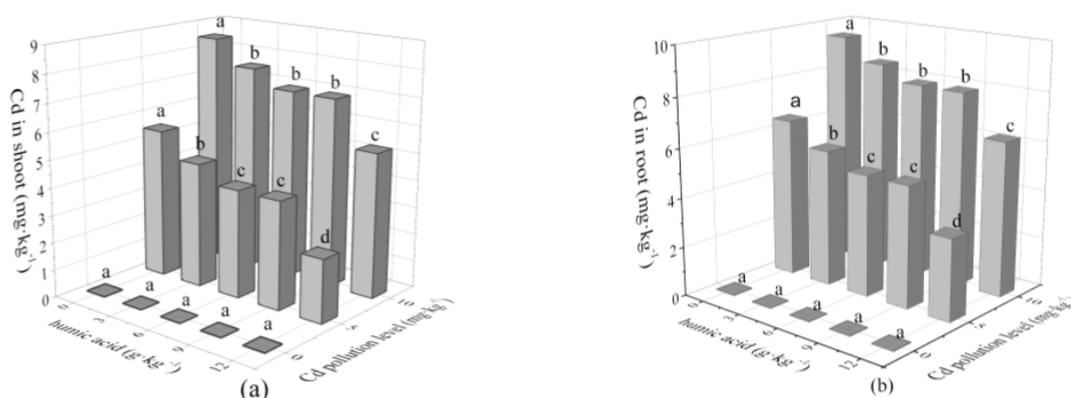


was introduced to the plastic pots ( $12 \times 10$  cm) with the mass of 330 g·pot<sup>-1</sup>. CdCl<sub>2</sub> solution was added to achieve the Cd pollution levels (0, 5 and 10 mg·kg<sup>-1</sup>). Under each pollution level, the humic acid was added in soil as powder and the concentrations were 0, 3, 6, 9, and 12 g·kg<sup>-1</sup>. The treatments were conducted in triplicate. The holes on the bottom were sealed using glass cement. The N, P and K were added in the soil to reach the following levels: 3.067 g·kg<sup>-1</sup>, 1.027 g·kg<sup>-1</sup>, 0.157 g·kg<sup>-1</sup>, respectively [6]. The pakchoi (*Brassica chinensis* L.) was grown in the pots for 40 days. The moisture content in the soil was maintained at about 75% of the water-holding capacity using distilled water [7]. The plants were kept under the condition of 28°C-14h and 15°C-10h cycles [8]. After harvest, the plant and soil samples were dried and sieved using 1-mm nylon mesh for further analysis.

**Analytical Procedure.** The prepared plant samples were digested with nitric acid, perchloric acid and hydrofluoric acid to assay the Cd concentrations which were determined with Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Soil UE activity was determined by the method of C.S. Yan [9], and the results were expressed as the concentration of NH<sub>4</sub><sup>+</sup> released by urease enzymatic hydrolysis of urea (mg NH<sub>3</sub>-N·g<sup>-1</sup> soil). Soil ALP activity was determined by the method of M.A. Tabatabai and J.M. Bremner [10], and the results were expressed as the concentration of p-nitrophenol (PNP) produced by the enzymatic reaction (mg PNP·g<sup>-1</sup> soil). Soil CAT activity was measured by back-titrating residual H<sub>2</sub>O<sub>2</sub> with KMnO<sub>4</sub> [11], and the results were expressed as the volume of 0.02 mol·L<sup>-1</sup> KMnO<sub>4</sub> solution consumed in the titrating process (mL KMnO<sub>4</sub>·g<sup>-1</sup> soil). All the processes were conducted in triplicate. The results were expressed as the means of replicates. The data were graphed and analyzed with Origin 9.1 and SPSS Statistics 20.0 software.

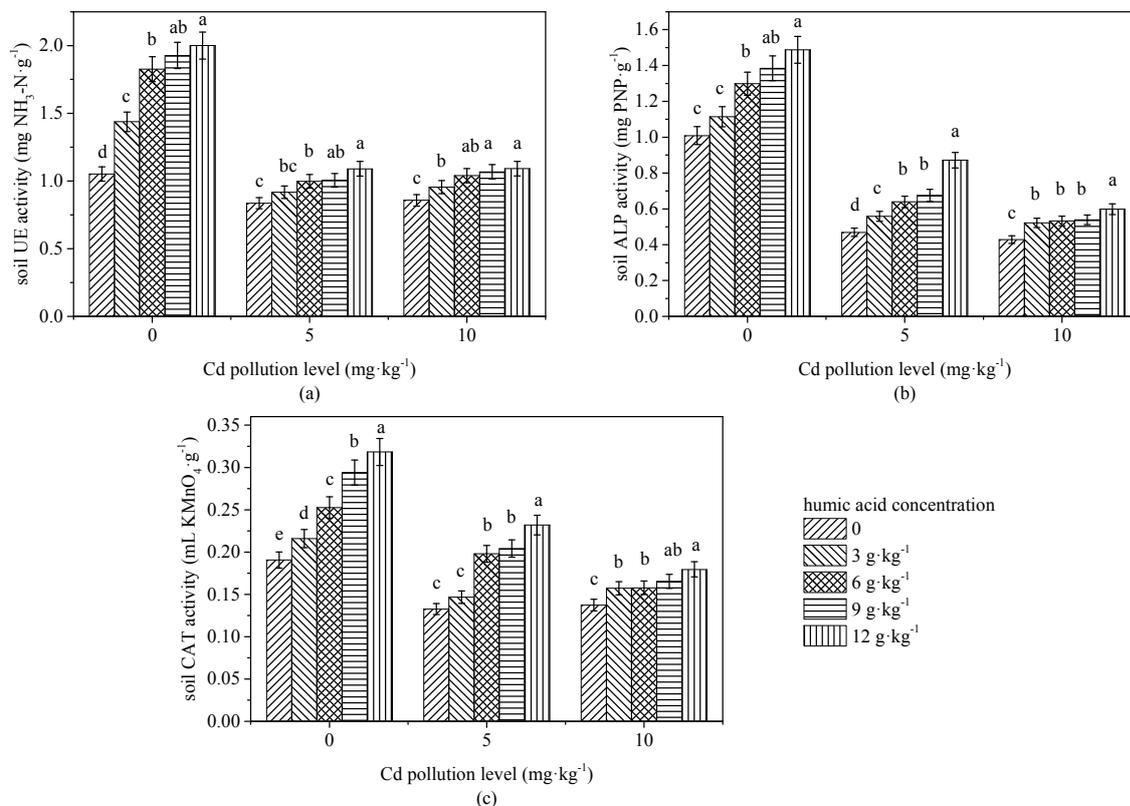
### 3. Results and discussion

**Cd Concentration in the Plant Tissues.** The Cd concentrations in plant tissues are shown in Fig 1. With exogenous Cd in soil, Cd levels in the tissues of the pakchoi significantly increased ( $P < 0.01$ ), and Cd concentrations in the roots were significantly larger than those in the shoots ( $P < 0.05$ ). As can be seen in the figure, Cd concentrations in shoots and roots kept decreasing with the humic acid concentration augment (0 to 12 g·kg<sup>-1</sup>) by 33.42% to 57.87% ( $P < 0.05$ ), indicating a good efficacy for inhibiting Cd uptake by pakchois. Humic acid had a great affiliation to metal cations due to the existence of functional groups or ligands which could form chelates with metals [12]. Given the special molecular structure, humic acid had the potential to chelate with the free Cd<sup>2+</sup> in soil, resulting in lower phytoavailability.



**Fig 1.** Cd concentrations in plant tissues under humic acid treatments. (a) shoot; (b) root Results of Duncan Multiple Comparison are expressed as letters in the figure. Different letters in each group indicate a significant difference ( $P < 0.05$ ); the same letters indicate that differences were not significant. Same as below.

**Soil Enzyme Activities.** The activities of UE, ALP and CAT in soil are shown in Fig 2. As can be seen in the figures, the varying trends of the activities of the three kinds of enzymes were similar. When there was exogenous Cd in the soil, the activities of the enzymes significantly decreased ( $P < 0.05$ ). The results reflected the Cd toxicity to microorganisms [13, 14], which might result from the interacting with the enzyme-substrate complex, denaturing the enzyme protein or interacting with the protein-active groups [15]. The activities of the UE, ALP and CAT kept rising gradually with the humic acid concentration augment in the soil by 27.25% to 97.18%, 39.56% to 85.85% and 30.71% to 74.84%, respectively ( $P < 0.05$ ). Correlation matrix between Cd concentration in tissue and soil enzymes is shown in Table 1. The activities of UE, ALP and CAT in soil were all negatively correlated with the Cd accumulation levels in tissue ( $P < 0.01$ ).



**Fig 2.** Soil enzyme activities under various Cd and humic acid treatments. (a) UE; (b) ALP; (c) CAT

**Table 1.** Correlation matrix between Cd concentration in tissue and soil enzymes (\*\* $P < 0.01$ )

	Cd in tissue	UE	ALP	CAT
Cd in tissue	1			
UE	-0.699**	1		
ALP	-0.788**	0.850**	1	
CAT	-0.710**	0.902**	0.907**	1

The results indicated that the Cd phytoavailability in soil was an important factor influencing the enzyme activities. According to S.J. Sun et al. [16] and Y.P. Wang et al. [17], there was a negative correlation between heavy metals phytoavailability and heavy metals availability to microorganisms in

soil. Therefore, humic acid could reduce the Cd phytoavailability and toxicity to microorganisms in soil at the same time, leading to improvement of enzyme activities.

#### 4. Conclusion

Humic acid can influence the Cd phytoavailability in fluvo-aquic soil as well as the enzyme activities. When humic acid is applied, Cd phytoavailability kept decreasing with the concentration augment (0 to 12 g·kg<sup>-1</sup>), while the enzyme activities (UE, ALP and CAT) kept increasing. The enzyme activities are negatively correlated with the Cd uptake levels in plant, indicating the Cd toxicity to microorganisms is the reason for the disturbance to enzyme activities and humic acid can reduce the damage. In conclusion, humic acid is effective for Cd phytoavailability reduction in fluvo-aquic soil, which can also reduce the devastation to soil enzyme activities due to the Cd pollution.

#### Acknowledgements

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