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Toxicity and subcellular distribution of cadmium in wheat as affected by dissolved organic acids

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Abstract

We aim to investigate the effects of humic acid (HA) and citric acid (CA) on the toxicity and subcellular distribution of Cd in wheat. Results show that the toxicity and uptake of Cd decreased with increasing HA. The EC_{50} values of Cd increased from 3.36 μmol/L to 4.96 and 7.33 μmol/L at 50 and 250 mg/L HA, respectively, but decreased to 1.39 μmol/L in the presence of CA based on free ion activity model (FIAM). HA decreased the relative subcellular distribution of Cd in the heat-denatured proteins (decreased from 54% to 33%) but increased Cd in the heat-stable proteins in root (from 25% to 50%) at 7.61 μmol/L $\{Cd^{2+}\}$ (free Cd activity), which resulted in decreasing Cd toxicity. However, CA increased Cd toxicity due to the increased internalization of Cd although the relative subcellular distributions of Cd exhibited a decrease in the heat-denatured proteins and increase in the granule fraction compared to the control at high-level Cd. The FIAM could not predict the toxicity of Cd in the presence of organic acids. Alternatively, the internal Cd accumulation and subcellular Cd concentration were better to describe the toxicity of Cd to wheat.

Key words: toxicity; cadmium; organic acid; subcellular distribution
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Introduction

The toxicity of metals in environment is generally dependent on their chemical speciation, which is related to the physicochemical characteristics of environmental medium, such as cations, pH, and metal complexation (Campbell and Evans, 1987; Davies et al., 1993; Bervoets and Blust, 1999; Fraysse et al., 2000). Therefore, the total metal concentration is not a good predictor of its bioavailability in many cases. The steady-state models such as the free ion activity model (FIAM) and the biotic ligand model (BLM), as an extension of the FIAM, are able to more accurately predict trace metal bioavailability (Campbell et al., 2002). According to the FIAM, the activity of free metal ion in solution dominates its interaction with cellular surface sites and hence its bioavailability (Morel, 1983). Although they are widely used under laboratory conditions, these equilibrium models are not always able to quantify metal bioavailability in the presence of organic ligands (Lamelas et al., 2009). For example, Al uptake by freshwater algae *Chlorella pyrenoidosa* (Parent et al., 1996) and juvenile Atlantic salmon *Salmo salar* (Roy and Campbell, 1997) were overestimated, while Pb uptake by *C. kesslerii* in the presence of both humic and fulvic acids was underestimated by the FIAM (Slaveykova et al., 2003). In addition, the BLM and FIAM just consider the chemical factors, but they did not take into account the physiological factors. Actually, to evaluate the bioavailability of heavy metal should consider the influences of biological and geochemical factors because the bioavailability of heavy metals is the outcome of several processes: uptake, distribution, storage, and excretion (Rainbow, 2002). The subcellular partition model (SPM) considers the geochemical factors and the physiological factors (Wallace et al., 2003). Furthermore, many publications reported that SPM was a useful model to describe the bioavailability of metal to organisms (Wang and Wang, 2008a, 2008b; Lavoie et al., 2009).

Humic acid is complex aromatic macromolecules with a wide variety of functional groups such as carboxylic and phenolic alcoholic and amino groups (Stevenson, 1982), and is often present at concentrations many orders of magnitude higher than that of the trace metals (Boggs et al., 1985). The citric acid as a small organic ligand is one of the root exudates on rhizosphere, and is commonly found in soil. These organic ligands react with metal ions in the solution and dominate the complexation characteristics (Turner et al., 1986). Subsequently, it affects the bioavailability of heavy metal. Although the effects of organic acid on metal toxicity have been studied through chemical speciation, little is known about the physiologic

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mechanisms of uptake and toxicity of heavy metal in the presence of organic acid. Therefore, the aim of this study was to investigate the effects of organic ligands, taking humic acid and citric acid as examples, on the bioavailability and toxicity of Cd and the subcellular distribution of Cd in wheat (*Triticum aestivum*) root.

1 Materials and methods

1.1 Test organism and experimental conditions

The tested wheat seeds (*Triticum aestivum* L. Yang line 16) were provided by the Agricultural Academy of Sciences, Yangzhou, China. Seeds were surface sterilized in 0.1% NaClO solution for 15 min and rinsed with deionized water. Germination was performed in the dark on filter papers which were moistened with culture solution for 48 hr at 25°C.

The culture solutions contained 0.25 mmol/L $Ca(NO_3)_2$, 0.25 mmol/L $MgSO_4$, 0.25 mmol/L KNO_3, and 0.08 mmol/L KH_2PO_4 (Li et al., 2011b). Cadmium was added as chloride salt into the culture solution in final concentrations ranging from 0.50 to 50 μmol/L. The solution was buffered at pH 6.0 with 2.0 mmol/L MES ($C_6H_{13}NO_4S$, 2-[N-morpholino] ethane sulfonic acid). The humic acid (HA) stock solution of 1 g/L was prepared by dissolving the HA sodium salt (technique grade) from Aldrich Sigma (USA) and stored at 4°C in the dark. The DOC values of different concentrations of HA were measured by the TOC analyzer (Multi N/C 3000, Analytikjena, Germany). Before measuring DOC, the HA solution should be filtrated through 0.45 μmol/L filter. The citric acid (CA) stock solution (44.2 mmol/L) was also prepared and stored at 4°C in the dark. During the exposure processes, the solution remained unstirred. Before and after the exposure, Cd concentration in the test solutions was determined by Flame Atomic Absorption Spectrometry (F-AAS; Hitachi Z-2000, Japan). The range of Cd determination was 0.025–2 mg/L for F-AAS. Standard Cd solution was bought from Ministry of Environmental Protection of the People's Republic of China. A range of concentration (from 0.025 to 2 mg/L) was prepared for standard curve by diluting the stock standard Cd solution with super pure water containing 1 mol/L HNO_3. The standard error was lower 5%. Preliminary tests showed that the depletion of Cd in the solution was negligible.

All chemicals except HA sodium salt used were analytical grade or much higher, and bought from Sinopharm Chemical Reagent Co. Ltd., China. The glassware used for toxicity experiments were cleaned, followed by immersion in an acid bath containing 10% HNO_3 for 24 hr and rinsing with deionized water for several times prior to use.

1.2 Toxicity experiments

The 72-hr root elongation toxicity tests were performed following ISO Guideline 11269-1 (ISO, 1993). Six uniformed seedling, after seed germination, were transferred to a beaker with a volume of 500 mL test solution containing six levels of total Cd in the absence or presence of HA or CA for 72 hr (Table 1). To investigate the effect of humic acid or citric acid on Cd toxicity in wheat root, 50 mg/L HA, 250 mg/L HA and 442 μmol/L CA according to literatures, respectively, was added into the solution containing different concentrations of Cd. Meanwhile, for the control, four treatments were performed. Six seedlings were transferred to the culture solution in the absence or presence of HA solution or CA solution.

The beakers were placed randomly in a growth cabinet with 80% humidity and the temperature was maintained at 25°C. Each treatment was run in triplicates. At the end of the experiment, the roots in each treatment were rinsed with deionized water, and then washed with 25 mL of 10 mmol/L ethylenediaminetetraacetic acid (EDTA) for 5 min to remove the surface-bound (adsorbed) Cd from the roots. Then, the roots were rinsed with deionized water again and the length and fresh weight of roots were measured. Finally, the roots were dried at 70°C for obtaining the dry weight, and digested with 5.0 mL of concentrated pure nitric acid. Cd was measured by the Flame Atomic Absorption Spectrometry (F-AAS; Varian 220Z).

To investigate the effect of HA and CA on the subcellular distribution of Cd, another 72-hr root elongation toxicity tests were performed in the presence or absence of HA or CA. In this experiment, two levels of $\{Cd^{2+}\}$ were investigated (Table 2). The following procedures were the same as above. At the end of the exposure, the root was frozen at –70°C for analyzing the subcellular distribution of Cd.

Table 2 Initial organic acid and total Cd concentrations in the exposure solution at constant free Cd^{2+} activity ($\{Cd^{2+}\}$) for each experiment

Test no.	Experimental condition		
		Organic acid	Total Cd concentration
1	$\{Cd^{2+}\}$ 0.763 μmol/L	0	1.0 μmol/L
2		50 mg/L HA	1.62 μmol/L
3		250 mg/L HA	5.37 μmol/L
4		442 μmol/L CA	3.07 μmol/L
5	$\{Cd^{2+}\}$ 7.61 μmol/L	0	10 μmol/L
6		50 mg/L HA	13.2 μmol/L
7		250 mg/L HA	32.5 μmol/L
8		442 μmol/L CA	29.3 μmol/L

Table 1 Chemical composition of the exposure solution used in different bioassay sets

Bioassay set	Cd concentration (μmol/L)	Humic acid (mg/L), or citric acid (μmol/L)	Concentration of other cations keeping at constant (mmol/L)	pH
Cd-set	1.0, 2.5, 5.0, 10, 25, 50	0	0.25 Ca, 0.25 Mg, 0.33 K	6.00
Humic acid-set	1.0, 2.5, 5.0, 10, 25, 50	50, 250	0.25 Ca, 0.25 Mg, 0.33 K	6.00
Citric acid-set	1.0, 2.5, 5.0, 10, 25, 50	442	0.25 Ca, 0.25 Mg, 0.33 K	6.00

1.3 Subcellular distribution of Cd in wheat roots

The subcellular distribution of Cd in wheat roots was performed at 4°C according to Wang and Rainbow (2006) with some modifications. In brief, 0.2 g root sample from each treatment was ground to powder with liquid nitrogen using a mortar and pestle, and homogenized in 5.0 mL of buffer solution containing 0.25 mol/L sucrose, 1.0 mmol/L dithioerythritol, and 50 mmol/L Tris-HCl (pH 7.5) (Weigel and Jäger, 1980). The homogenate was centrifuged at 2500 $\times g$ for 15 min. The resulting supernatant (S1) and pellet (P1) were separated. The 2.0 mL ultra-pure water was added in P1 and kept in a water bath at 100°C for 2 min, followed by adding 2.0 mL NaOH (1.0 mol/L) and heating again at 70°C for 1 hr, and then was centrifuged at 10,000 $\times g$ for 15 min. The pellet contained granule (MRG) and the supernatant contained the cellular debris. Meanwhile, S1 was centrifuged at 100,000 $\times g$ for 60 min to get the pellet (organelles) and supernatant (S2). S2 containing the cytosol fraction was heat denatured at 80°C for 10 min and cooled on ice for 60 min, followed by centrifugation at 50,000 $\times g$ for 15 min. The resulted pellet contained heat denatured proteins (HDP) and the supernatant contained the heat-stable proteins (HSP, MT-like proteins). The five fractions were digested with 5.0 mL pure concentrated HNO_3. Then Cd was measured by the Flame Atomic Absorption Spectrometry (F-AAS; Hitachi Z-2000, Japan). The presumed metal-sensitive fraction (MSF) was defined as organelles+HDP, and the biological detoxification fraction (BDM) were defined as HSP + MRG. The total recovery of the five different subcellular fractions was greater than 95% in preliminary experiment, suggesting that the separation was satisfactory in this study.

1.4 Data analysis

The solution Cd speciation was calculated using visual MINEQL (version 2.51) chemical equilibrium program (US Environmental Protection Agency, Athens, GA, USA) by inputing the concentration of different cations and anions in the solution, pH, and temperature. Stability constants were updated using the data from NIST (National Institute of Standards and Technology), and the equilibrium phases in the speciation calculation included atmospheric CO_2. The free Cd ions in the presence of HA were calculated according to the NICA-Donnan model of Visual MINTEQ. In the parameters, we presumed the DOM:DOC = 1:1 and other parameters according to ppha.NPF.txt in the NICA-Donnan model (which suggested that 100% DOM was humic acid). The concentration of DOC is the measured value according to TOC analyzer.

Relative root elongation (RRL, %) was calculated using Eq. (1):

$$RRL = ((RL_T - RL_S)/(RL_C - RL_S)) \times 100\% \quad (1)$$

where, RL_T (cm) represented the mean root length (RL) in the presence of toxicants (i.e., Cd), RL_C (cm) represented RL in the corresponding toxicant-free control, and RL_S (cm) represented RL in toxicant sufficient to saturate growth-inhibitory processes. The RL_S is nearly equal to RL at the time of seedling transfer to the test media.

The 72-hr EC_{50} values expressed as the median effective concentration of total, free activity of Cd and the intracellular Cd denoted EC_{50}[Cd], $EC_{50}\{Cd^{2+}\}$ and $EC_{50}[Cd]_{intra}$, respectively, which could result in 50% lethal effect of the wheat seedlings, were calculated from the observed RRL at each Cd concentration by fitting a sigmoid dose-response curve.

$$RRL = (100/(1 + 10^{(\log EC_{50} - X)b})) \times 100\% \quad (2)$$

where, X (μmol/L) is the logarithm of concentration of total Cd or free activity of Cd, intracellular Cd; b is the slope of the curve.

Significant differences among the different subcellular distribution of Cd in wheat were determined by one-way ANOVA using a least significant difference test (LSD test). Significant difference was accepted at $p < 0.05$. All the data were the mean values which were calculated with triplicates.

2 Results

2.1 Effect of humic acid on the toxicity and uptake of Cd in wheat root

The addition of Cd caused significant reduction in root length, and the relative root length was fitted to the dose-response curve (Fig. 1). The values of EC_{50} for the total Cd and free ion activity of Cd were 4.34 and 3.36 μmol/L, respectively. In the control, the addition of HA significantly increased the root length. HA also alleviated the toxicity of Cd. The values of EC_{50} for total Cd increased to 8.97 and 31.5 μmol/L at 50 and 250 mg/L HA, respectively (Fig. 1a). In addition, HA also increased the values of EC_{50} to 4.96 μmol/L at 50 mg/L HA and 7.33 μmol/L at 250 mg/L HA based on the free activity of Cd (Fig. 1b).

The uptake of Cd in the root increased from 1077 to 4133 μg/g with the solution Cd varied from 1.0 to 50 μmol/L in the control. The addition of HA decreased the uptake of Cd in the root (Fig. 2a). When 250 mg/L HA was added, the uptake of Cd in the root was decreased to a half. On the base of free ion activity of Cd, HA also decreased the uptake of Cd in root (Fig. 2b).

The relationships between the combined data of relative root length and the free ion activity of Cd in solution and the intracellular Cd in the root were established respectively in the absence and presence of HA (Fig. 3). The intracellular Cd in root was better to indicate the Cd toxicity.

2.2 Effect of citric acid on the toxicity and uptake of Cd in wheat root

In the control, the addition of CA decreased the root length. Based on total Cd, CA slightly decreased the toxicity of Cd (Fig. 4a). However, CA enhanced the toxicity of Cd on the base of free Cd ion activity and the value of EC_{50} decreased to 1.39 μmol/L (Fig. 4b). Similarly, the addition of CA slightly decreased the uptake of Cd based on the

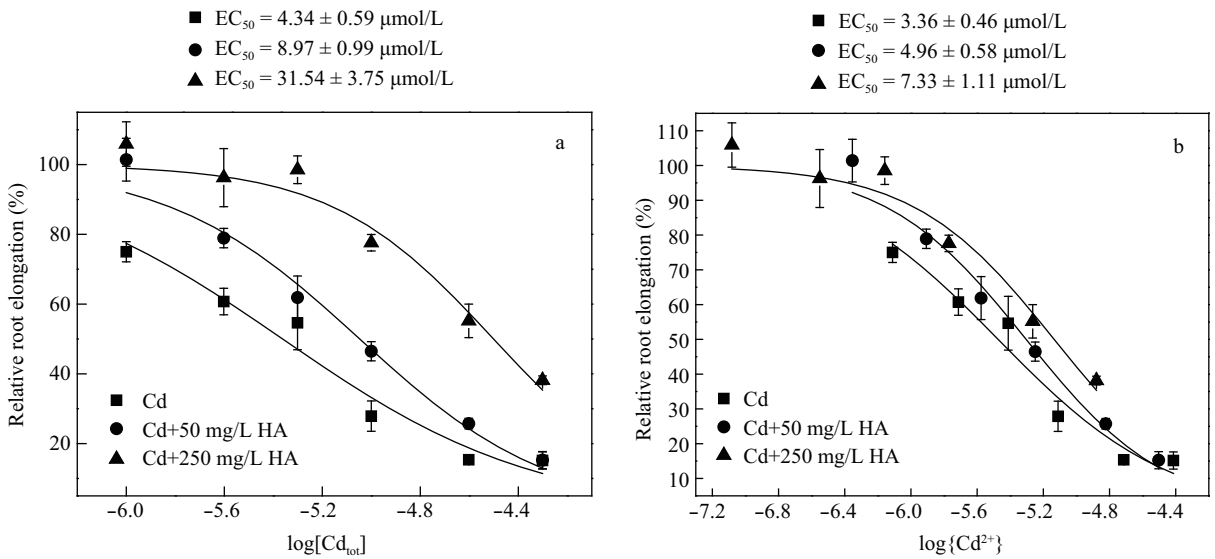

Fig. 1 Relative root length plotted with total Cd concentration (a) and free Cd ion activity (b) in solution in the presence or absence of humic acid (HA). The solid lines were fitted with the dose-response curve.

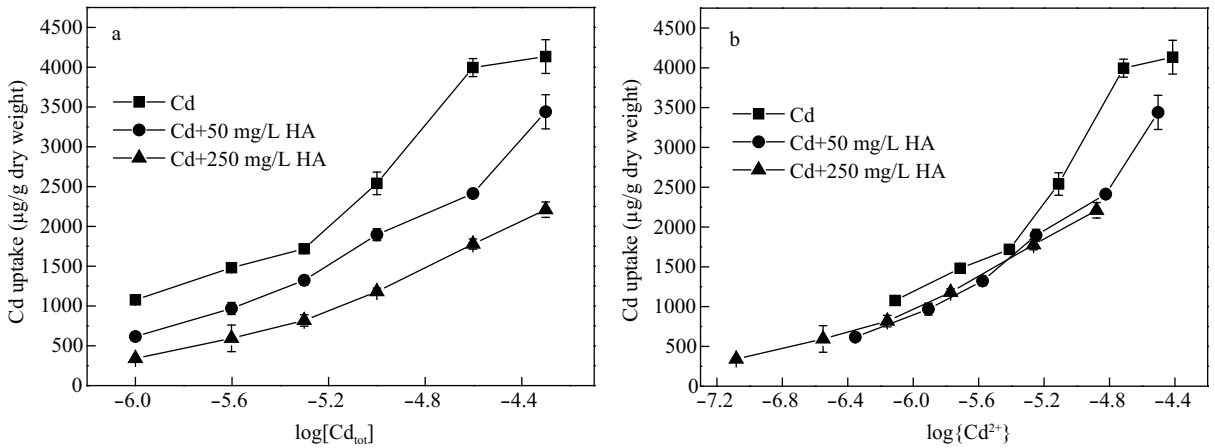

Fig. 2 Cd uptake plotted with total Cd concentration (a) and free Cd ion activity (b) in solution in the presence or absence of humic acid (HA).

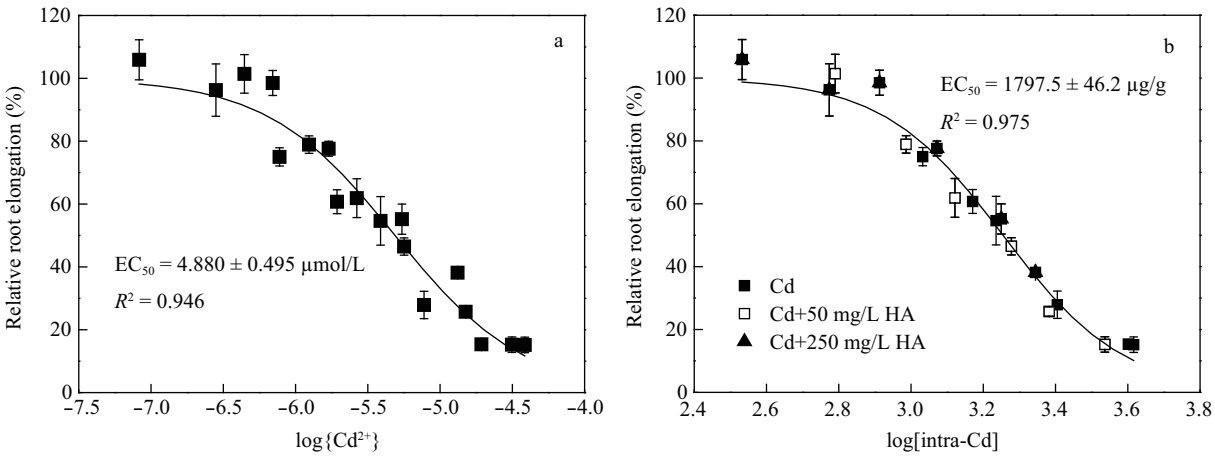

Fig. 3 Relative root length plotted with free Cd ion activity (a) and total cellular Cd (b) in the presence and absence of humic acid (HA). The solid lines were fitted with the dose-response curve.

total Cd while increased the uptake of Cd based on the free Cd ion activity (Fig. 5). Therefore, the intracellular Cd was better to correlate with the relative root length than free activity of Cd (Fig. 6). In addition, we observed that the pH in the exposure solution increased from 6.0 to 6.85 at the end of experiment in the presence of CA, while the pH was kept at 6.0 in the absence of CA or in the presence of HA.

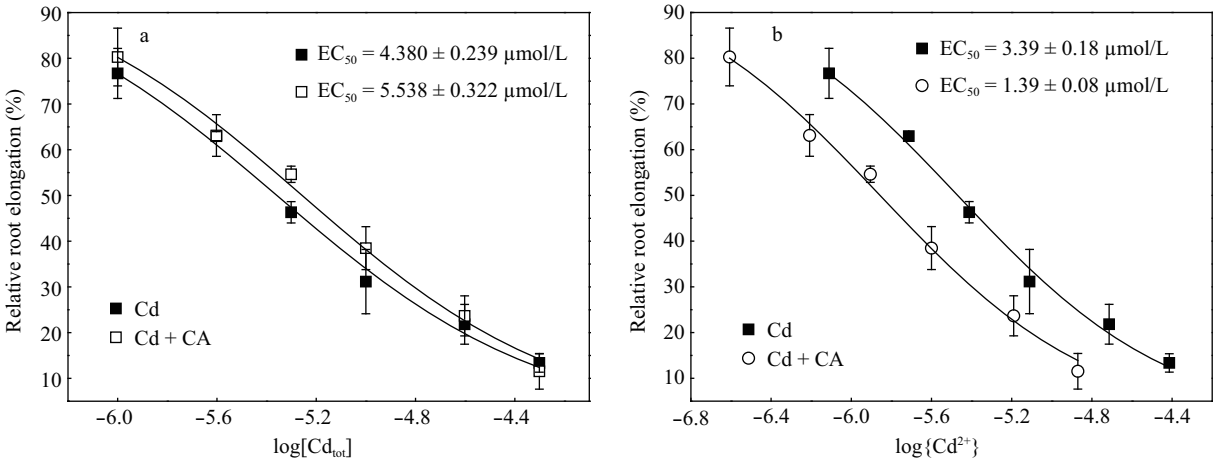

Fig. 4 Relative root length plotted with total Cd concentration (a) and free Cd ion activity (b) in solution in the presence or absence of citric acid (CA). The solid lines were fitted with the dose-response curve.

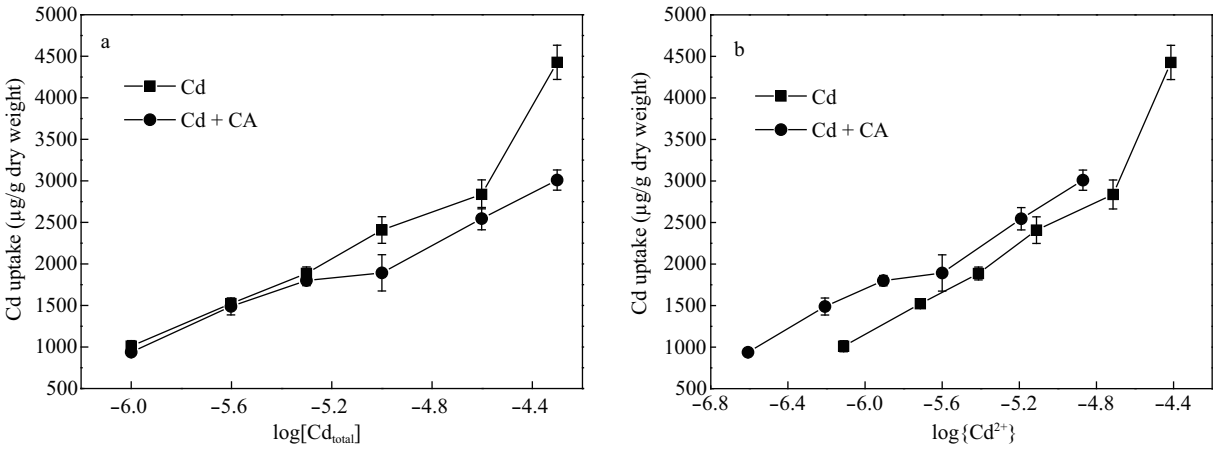

Fig. 5 Cd uptake plotted with total Cd concentration (a) and free Cd ion activity (b) in solution in the presence or absence of citric acid (CA).

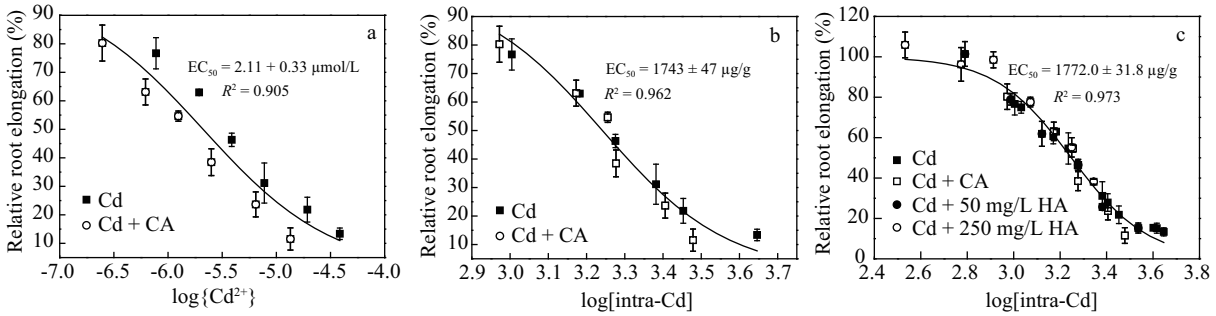

Fig. 6 Relative root length plotted with free Cd ion activity (a) and total cellular Cd (b) in the presence and absence of citric acid and (c) in the presence of humic acid (HA) and citric acid (CA). The solid lines were fitted with the dose-response curve.

2.3 Effects of humic acid and citric acid on the subcellular Cd distribution in root

When the experiment was performed at the same free Cd ion activity, the measured relative root length and the uptake of Cd in root were consistent with the results in the first experiment. As shown in Fig. 7, the addition of HA decreased the uptake and toxicity of Cd while the addition of CA increased the uptake and toxicity of Cd.

Cd was mainly bound to HDP, HSP, and cell debris fractions. Cd in the MRG fraction was the least (Fig. 8). The relative subcellular distribution of Cd decreased and increased in the HSP and HDP, respectively, with increasing Cd in the exposure solution in control. At the low level of free Cd activity, both HA and CA did not change the relative subcellular distribution of Cd. At the high level of free Cd ion activity, the percentage of Cd in the HSP fraction increased and in the HDP fraction decreased with increasing HA compared to the control. In the presence of CA, the percentage of Cd had a slight decrease in HDP and increase in MRG and cell debris fractions at high level free Cd ion activity.

The correlation between relative root length and the Cd concentration in subcellular fractions in the presence and absence of HA and CA were established. As shown from the Table 3, result shows that the Cd in organelle fraction and in HDP fraction were well correlated with Cd toxicity.

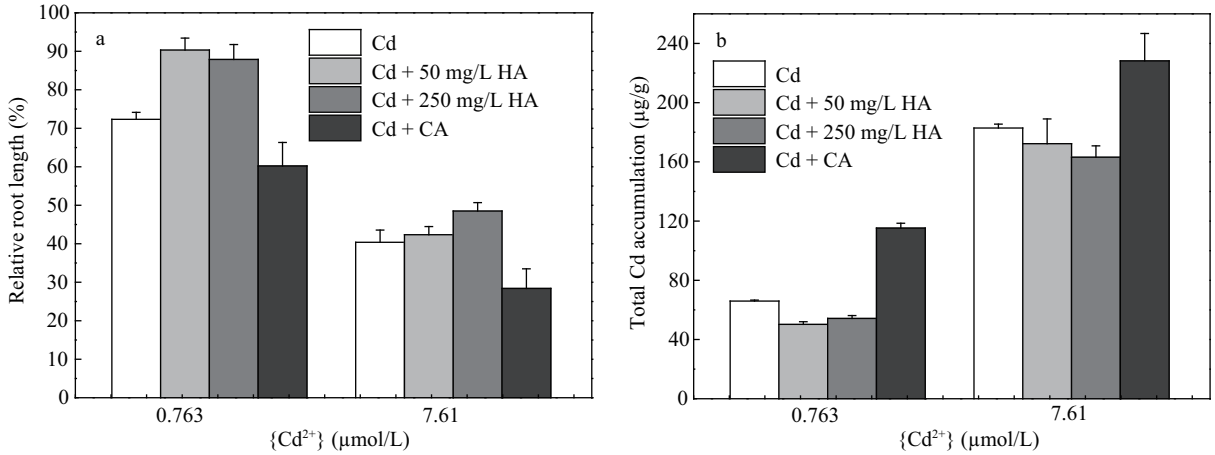

Fig. 7 Relative root length (a) and the Cd uptake in root (b) at two levels of free Cd ion concentrations in the presence or absence of citric acid (CA) or humic acid (HA).

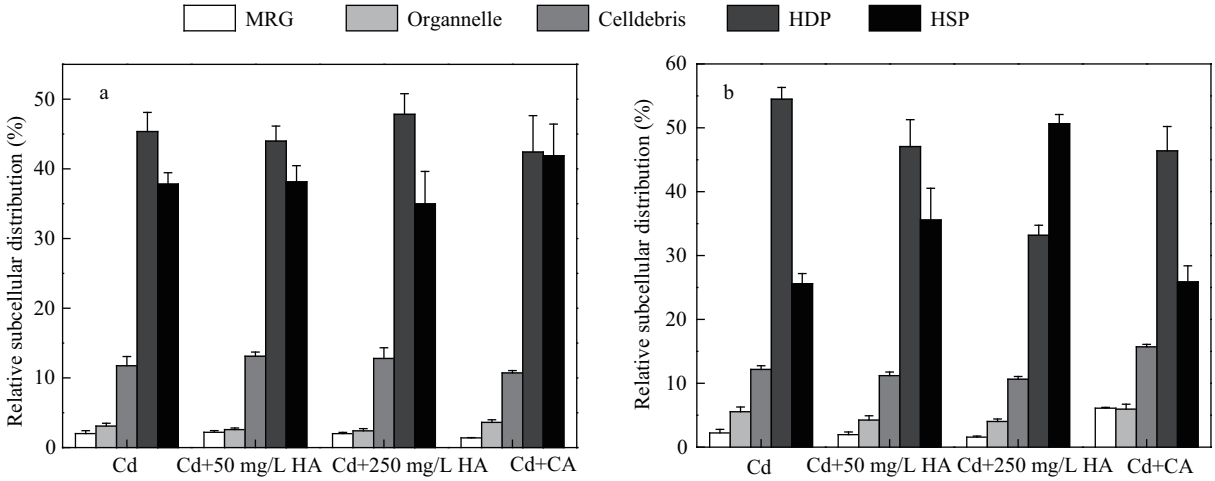

Fig. 8 Relative subcellular distribution of Cd in root at free Cd ion concentration of 0.763 (a) and 7.61 μmol/L (b) in the presence or absence of citric acid (CA) or humic acid (HA).

Table 3 Correlation between relative root length and the Cd concentration in subcellular fractions in the presence and absence of organic acid

Correlation	MRG	Organelle	Cell debris	HDP	HSP	Total
RRL	–0.668**	–0.915**	–0.897**	–0.903**	–0.821**	–0.97**

MRG: metal-rich granules; HSP: heat-stable proteins; HDP: heat-denatured proteins; Total: the cellular accumulation; RRL: relative root length.

3 Discussion

3.1 Effect of humic acid on the toxicity, uptake and subcellular distribution of Cd in wheat root

Humic acid has potential effects on plant growth, toxicity, and bioavailability of heavy metals in a soil-plant system. Jindo et al. (2012) reported that humic acids promoted root growth by releasing auxin-like plant growth promotors and enhancing proton pump activity. The enhanced proton pump activity increased the uptake of nutrients by enhancing the electrochemical proton gradient that drives ion transport across cell membranes. Vigenault et al. (2000) also reported that HA increased membrane permeability due to the enhancement of negative algal surface charge and affected the passive uptake of chemical species. In this study, the addition of HA promoted the root length in the control which was consistent with the literature as shown above. However, the presence of HA decreased the uptake of Cd in the root on the base of total Cd concentration and free Cd ion activity. Thus the increase in electrochemical proton gradient or the surface charge on the root surface seems to play an insignificant role in the uptake of Cd. Also, Lamelas and Slaverykova (2007) reported that the changes in algal surface charge play a limited role in the uptake of metals in the presence of HA. Two reasons may explain the decrease in the uptake of Cd. First, the complexing of Cd with HA reduced the solution free Cd ion concentration, resulting in decreasing uptake of Cd. Second, the HA adsorbed on the root surface and decreased the uptake sites for the internalization of Cd, and thus decreased the uptake of Cd.

In addition, the uptake of Cd was not different between HA at 50 and 250 mg/L based on FIAM, but the toxicity of Cd still decreased with increasing HA in this study (Fig.1b) due to the possible physiologic process of Cd in root. After its uptake by plant, Cd is either excreted, or detoxified, and then sequestered in subcellular compartments, resulting in

accumulation and toxicity to plants. Actually, only Cd in the metal-sensitive fraction (organelle and HDP fractions) could result in toxicity to organisms (Wallace et al., 2003), while Cd in the biological detoxify fraction (granule and HSP fractions) initiated detoxification for heavy metal to organisms. In this study, we observed that the percentage of Cd increased in the HSP fraction and decreased in the HDP fraction with increasing HA at the high level of free Cd ions in solution, which implied that Cd was regulated by HA according to the SPM. Therefore, the presence of HA decreased the uptake and toxicity of Cd by decreasing the free Cd ion concentration accessible to organism and changed the subcellular distribution of Cd.

3.2 Effect of citric acid on the toxicity, uptake and subcellular distribution of Cd in wheat root

The addition of CA decreased the root length in the control because citrate acid was possibly adsorbed on the roots or internalized. Previous papers (Jones and Darrah, 1995; Bell et al., 2003) also reported the root uptake of anionic citrate and other low-molecular-weight organic anions. The uptake and toxicity of Cd decreased on the base of total Cd concentration in solution in the presence of CA due to the complexing of Cd with CA which reduced the solution free Cd ion activity. However, the presence of CA increased the uptake and toxicity of Cd to wheat based on the solution free Cd ion activity. This result was consistent with previous reports (Berkelaar and Hale, 2003; Panfili et al., 2009). There are many reasons to be related. First, the dissociation of the Cd-citrate complex supplied free Cd ions on the root surface resulting in increased uptake of Cd, which is accepted mostly. Second, Senden et al. (1994) observed an increase of adsorbed citrate on tomato xylem cell walls, and Cd was complexed by citrate in the cell walls (Senden et al., 1995). Thus the adsorbed citrate decreased Cd transferring to cytosolic and increased the percentage of Cd in cell debris fraction at high level of Cd^{2+} in the presence of CA in this study. The increase in percentage of Cd in cell debris fraction was just account for 3%, and thus the adsorbed citrate seems to play a limit role in the increase in the uptake of Cd. Third, the Cd-citrate complex was directly taken up as suggested by Berkelaar and Hale (2003) and Campbell et al. (2002). And Krishnamurti et al. (2004) also reported that the toxicity of the Cd-citrate complex was more toxic than Cd^{2+} in root. In the last, the increased pH decreased the competition of H^+ with Cd^{2+} for the uptake sites on the surface root and enhanced the uptake and toxicity of Cd to wheat according to the BLM.

Our previous study (Li et al., 2011a) suggested that the relative Cd distribution decreased in the HSP fraction and increased in the HDP fraction with increasing root Cd. In this study, a similar result was observed in the control. Also, in the presence of CA the percentage of Cd in the HSP fraction decreased with increasing solution free Cd ion concentration. However, it was not the case in the presence of CA although the internalization of Cd was enhanced compared to the control. This result indicated that CA posed a similar function of HA which could regulate Cd in wheat. What's more, although the relative distributions of Cd in the presence of CA were not different compared to the control at low-level free Cd ion, the increased internalization of Cd could also enhance its toxicity in the process of accumulation of Cd. In addition, the relative distribution of Cd had a decrease in the HDP fraction and increase in the MRG fraction in comparison to the control at high level of Cd, which should generate detoxification according to the SPM. However, the concentrations of Cd in the five subcellular fractions in the presence of CA were more than those in the control and resulted in increased toxicity of Cd in the presence of CA.

3.3 Prediction of Cd toxicity

The free ion activity model, which assumes that metal uptake across the plasma membrane, is a rate-limiting process, and the rate of metal uptake from a solution is directly proportional to the free metal ion concentration in the bulk solution. This model has been frequently used to predict the bioavailability and toxicity of metals (Campbell, 1995). For the effect of organic matter on the uptake of Cd, different and even controversial results have been reported in the literatures. Van Ginneken et al. (2001) demonstrated that Cd uptake by carp was in accordance with the FIAM. However, Höss et al. (2001) reported that DOM increased the bioavailability and toxicity of Cd to the nematode *Caenorhabditis elegans*. And Roditi et al. (2000) found that DOM derived from algae provided a nutritional supplement to zebra mussels and the zebra mussels adsorbed some dissolved metals that were complexed by the DOM. Some reports indicated that DOM-bound Cd was partially available for uptake but that difference in uptake efficiency depended on molecular weight, binding characteristics and concentration of the DOM (Voets et al., 2004). These examples concerning invertebrates and plants suggest that the FIAM is not always valid.

In the present study, although we observed that the free Cd ion could explain the toxicity of Cd to wheat to some extent, we observed that the intracellular Cd was better to predict the toxicity of Cd than the FIAM, since the relative root length were much more dependent on the intracellular Cd concentration, and sigmoid relationships were shown between the relative root length and the intracellular Cd concentrations (R^2 = 0.973) (Figs. 3 and 6). Also, other papers reported that intracellular metal was a better predictor for the toxicity of heavy metals (Zeng et al., 2009; Penttinen et al., 2011). The intracellular metal as a result of bioaccumulation is an explicit expression of a chemical's bioavailability and thus could be a better surrogate for dose at the site of toxic action and a more constant measure of toxicity than the conventionally used exposure concentration, which is sensitive to bioavailability-modifying factors (Meador et al., 2008).

The bioaccumulation is the outcome of several processes: uptake, distribution, storage, and excretion (Rainbow, 2002). Similar to the total metal concentration in solution, only a portion of the bioaccumulation of metal is biologically available for interaction with sites of toxic action.

The subcellular partitioning of metal in organism implied more significance to understand the metal toxicity. In this study, Cd concentration in the organelle and HDP fractions were well correlated with the relative root length (Table 3). Although the relationship was less than the intracellular Cd, the subcellular distribution of Cd could enough explain the toxicity of Cd in the presence of HA and CA.

4 Conclusions

We can conclude that CA and HA complexed with solution free Cd ions and decreased free Cd ion concentration. Subsequently, the uptake and toxicity of Cd to wheat were alleviated based on the total Cd concentration. The uptake of Cd also decreased in the presence of HA based on the FIAM. The presence of HA changed the relative subcellular distribution of Cd in root which could result in deceased toxicity to wheat based on the FIAM. In the presence of CA, the uptake and toxicity of Cd to wheat increased based on FIAM. The relative distribution of Cd had a decrease in the HDP fraction and increase in the MRG fraction in comparison to the control at the high level of Cd, which should generate detoxification according to the SPM. However, the toxicity of Cd in the presence of CA was increased due to the increased internalization of Cd. FIAM is not enough to predict the bioavailability and toxicity of Cd in the presence of organic acid. The internal accumulation of Cd and the subcellular Cd were better to describe the toxicity of Cd to wheat.

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