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EDTA Influences Time and Concentration Dependent Cadmium Uptake Characteristics of Indian Mustard

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Various chelates, including EDTA, have been used to increase the solubility of soil metals and have been reported to have significant effects on the phytoextraction of several heavy metals, especially Pb in Indian Mustard (Huang et al., 1997; Blaylock et al., 1997). Recent studies showing that EDTA and Pb are taken up by Indian Mustard and suggest that Pb is translocated in the plant as Pb-EDTA complex both in hydroponic systems (Vassil et al., 1998) and in soil (Epstein et al., 1999). However, in our previous study on EDTA induced Cd phytoextraction, we found the concentration of soluble Cd increased markedly in soil treated with EDTA whereas plant shoot uptake and total Cd uptake did not increase accordingly (Jiang et al., 2003). Similar effects were found on other nutrient elements such as Ca, Zn, Mg and Cu (Jiang et al., 2004). Thus, EDTA treatment did not give substantially increased Cd removal by the plants but rendered a larger fraction soil Cd vulnerable to loss processes with consequent potential environmental risk.

The apparent contradictions in the results of studies reported by different laboratories may be attributable to phytotoxicity both in soil and hydroponic systems (Vassil et al., 1998; Jiang et al., 2003). It was proposed from our previous pot experiment that Cd-EDTA complex was not ready to be taken up through root plasma membranes compared to free Cd ion while plant growing normally. Hydroponic experiments on the influence of EDTA on Cd uptake kinetics by Indian Mustard were carried out to confirm this idea.

MATERIALS AND METHODS

Before use, all containers and quartz sand were soaked in 15 % HNO_3 for 24 h and subsequently washed with distilled water. Additionally, the quartz sand was sterilized at 160 °C for 2 h. Seeds of Indian mustard (*Brassica juncea* L. Czern. Var. 426308) were soaked in distilled water at 25 °C for 12 h and germinated on moist filter for 48 h before they were transplanted into moist quartz sand. Hoagland nutrient solution was added every day. Briefly, the nutrient solution consisted of 5.0

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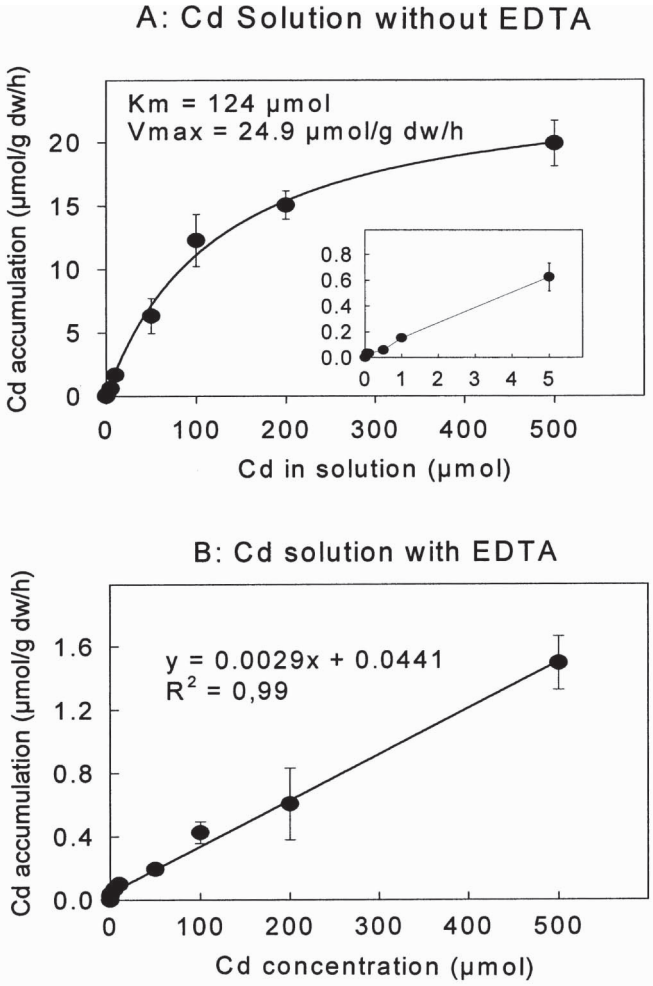

Figure 1. Kinetics of Cd uptake in roots of 40 day-old *B. juncea* seedlings. Seedlings were exposed to A, 0.05-500 µM Cd for 3h, and B, 0.05-500 µM Cd-EDTA for 3h, and subsequently washed in 1 mM $Ca(NO_3)_2$ for 15min. The data for the lower concentration range of A is presented as inlet. Bars: standard errors.

mM $Ca(NO_3)_2x4H_2O$, 5.0 mM KNO_3, 2.0mM $MgSO_4x7H_2O$, 1.0 mM KH_2PO_4, and trace elements (mg L^{-1}): B 0.5, Mn 0.5, Zn 0.05, Cu 0.02, Mo 0.01. 0.1 mM Fe was added as Fe-citrate solely. The plants were grown in glasshouse at 15/25 °C

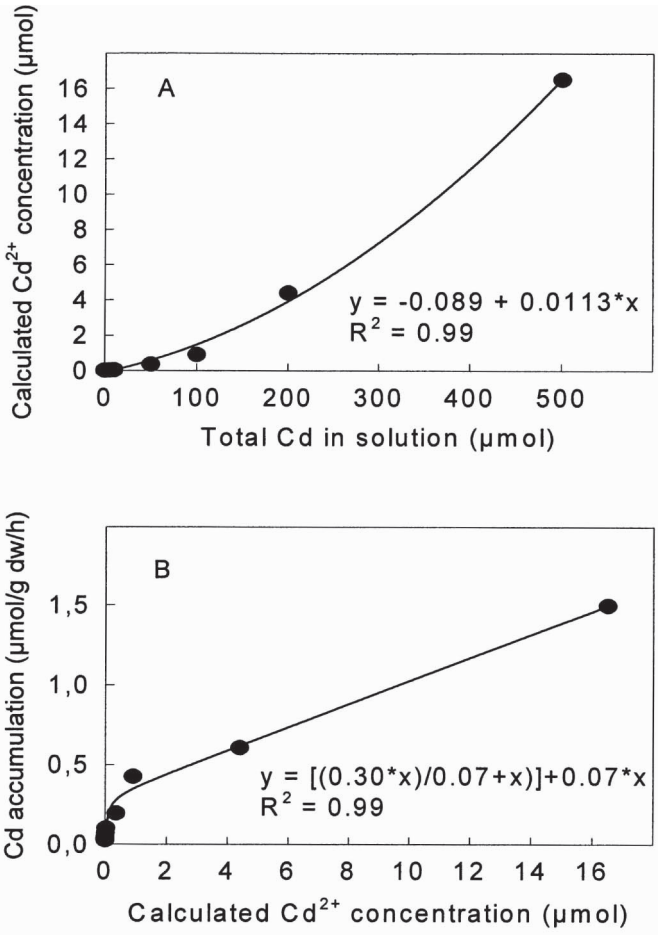

Figure 2. A, correlation between total Cd in solution and concentration of free Cd^{2+} calculated by MINEQL+. B, correlation between calculated concentration of free Cd^{2+} and Cd accumulation in *B. juncea* roots. The curve fit revealed a Michaelis Menten kinetics including a linear component. Bars: standard errors.

(night/day) for 40 d. Subsequently, the plants were taken out and quartz sand was washed off the roots by gentle rinsing with distilled water. The plants were transplanted into potteries (1.2 L), two per pot, with 1000 ml $Cd(NO_3)_2$ solution or Cd-EDTA solution of different concentration. The concentrations of Cd (µmol L^{-1})

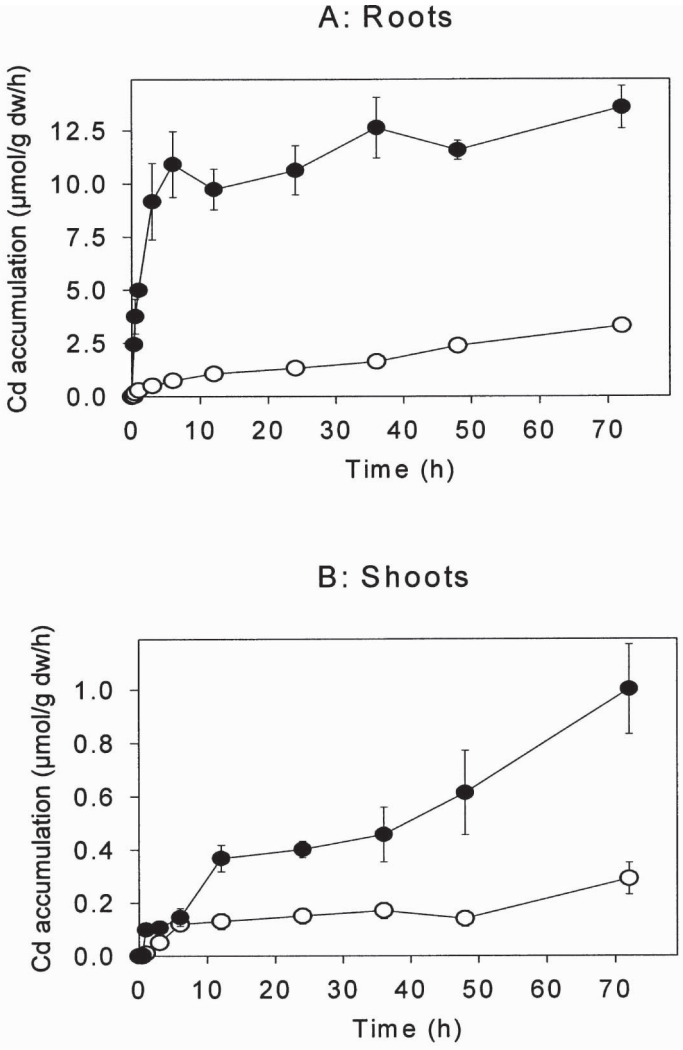

Figure 3. Cd accumulation in roots (A) and shoots (B) of 40-day-old *B. juncea* seedlings exposed to either 10 µM Cd $(NO_3)_2$ (black circles) or 10 µM Cd $(NO_3)_2$ + 10µM Na_2-EDTA (white circles) in distilled water for 72 h, and subsequently washed in 1mM $Ca(NO_3)_2$ for 15min. Bars: standard errors.

added as $Cd(NO_3)_2$ were 0.05, 0.1, 0.5, 1.0, 5.0, 10.0, 50.0, 100, 200, 500, respectively. 0.05 µM Cd-EDTA solution was gained by mixing 500 ml 0.1µM

$Cd(NO_3)_2$ with 500 ml 0.1 μM Na_2-EDTA solution, and other Cd-EDTA solutions of the same concentrations as $Cd(NO_3)_2$ respectively were gained in the same way. There were 20 treatments, each one in triplicate. The uptake experiment was set up in the greenhouse at 16 °C for 3 h, and then the plants were harvested. The seedlings were desorbed in 1.0 mmol L^{-1} $Ca(NO_3)_2$ solution for 15 min to remove the Cd sorbed on root cellular anionic binding sites (Salt et al., 1997). The shoots were cut at the shoot-root junction. Both shoots and roots were washed with distilled water, and dried at 105 °C for 2 h, then at 70 °C for 24 h. The oven-dried samples were ground and digested with reagent grade HNO_3-$HClO_4$, and analyzed for Cd by graphite furnace atomic absorption spectrometry (GFAAS, Hitachi Z-8200).

For the time-dependent dynamics experiment, materials and methods were the same as described above except that only one concentration of 10.0 μmol L^{-1} $Cd(NO_3)_2$ and 10.0 μmol L^{-1} Cd-EDTA solution was used. The treatment times were 0 min, 10 min, 30 min, 1 h, 3 h, 6 h, 24 h, 36 h, 48 h, and 72 h. There were twenty treatments (with and without EDTA treatment) and each one was triplicate. The speciation of ionic components in the solution including the concentration of free Cd^{2+} was calculated using MINEQL+ 4.0.

RESULTS AND DISCUSSION

Figure 1A displays the concentration dependent Cd accumulation in roots of *Brassica juncea*. Cd accumulation in roots follows a saturable curve that exhibits Michaelis-Menten enzyme kinetics. The calculation using SigmaPlot (Chicago, IL, USA) shows that when *B. juncea* seedlings were exposed to 0.05-500 μM Cd for 3 h, the saturable Cd^{2+} influx was characterized by a K_m of 124 μmol and a maximal influx (V_{max}) of 24.9 μmol g dw^{-1} h^{-1}. The inlet figure in Figure 1A shows the calculated Km and Vmax from 0.05 μmol L^{-1} to 5.0 μmol L^{-1}. Here, Vmax was 22.7 ± 17.1 μmol g dw^{-1} h^{-1} and Km was 3.5 ± 2.2 μmol.

Cadmium is not known to be an essential micronutrient to any plant (Marschner, 1995). Previous studies have shown great differences in Cd uptake among different plant species and even different cultivars. Several investigations into the kinetics of Cd^{2+} uptake have been conducted and saturation kinetics are often observed, with quite different Km and Vmax values (Cataldo et al., 1983; Costa and Morel, 1993;

Homma and Hirata, 1984; Mullins and Sommers, 1986). These results suggest the process of Cd uptake across the plasma membrane is a transport-mediated process. Further studies conducted by Salt et al. (1997) and Hart et al. (1998) show that the Cd uptake curve could be dissected into linear and saturable components. The

saturable component likely represented carrier-mediated Cd influx across root-cell plasma membranes, whereas linear Cd uptake represented cell wall binding of Cd.

We obtained the similar curve in this study (Figure 1). Application of equimolar EDTA to the Cd solution changed Cd uptake characteristics in roots significantly (Figure 1B). A linear correlation was observed between Cd uptake and the Cd concentration in solution ($y = 0.0029 x + 0.0441$; $n = 10$, $r = 0.99$) instead of saturable curve without EDTA (Figure 1A). Maximum Cd influx for 500 µmol Cd solution was 1.49 µmol g dw^{-1} h^{-1}, compared to 19.9 µmol g dw^{-1} h^{-1} for the non-EDTA treatment. The concentration of free Cd^{2+} in solution was calculated using MINEQL+. More than 99 % of Cd were present in complexed form as Cd-EDTA. The correlation between total Cd and free Cd^{2+} is presented in Figure 2A. The correlation between total Cd and free Cd^{2+} is not linear, but follows a polynomic curve. If Cd accumulation in *B. juncea* roots is correlated with the calculated free Cd^{2+} concentration, it is clearly shown that plant uptake follows the Michaelis Menten model including a linear component (Figure 2B). Figure 3A displays the time dependent Cd accumulation in roots of *Brassica juncea* from a 10 µM Cd solution and a 10 µM Cd + 10 µM EDTA solution. Without application of EDTA, Cd accumulation in roots follows a hyperbolic saturation curve with high uptake rates within the first 6 hours and with little further increase in the following 70 hours. Root Cd concentration increased from 0.03 µM g dw^{-1} to 10.9 µM g dw^{-1} after 6 hours and reached 13.6 µM g dw^{-1} after 72 hours. Application of equimolar EDTA to the Cd solution changes Cd uptake characteristics in roots significantly. Cd concentration increased linearly reaching 3.30 µM Cd g dw^{-1} after 72 hours.
Cd accumulation in shoots is presented in Fig 3B. As for the roots, Cd accumulation was significantly lower for EDTA-treated plants. Without EDTA, Cd concentration increased almost linearly reaching 1.00 µmol g dw^{-1} after 72 hours. For the treatments with EDTA, increase of Cd concentration was similar to the non-EDTA treatment for the first 30 minutes, but showing little further increase thereafter and just reaching 0.29 µmol g dw^{-1} after 72 hours.

Application of EDTA decreased Cd accumulation by *B. juncea* dramatically in the present experiments. This finding is shown by both concentration-dependent and time-dependent uptake experiments. The result is consisted with other reports that application of EDTA to the soil has been observed to reduce the uptake of Mn (Denduluri, 1994a), Pb (Denduluri, 1993; 1994b), Cd (Wolterbeek et al., 1988), Ni (Albasel and Cottenie, 1985) and Zn (Laurie et al., 1991) by plants. The results suggest that complexation of Cd in nutrient solution decreased the bioavailability. It is concluded that only free Cd^{2+} was available for plant uptake and only a small fraction of complexed Cd was splitted and could be taken up by the plant.

Complexed Cd could be splitted through the action of reducing agents released from the root (in the case of Fe and dicotyledonous plants), or through binding to receptors on the root membrane. This theory is the basis for the chelate-buffered hydroponic systems being used in plant nutrition research (Barber and Elgawhary, 1974; Chaney et al., 1989; Hodgson, 1968; Wallace and Wallace, 1992).

Our findings may have significance for the design of EDTA-induced phytoextraction or using EDTA in nutrient solution. Since EDTA may decrease the bioavailability of metals, availability of nutrients or pollutants might be affected in an unexpected and unwanted way. Cd uptake by Indian Mustard follows a saturable curve that exhibits Michaelis-Menten enzyme kinetics. Application of EDTA decreased Cd accumulation by *B. juncea* dramatically in both concentration-dependent and time-dependent uptake experiments. Phytotoxicity cannot be discounted during the process of the stimulation of Pb uptake and translocation by EDTA.

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