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Soil Cd availability to Indian mustard and environmental risk following EDTA addition to Cd-contaminated soil

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Abstract

A pot experiment was conducted to investigate the influence of EDTA on the extractability of Cd in the soil and uptake of Cd by Indian mustard (*Brassica juncea*). Twenty levels of soil Cd concentration ranging from 10 to 200 mg kg⁻¹ were produced by spiking aliquots of a clay loam paddy soil with Cd(NO₃)₂. One week before the plants were harvested EDTA was applied to pots in which the soil had been spiked with 20, 40, 60...200 mg Cd kg⁻¹. The EDTA was added at the rate calculated to complex with all of the Cd added at the 200 mg kg⁻¹ level. Control pots spiked with 10, 30, 50...190 mg Cd kg⁻¹ received no EDTA. The plants were harvested after 42 days' growth. Soil water- and NH₄NO₃-extractable Cd fractions increased rapidly following EDTA application. Root Cd concentrations decreased after EDTA application, but shoot concentrations increased when the soil Cd levels were >130 mg kg⁻¹ and Cd toxicity symptoms were observed. The increases in soil solution Cd induced by EDTA did not increase plant total Cd uptake but appeared to stimulate the translocation of the metal from roots to shoots when the plants appeared to be under Cd toxicity stress. The results are discussed in relation to the possible mechanisms by which EDTA may change the solubility and bioavailability of Cd in the soil and the potential for plant uptake and environmental risk due to leaching losses to groundwater.

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1. Introduction

Phytoextraction, the use of plants to remove toxic metals from soils, is emerging as a potentially cost-effective and environmentally sound remediation technique for metal-contaminated soils (McGrath, 1998), and there are two general strategies currently under investigation. The first is the use of hyperaccumulator plant species with exceptionally high metal-accumulating capacities but with relatively low yields ('natural

phytoextraction'). The second is the use of high yielding crop plants that can take up relatively large amounts of metals only when the mobility of the metals in the soil is elevated by the application of chemical agents ('chemically assisted phytoextraction'). 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 (Blaylock et al., 1997; Huang et al., 1997). Wallace et al. (1974) first suggested that the metal-EDTA complexes formed in the soil could increase metal solubility and promote diffusion, and thus increase the potential for plant uptake. However, Halvorson and Lindsay (1977) found that chelates in the soil lowered metal toxicity and uptake. EDTA was also reported to

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be a useful amendment for lowering the toxicity of Mn (Denduluri, 1994a) and Pb (Denduluri, 1993, 1994b) to okra grown in a soil irrigated with sewage. Moreover, application of EDTA to the soil has been observed to lead to decreases in the uptake of Cd (Wolterbeek et al., 1988), Ni (Albasel and Cottenie, 1985) and Zn (Laurie et al., 1991) by plants.

Some of the apparent contradictions in the results of studies reported by different laboratories may be attributable to phytotoxicity of metals or EDTA. In this paper we report a pot experiment in which the effects of EDTA applied to a clay loam soil on Cd uptake by Indian mustard were studied. Twenty levels of soil Cd ranging from 10 to 200 mg kg⁻¹ in 10-mg kg⁻¹ increments were produced by adding Cd(NO₃)₂ to triplicate aliquots of the soil prior to plant growth. EDTA was applied to the soil one week before the plants were harvested, and control pots were established with no EDTA treatment. The higher Cd levels were included to induce Cd phytotoxicity in an attempt to elucidate the potential role of metal toxicity in plant Cd uptake in EDTA-amended soil.

2. Materials and methods

A clay loam paddy soil (0–20 cm) developed from lacustrine sediments was air-dried and ground to pass through a 2-mm nylon mesh sieve. Selected properties of the soil were as follows: pH (H₂O) 7.40; organic matter 36.3 g kg⁻¹; total N 2.25 g kg⁻¹; total P 0.75 g kg⁻¹; total K 17.4 g kg⁻¹; total Cd 0.07 mg kg⁻¹; free Fe₂O₃ 16.2 g kg⁻¹; CEC 216 mmol kg⁻¹. Soil subsamples were mixed with appropriate amounts of analytical reagent (AR) grade solid Cd(NO₃)₂ in order to add 10–200 mg Cd kg⁻¹ of soil (oven dry basis) in increments of 10 mg kg⁻¹, resulting in 20 soil Cd levels. Basal fertiliser was applied as AR KH₂PO₄ to give 80 mg P and 100 mg K kg⁻¹. Nitrogen was added as NH₄NO₃ to give the same N application rate to each treatment. The metal and fertiliser salts were thoroughly mixed with the soil. The mixture was then adjusted with distilled water to 60% of water holding capacity (WHC) and maintained at this moisture content for two days. The moistened subsamples were mixed thoroughly again and aliquots (equivalent to 350 g oven dry basis and containing ~80 g of added water to reach 60% WHC) were transferred to acid-washed plastic pots and sown with seeds of *Brassica juncea*. After emergence, the seedlings were thinned to two per pot. There were three replicates of each treatment in a randomised block design. During the period of plant growth, all pots were weighed and adjusted daily to 60% WHC with distilled water. The plants were grown in a glasshouse for 42 days. One week before harvest, half of the Cd treatments (20, 40, 60... 200 mg Cd kg⁻¹) were supplemented with 25 ml 1.78

mmol l⁻¹ EDTA from the base of each pot. The application rate of EDTA used was calculated to be the stoichiometrically exact amount to complex with the Cd in the soil spiked with 200 mg Cd kg⁻¹, the same as was used in previously reported experiments (Luo et al., 1999; Wu et al., 2000). Alternate Cd treatments (10, 30, 50...190 mg Cd kg⁻¹) were controls that received no EDTA. At harvest, the shoots were cut at the shoot–root junction. Both shoots and roots were washed with distilled water and dried at 70 °C for 48 h. The oven-dried samples were weighed, ground and subsamples digested with HNO₃–HClO₄. The resulting solutions were analysed for Cd concentration by atomic absorption spectrophotometry with a graphite furnace (GFAAS) using a model Z-8200 spectrophotometer.

Soil samples were collected from the pots after harvest and analysed for: (1) water-extractable Cd: distilled water was added to give a 1:1 (w/v) soil:solution ratio, the suspension was shaken at 120 rpm for 1 h and centrifuged at 3500 rpm for 10 min, then the supernatant was filtered through Whatman No. 41 filter paper and passed through a 0.45 µm membrane filter under negative pressure (1 bar); (2) NH₄NO₃-extractable Cd: 1 M NH₄NO₃ was added to give a 1:2.5 (w/v) soil:solution ratio, the suspension was shaken at 120 rpm for 1 h and the supernatant was filtered through Whatman No. 41 filter paper; and (3) EDTA-extractable Cd: EDTA (0.05 M, pH 7.0) was added to give a 1:5 (w/v) soil:solution ratio, the suspension was shaken at 120 rpm for 1 h and the supernatant was filtered through Whatman No. 41 filter paper. All the filtrates were analysed for Cd by GFAAS. The results are presented as the mean values of three replicates of each treatment together with the standard errors of the means (SEM).

3. Results

3.1. Influence of EDTA on plant growth

Fig. 1 shows the influence of EDTA treatment one week before harvest on shoot and root dry matter yields of plants grown in pots for 42 days. Both root and shoot yields were lower in EDTA treated pots with soil Cd concentrations of up to about 100 mg kg⁻¹, and the effect was more pronounced on the shoots. At soil Cd concentrations above about 100 mg kg⁻¹ there were no differences between yields of EDTA treated and control plants, and root and shoot yields of all plants declined with increasing soil Cd concentration.

3.2. Influence of EDTA on soil Cd extractability

Fig. 2 shows the influence of EDTA application on soil Cd extractability immediately after harvest. The

Fig. 1. Influence of EDTA application to the soil one week before harvest on shoot and root yields of Indian mustard after 42 days' growth. Bars: SEM.

Fig. 2. Influence of EDTA applied to the soil one week before harvest of Indian mustard on (A) H_2O -extractable; (B) NH_4NO_3 -extractable; and (C) EDTA-extractable Cd in the soil immediately after harvest. Symbols: ($\blacktriangle$) control; ($\square$) EDTA added. Bars: SEM.

concentration of water-extractable Cd increased sharply with increasing Cd application rate in the presence of EDTA but remained very low ($<0.2 \text{ mg kg}^{-1}$) in the unamended controls. The concentration of NH_4NO_3 -extractable Cd also increased with increasing Cd application rate when EDTA was present and the controls showed a negligible increase at higher Cd application rates. In contrast, the increase in EDTA-extractable Cd with increasing Cd application rate occurred equally in both EDTA-treated and unamended control pots.

3.3. Influence of EDTA on plant Cd concentration and uptake

The effects of EDTA on plant shoot and root Cd concentrations are shown in Fig. 3. There were upward trends in Cd concentration in both shoots and roots with increasing soil Cd level. However, root Cd concentrations were consistently higher in the controls than in the EDTA-treated pots, but shoot concentrations were consistently lower in the controls.

Shoot Cd concentrations increased dramatically in EDTA-treated pots between 150 and 200 mg kg^{-1} Cd application rates, and the differences between these and the controls widened accordingly. Root and shoot Cd uptake values followed similar trends to the Cd concentrations in the plants (Fig. 4). Plant (root + shoot) Cd uptake by *B. juncea* was very similar in EDTA-treated and control soils with added Cd concentrations below about 150 mg kg^{-1} . Above this concentration of soil Cd, EDTA-treated pots showed a sharp increase in total plant Cd uptake with increasing Cd application rate.

4. Discussion

Although differences were observed in root and shoot yields between EDTA-treated pots and controls, the differences were small and had little influence on the amounts of Cd taken up by the roots and shoots. However, the sharp decline in shoot yield of EDTA-treated plants at higher soil Cd levels together with the

Fig. 3. Influence of EDTA applied to the soil one week before harvest on Cd concentrations in the roots and shoots of Indian mustard after growth for 42 days. Bars: SEM.

Fig. 4. Influence of EDTA application to the soil one week before harvest on Cd uptake by shoots and roots of Indian mustard after 42 days' growth. Bars: SEM.

occurrence of visual symptoms indicates that Cd phytotoxicity occurred at the higher Cd application rates. The experiment could have been better designed for studying the effects of EDTA by adding the Cd in 20 mg kg^{-1} increments to give six replicate pots, three for EDTA treatment and three controls, at each soil Cd level. The pairs of mean values could then have been compared by a statistical method such as the *t*-test or least significant difference. Nevertheless, the design adopted does allow comparison between trends in the data from the EDTA-treated and control pots.

The concentrations of both H_2O - and NH_4NO_3 -extractable Cd increased markedly in soil treated with EDTA (Fig. 2). However, plant shoot uptake and total Cd uptake did not increase accordingly, especially at low soil Cd concentrations (Fig. 4). The results indicate that Cd absorbed by *B. juncea* was not directly related to the Cd concentration in the soil solution. However, over the Cd concentration of 150 mg kg^{-1} , EDTA addition resulted in a decrease in plant yield (Fig. 1) and an increase in shoot Cd concentration (Fig. 3). Salt et al.

(1997) reported that the roots of *B. juncea* had a greater capacity to accumulate Cd, both intracellularly and adsorbed to the cell walls, than the shoots. This may explain, at least in part, the observed decrease in Cd accumulation in the treatments receiving EDTA. A contributing factor may have been desorption of Cd from cell walls by EDTA, and because of the large amount of Cd adsorbed to the cell walls the total amount of Cd taken up by the plants increased slightly, but did not correspond to the increased concentration of Cd in the soil solution. Thus, EDTA may have stripped non-specifically bound Cd from root cell walls, making it appear that the roots took up less Cd. In addition, added EDTA is also likely to have chelated soil-bound Cd, and the EDTA–Cd complex would then have been easily extracted by water from the soil samples, but would not have been taken up readily through root plasma membranes, particularly at low concentrations. When our soil water-extractable and NH_4NO_3 -extractable Cd values were compared with shoot Cd uptake by

linear correlation, they were both highly correlated with coefficients (r) of 0.931 ($P < 0.001$) and 0.958 ($P < 0.001$) respectively. Thus, both extractants (and especially NH_4NO_3) were very good indicators of plant-available Cd.

The occurrence of some stimulation of Cd translocation from roots to shoots by EDTA when Cd levels were high enough to induce phytotoxicity cannot be discounted. One possible mechanism, suggested by Vassil et al. (1998) for Pb, is that some of the soil-bound Cd was chelated by EDTA in the soil, as discussed above. Some of the soluble Cd–EDTA complex in the soil solution would then have been transported through the plant via the xylem to the shoots.

Indian mustard did not show any evidence of Cd hyperaccumulation in this experiment, with maximum shoot concentrations of $<400 \text{ mg kg}^{-1}$, even with EDTA application to the soil. Furthermore, EDTA treatment did not substantially enhance shoot Cd uptake compared with unamended controls, although some enhancement of translocation from roots to shoots may have occurred at higher soil Cd levels at which Cd phytotoxicity (and lower shoot yields) occurred. Since soil water-extractable Cd increased at least 400-fold following EDTA application, a more important concern may be alternative pathways for the soil Cd fraction rendered soluble by EDTA. In the field, dramatic increases in soil solution Cd could result in contamination of groundwater with consequent environmental impacts. Thus, the practical use of chelates for enhancement of metal removal in phytoremediation strategies may require careful site-specific evaluation to minimise the risk of serious secondary environmental contamination.

5. Conclusions

Indian mustard did not exhibit hyperaccumulation of Cd under the conditions of this experiment, although the maximum shoot uptake values obtained ($>300 \text{ mg kg}^{-1}$) could be useful in Cd removal by bioavailable element stripping in which the objective is to remove the bioavailable metal fraction. However, these uptake values were achieved at the higher soil Cd levels investigated, at which there was also evidence of Cd phytotoxicity. EDTA addition to the soil did not increase plant Cd uptake, but appeared to stimulate translocation of Cd from roots to shoots. Some of this apparent stimulation of translocation may have been due to non-specific stripping of Cd from root cell walls. EDTA application led to substantial increases in soluble Cd in the soil. 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.

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