POT EXPERIMENT TO STUDY THE UPTAKE OF CD AND PB BY THREE INDIAN MUSTARDS (*BRASSICA JUNCEA*) GROWN IN ARTIFICIALLY CONTAMINATED SOILS

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*Most of the metals-contaminated and fallow lands in Taiwan are a result of irrigation with illegal effluent of factories. Phytoextraction methods can be applied to reach the target of fallow-lands reuse and earn more incomes for farmers. In many studies, Indian mustards (*Brassica juncea*) were planted in the metal-contaminated soils to study their suitability in phytoextraction. However, the total removal of metals by plants was quite different between accessions. In this pot study, three accessions of *B. juncea* (cv. 182921, cv. 211000, and cv. 426308) were planted in artificially Cd- or Pb-contaminated soils to investigate the differences between them. EDTA was applied to study its effect in increasing the bioavailability of Cd and Pb and their uptake by these Indian mustards. Experimental result showed that three accessions of Indian mustard can accumulate a high concentration of Cd and Pb when growing in the artificially Cd- and Pb-contaminated soils. Their shoot Cd or Pb concentrations were significantly enhanced, resulting from the application of EDTA. Among the three accessions, *B. juncea* cv. 211000 accumulated the highest concentrations of Cd and Pb in their shoots compared with *B. juncea* cv. 182921 and cv. 426308, but its total removal was the lowest due to its lower biomass.*

KEY WORDS: metals-contaminated soils, phytoextraction, Indian mustard, Cd, Pb

INTRODUCTION

Phytoextraction is a soil cleanup technology that uses the ability of metal-accumulating plants to extract metals from contaminated soil with their roots and to concentrate these metals in above-ground plant parts (Chaney *et al.*, 1997; Lai and Chen, 2004, 2005, 2006, 2007; Salt *et al.*, 1995). The metal-accumulating plant material can be safely harvested and removed from the site without the extensive excavation, disposal costs, and loss of topsoil associated with traditional remediation practices. The success of phytoextraction is dependent on several factors, including the availability of metals in the soil to plant. Several workers using chemical agents (such as EDTA, EDDS, NTA, and

CA, etc.) to enhance the availability of metals and subsequently their uptake by the plants are called induced phytoremediation (Huang *et al.*, 1997; Lai and Chen, 2004, 2005, 2006, 2007; Tandy, Schulin, and Nowack, 2006). These agents significantly increase the uptake of metals and thus decrease the period needed for remediation. Some of the chelating agents (*i.e.*, EDTA and HEDTA) were resisted the decomposition by organisms or microorganism. For example, results of previous studies have shown that the EDTA-metal complexes can exist in water-soluble forms for many months (Lombi *et al.*, 2001; Means, Kucak, and Crerar, 1980; Meers *et al.*, 2005). Although these complexes can be adsorbed by soil particles, soils that have a sandy texture or a groundwater table that has a low depth may have a high risk of groundwater contamination.

Several species of garden flowers were planted in the metals-contaminated soils of northern Taiwan approximately 20 years ago to assess the phytoextraction capacity of marketable plants (Chen and Lee, 1997). Researchers found that some of these garden flowers could accumulate a high concentration of metals in their shoots without showing any withered symptoms. When rainbow pink (*Dianthus chinensis*), one of the garden flowers used, was grown in a Cd-contaminated site in northern Taiwan for a period of 5 weeks. These plants accumulated 115 mg kg⁻¹ of Cd in their shoots, which is a 73.7-fold increases in comparison to conditions before planting. Cadmium concentration in the shoots of rainbow pink can reach the threshold (100 mg Cd kg⁻¹) of a Cd hyperaccumulator proposed by Baker *et al.* (2000). From the result an in-situ experiment, rainbow pink was demonstrated and used for the phytoextraction of Cd-contaminated soil in Taiwan. Due to the low biomass of this hyperaccumulator compared with normal plants, some studies used high-biomass plants to increase the uptake of metals (Ebbs *et al.*, 1997; Huang *et al.*, 1997; Tandy *et al.*, 2006; Wu, Hsu, and Cunningham, 1999). These plants include corns, sunflowers, and Indian mustards. Among these three plants used in previous studies, Indian mustards could accumulate high Cd concentration in their shoots when growing in the artificially Cd-contaminated soils (Dushenkov *et al.*, 1995; Ebbs *et al.*, 1997; Guo and Marschner, 1995; Kumar *et al.*, 1995). The uptake capacity of Cd of different accessions of Indian mustards was not identical even when grown in the same metal-contaminated soil. However, only one accession of Indian mustards was used in each study to investigate their uptake of metals in enhanced phytoextraction (Irtelli and Navari-Izzo, 2006; Jiang *et al.*, 2003; Quartacci *et al.*, 2006; Van Engelen *et al.*, 2007; Vassil *et al.*, 1998; Wu *et al.*, 2004).

The objective of this study was to compare the differences in phytoextraction capacity between three accessions of *B. juncea* (cv. 182921, cv. 211000, and cv. 426308) grown on artificially Cd- or Pb-contaminated soils in Taiwan. Emphasis was also put on the accumulation of metals and its translocation pattern in plants with and without the application of EDTA.

MATERIALS AND METHODS

Soil Characteristics

The Cd-contaminated soils (0–20 cm) used in this pot experiment were collected from the northern part of Taiwan, and the contamination was due to the continuous irrigation of metals-contaminated wastewater. The samples were air dried, ground, passed through a 2-mm sieve, and stored in plastic vessel for further analysis. The pH was measured using glass electrodes in a water suspension with a soil-to-water ratio of 1:1 (McLean, 1982).

The soil's particle-size distribution was measured using a pipette method (Gee and Bauder, 1986). Organic carbon was determined by using the Walkley–Black wet combustion method (Nelson and Sommers, 1982) and the cation exchange capacity (CEC) was measured using the ammonium acetate method (Rhoades, 1982). For total metals analysis, soil was digested in aqua regia. Digested samples were filtered with Whatman filter papers (No. 42, Whatman International Ltd., England) and analyzed with an atomic absorption spectrometer (AAS) (Hitachi 180-30 type Hitachi Ltd., USA) for metals analysis (EPA/ROC, 2002).

Phytoextraction Capacity of Indian Mustards

Soils used for the pot experiment to study the phytoextraction capacity of three Indian mustards were sampled from the same Cd-contaminated site as described in the soil characteristics section and artificially added with solutions of $\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ or $\text{Pb}(\text{NO}_3)_2$ to make the total concentration 25 and 50 mg Cd kg^{-1} (coded as Cd 25 and Cd 50) or 500 and 1,000 mg Pb kg^{-1} (coded as Pb 500 and Pb 1000), respectively (as individual element concentration). Each pot (16 cm in diameter and 19 cm in height) contained 3 kg (dry weight) of artificially Cd- or Pb-contaminated soils and then 15 seeds for each *B. juncea* (cv. 182921, cv. 211000, and cv. 426308) taken from the United States Department of Agriculture Northern Central Regional Plant Introduction Station were sown in each pot. Two rhizosphere soil moisture samplers (RSMS; Eijkelpamp Agrisearch Equipment Comp., The Netherlands) were put in each pot at a depth of approximately 10 cm below the soil surface. The pot experiment was conducted in the phytotron (day/night = 30/25°C) with three replicates and kept with soil's water content at 60% of the water-holding capacity (WHC) by adding deionized water every 2 d. After germination, we kept five seedlings in each pot and removed surplus seedlings because the diameter of each pot is only 16 cm. After growing for 35 d, the shoots of plants were harvested, rinsed with tap water and deionized water, oven dried at 60°C for 72 h, ground into <0.5 mm, digested with the $\text{H}_2\text{SO}_4/\text{H}_2\text{O}_2$ method (Harmon and Lajtha, 1999), and then used to determined for the Cd and Pb concentrations with an AAS (Hitachi 180-30 type).

In another experiment, 15 seeds of the same *B. juncea* were sown in the pot, in soils that were artificially contaminated with Cd- or Pb as described above. The pot experiment was conducted in the phytotron (day/night = 30/25°C) with three replicates and at 60% of WHC by adding deionized water every 2 d. After germination, five seedlings were kept in each pot and the surplus seedlings were removed. Solutions of 5 mmol EDTA kg^{-1} soil were applied to each pot after growing for 28 d. The shoots of plants were harvested at day 7 after applying EDTA and then treated with the same procedures as described above to determine the Cd or Pb in the shoots of plants. Soil solutions before seeding and at days 7, 14, 21, 28, and 35 after seeding were sampled with RSMS and the Cd and Pb concentrations were determined with an ICP/OES (Perkin Elmer 2000DV, Perkin Elmer, Inc., USA).

Statistics

The variance and significance of the differences among concentrations of Cd and Pb in the soil solutions and in the plants were analyzed by analysis of variance. Statistical significance was defined as the level of $p = 0.05$.

RESULTS AND DISCUSSION

Soil Characteristics

The soil texture was silty clay with a moderate CEC of $13.9 \text{ cmol}_{(+)} \text{ kg}^{-1}$ soil, its pH was 4.9 and the organic carbon was 16.9 g kg^{-1} . The initial un-contaminated total metal concentrations (mg kg^{-1}) in the contaminated soil were 2.58 ± 0.08 (Cd), 80.0 ± 5.3 (Zn), and 31.3 ± 2.1 (Pb), respectively.

Phytoextraction Capacity of Indian Mustards

Tables 1 and 2 show the concentrations of Cd and Pb in the soil solution or in soil extracted with different extractants in Cd- or Pb-contaminated soils. For these soils, 0.05 M EDTA and 0.005 M DTPA could extract approximately 55–96% and 65–90% of the total Cd and Pb concentration determined with aqua regia method. Those fractions of Cd and Pb in soil extracted by EDTA and DTPA were available for plant uptake (Kabata-Pendias and Pendias, 2001). The results revealed that these two extracts may have a strong capacity for extracting the metals in artificially contaminated soils or that most of the Cd and Pb in the study soil were in the labile fractions.

After growing in the initial soil (control treatment of Cd) for 35 d, the shoot Cd concentrations in three Indian mustards ranged from 38 to 44 mg kg^{-1} (Figure 1) with transfer coefficients (TC; metal concentration ratio of shoot/soil) of 12–14. Results showed that after growing in the artificially Cd-contaminated soils (Cd 25, 50) for 35 d, different Indian mustards could accumulate 120–210 mg Cd kg^{-1} in their shoots and that there was no significant difference between three accessions (Figure 1). However, their TC value decreased from 12–14 (initial soil) to 3.4–6.2 (Cd 25, 50). The accumulated concentration of Cd in Indian mustards was approximately 4- to 6-fold higher than earlier reported by Wu

Table 1 Cd concentration in soil solution and in soil digested with aqua regia, or extracted by 0.05 M EDTA or 0.005 M DTPA after planting Indian mustards for 35 d

Treatment	Soil solution (mg L ⁻¹)	Analytic methods		
		mg kg ⁻¹		
		Aqua regia	0.05 M EDTA	0.005 M DTPA
<i>B. juncea</i> cv. 182921				
Control	0.13 ± 0.03	3.18 ± 0.29	1.98 ± 0.02	1.72 ± 0.02
Cd 25	0.75 ± 0.21	25.0 ± 1.0	22.7 ± 0.9	20.2 ± 1.0
Cd 50	0.98 ± 0.10	49.0 ± 2.5	46.9 ± 3.2	41.3 ± 3.7
<i>B. juncea</i> cv. 211000				
Control	0.10 ± 0.02	3.24 ± 0.18	2.04 ± 0.44	1.76 ± 0.03
Cd 25	0.77 ± 0.09	27.1 ± 0.6	24.3 ± 0.5	21.7 ± 0.4
Cd 50	0.92 ± 0.30	49.4 ± 0.6	44.0 ± 2.1	40.2 ± 0.6
<i>B. juncea</i> cv. 426308				
Control	0.07 ± 0.02	3.18 ± 0.14	2.22 ± 0.05	1.42 ± 0.03
Cd 25	0.92 ± 0.69	28.4 ± 1.3	19.6 ± 5.8	17.5 ± 0.6
Cd 50	1.16 ± 1.09	51.8 ± 3.7	28.3 ± 2.7	36.1 ± 3.4

#:mean $\pm$ standard deviation ($n = 3$).

Table 2 Pb concentration in soil solution and in soil digested with aqua regia, or extracted by 0.05 M EDTA or 0.005 M DTPA after planting Indian mustards for 35 d

Treatment	Soil solution (mg L ⁻¹)	Analytic methods		
		mg kg ⁻¹		
		Aqua regia	0.05 M EDTA	0.005 M DTPA
<i>B. juncea</i> cv. 182921				
Control	0.02 ± 0.01 [#]	38.7 ± 2.0	9.13 ± 0.17	6.55 ± 0.07
Pb 500	1.35 ± 0.33	604 ± 5	529 ± 14	443 ± 11
Pb 1000	2.32 ± 0.44	1159 ± 34	1033 ± 20	888 ± 8
<i>B. juncea</i> cv. 211000				
Control	0.02 ± 0.01	35.5 ± 2.4	10.3 ± 1.3	6.54 ± 1.90
Pb 500	1.58 ± 0.30	612 ± 7	537 ± 29	466 ± 28
Pb 1000	3.26 ± 0.93	1197 ± 12	1072 ± 9	827 ± 60
<i>B. juncea</i> cv. 426308				
Control	0.03 ± 0.01	35.0 ± 1.4	10.2 ± 0.4	5.74 ± 0.30
Pb 500	1.06 ± 0.23	604 ± 10	537 ± 11	410 ± 16
Pb 1000	3.04 ± 1.67	1249 ± 1	1000 ± 200	807 ± 175

[#]: mean ± standard deviation (*n* = 3).

et al. (2004) and can reach the threshold (100 mg kg⁻¹) of a Cd hyperaccumulator (Baker *et al.*, 2000). Figure 2 shows the accumulated concentration of Pb in the shoots of three Indian mustards after growing in the artificially Pb-treated soils (Pb 500, 1000) for 35 d. In the initial soil without artificially spiked Pb, the Pb concentration in soil solutions was low

Figure 1 Shoot Cd concentration of three accessions of Indian mustard, which were harvested at day 35 after planting in the artificially Cd-contaminated soils. The probability level of significant difference is at *p* = 0.05. Replicates (*n*) = 3.

Figure 2 Shoot Pb concentration of three accessions of Indian mustard, which were harvested at day 35 after planting in the artificially Pb-contaminated soils. The probability level of significant difference is at $p = 0.05$. Replicates (n) = 3.

relative to the total concentration in soil. Both of the 0.05 M EDTA and 0.005 M DTPA extractable Pb concentration were $< 30\%$ of the total Pb concentration as determined by using the aqua regia method. Lead concentration in the shoots of Indian mustards was $< 20 \text{ mg kg}^{-1}$ with TC values of 0.2–0.6 due to its low availability in the initial soil. After planting in Pb 500 and Pb 1000 for 35 d, these Indian mustards could accumulate 45–210 mg Pb kg^{-1} in their shoots and *B. juncea* cv. 211000 had the highest Pb concentration ($p < 0.05$) in their shoots among these three accessions (Figure 2). However, their TC values were all < 0.2 due to its low concentration in soil solution relative to the total concentration in artificially Pb-contaminated soil.

There are good relationships between the Cd or Pb concentrations in soil solutions and those in the shoots of Indian mustards. The correlation coefficient (r) between the concentrations of Cd and Pb in the soil solutions and those in the shoots of Indian mustards was 0.77–0.82 ($p < 0.001$) and 0.92–0.93 ($p < 0.001$), respectively. There are good relationships between the Cd or Pb concentrations in soils extracted by 0.05 M EDTA or 0.005 M DTPA and those accumulated in the shoots of Indian mustards. The correlation coefficient (r) of shoot Cd versus 0.05 M EDTA and 0.005 M DTPA extractable Cd was in the range of 0.87–0.94 and 0.90–0.94 ($p < 0.001$). The correlation coefficient (r) of shoot Pb versus 0.05 M EDTA and 0.005 M DTPA extractable Pb was in the range of 0.88–0.95 and 0.88–0.94 ($p < 0.001$). The result of this study shows that soil solution, 0.05 M EDTA, and 0.005 M DTPA were suitable methods for predicting the accumulated Cd or Pb concentration in the shoots of Indian mustards.

EDTA-Enhanced Phytoextraction of Indian Mustards

The concentrations of Cd (Figure 3) and Pb (Figure 4) in the soil solutions of artificially Cd- or Pb-contaminated soil were significantly ($p < 0.05$) increased with the

Figure 3 Change of Cd concentrations in soil solutions of artificially Cd-contaminated soils. Three accessions of Indian mustard were harvested at day 7 after applying 5 mmol EDTA kg⁻¹ soil. Replicates (n) = 3.

application of 5 mmol EDTA kg⁻¹ soil. The application of EDTA significantly ($p < 0.05$) increased the Cd concentrations (mg L⁻¹) in the soil solutions of both the treatments from 1.2–1.5 and 3.3–4.5 to 14–24 and 28–37 (the increase was approximately 6.5- to 17-fold). Lead concentrations in the soil solutions were also found to be significantly ($p < 0.05$) increased in both the treatment of Pb (500 and 1000) from 1.6–2.1 and 4.9–5.2 to 480–580 and 570–880 (the increase was approximately 110- to 320-fold). Several reports are in

Figure 4 Change of Pb concentrations in soil solutions of artificially Pb-contaminated soils. Three accessions of Indian mustard were harvested at day 7 after applying 5 mmol EDTA kg⁻¹ soil. Replicates (n) = 3.

agreement with findings in this study (Huang *et al.*, 1997; Lai and Chen, 2004, 2005, 2006, 2007). The enhancement effect due to applying EDTA on Pb concentration in the soil solution was better than Cd due to the greater formation constant of EDTA-Pb ($\log K_f = 19.0$) relative to EDTA-Cd ($\log K_f = 17.4$). However, the high Cd and Pb concentrations in soil solutions resulted from the application of 5 mmol EDTA kg⁻¹ soil, which have a high potential for groundwater contamination (Jiang *et al.*, 2003; Lai and Chen, 2007).

Figure 5 Comparisons of Cd concentrations in the shoot of three accessions of Indian mustard, which were harvested at day 7 after applying 5 mmol EDTA kg⁻¹ soil. The probability level of significant difference is at $p = 0.05$. Replicates (n) = 3.

The application of 5 mmol EDTA kg⁻¹ soil significantly increased the Cd concentration in the shoots of *B. juncea* cv. 211000 (Figure 5) ($p < 0.05$). Their shoot Cd concentrations, when grown in Cd 25 and Cd 50, were significantly increased from

Figure 6 Comparisons of the Pb concentrations in the shoots of three accessions of Indian mustard, which were harvested at day 7 after applying 5 mmol EDTA kg⁻¹ soil. The probability level of significant difference is at $p = 0.05$. Replicates (n) = 3.

approximately 120–170 (without EDTA) to 200–330 mg kg⁻¹ after applying EDTA ($p < 0.05$) (Figure 5b). Because the Pb concentration in the soil solution was significantly increased after applying EDTA (Figure 4), the concentration accumulated in the shoots of three accessions of Indian mustard also significantly ($p < 0.05$) increased from 40–210

Figure 7 Comparisons of the biomass of three accessions of Indian mustard, which were grown in the artificially Cd-contaminated soils and harvested at day 7 after applying 5 mmol EDTA kg⁻¹ soil. The probability level of significant difference is at $p = 0.05$. Replicates (n) = 3.

(without EDTA) to 200–710 mg kg⁻¹ (in the EDTA treatment) (Figure 6). Compared with the other two accessions of Indian mustard, *B. juncea* cv. 211000 had higher uptake for Pb when grown in the Pb 500 and Pb 1000 with or without EDTA applied. However, it had lower biomass relative to *B. juncea* cv. 182921 and cv. 426308 (Figures 7 and 8) and thus had lower total removal of Cd and Pb when grown in the artificially Cd- or

Figure 8 Comparisons of the biomass of three accessions of Indian mustard, which were grown in the artificially Pb-contaminated soils and harvested at day 7 after applying 5 mmol EDTA kg⁻¹ soil. The probability level of significant difference is at $p = 0.05$. Replicates (n) = 3.

Pb-contaminated soils with or without EDTA applied. For most of the Indian mustards grown in the artificially Cd- or Pb-contaminated soils, the effect of applying 5 mmol EDTA kg⁻¹ soil on their biomass was not identical (Figures 7 and 8). EDTA seems to have had an adverse effect on the shoot biomass of Indian mustards grown in the Cd-contaminated soils, but the effect was not significant (Figure 7). The adverse effect of EDTA on the growth

of Indian mustards was also reported by previous studies (Jiang *et al.*, 2003; Van Engelen *et al.*, 2007).

EDTA-induced phytoextraction of three accessions of Indian mustards showed that the Cd and Pb concentrations in the soil solutions were significantly ($p < 0.05$) increased after applying 5 mmol of EDTA kg⁻¹ soil, especially for Pb. Although no leaching experiment was conducted in this study, the results of many researches have shown that the highly soluble concentration of metals after applying EDTA had the potential risk of impacting groundwater quality in sandy textural soils (Jiang *et al.*, 2003; Lai and Chen, 2007; Van Engelen *et al.*, 2007; Wu *et al.*, 2004). Biodegradable chelating agents, EDDS, were recommended by many researchers to be a substitute for EDTA (Luo *et al.*, 2006; Meers *et al.*, 2005; Tandy *et al.*, 2006). The increase of available fractions of Cd and Pb in soils after applying EDTA resulted in a significantly increased uptake in Indian mustards. Among the three accessions of Indian mustard used in this study, *B. juncea* cv. 211000 could accumulate higher concentration of Pb after growing in the artificially Pb-contaminated soils (500 and 1,000 mg kg⁻¹) for 35 d with or without applying EDTA as compared with *B. juncea* cv. 182921 and cv. 426308. However, its total removal of Pb was the lowest due to their lowest biomass among the three accessions.

CONCLUSION

In our opinion, the accumulated metals concentration in shoots, growing conditions, and their economic values are three major factors for selecting the plants used to phytoextract metals-contaminated soils in Taiwan. Results of a pot experiment showed that three accessions of Indian mustard can accumulate high concentration of Cd in their shoots without any injury. Among the three accessions of Indian mustard, *B. juncea* cv. 182921 and cv. 426308 removed higher amounts of Cd and Pb as compared with *B. juncea* cv. 211000 after growing in the artificially Cd- and Pb-contaminated soils for 35 d. They can be sown into the Cd-contaminated soils and then harvested by machine after planting for 1–2 mo. Overall, experimental results showed that three accessions of Indian mustard are suitable for phytoextraction of Cd-contaminated soils in Taiwan although their uptake capacity of Cd and Pb was different. The application of EDTA can significantly increase the metal concentration in soil solution of artificially metal-contaminated soils. However, results of previous studies showed that the high risk of groundwater contamination after applying EDTA was an unsolved problem due to the persistence of EDTA against the biodegradation of microorganism. Natural phytoextraction without applying chemical agents is a better way to treat of metal-contaminated soils as compared with EDTA-enhanced phytoextraction.

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