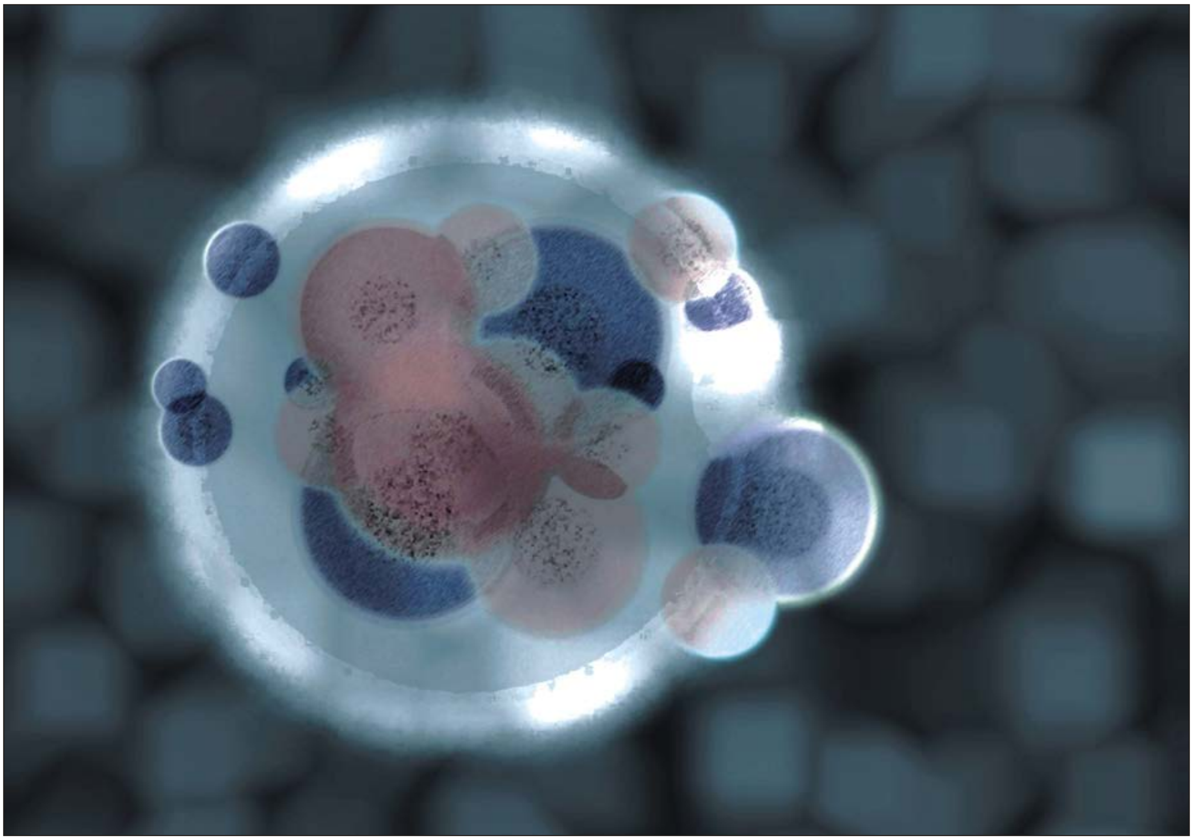

Journal of Biological Sciences

ISSN 1727-3048

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Journal of Biological Sciences 7 (4): 681-684, 2007
ISSN 1727-3048

Enhanced Uptake of Cadmium by Native Plant (*Artemisia princeps* var. *orientalis*) Using Ethylenediaminetetraacetic Acid

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Abstract: Phytoextraction using plants to clean up metals from the environment is an emerging technology for contaminated land restoration. Efficiency of phytoextraction is controlled by metal availability in the soil and the availability can be maximized by addition of chelates. The objective of this study was to determine Cd availability to native plant (*Artemisia princeps* var. *orientalis*) under hydroponic condition as influenced by different sources of ligands. Result showed that 1 mM MEDTA was more effective than oxalate or sulfate in mobilizing Cd in the contaminated soil into labile forms, resulting also in enhanced translocation of Cd from roots into shoots. Oxalate and sulfate had little effect on the Cd availability by *Artemisia princeps*. Plant seedlings accumulated relatively small amounts of Cd from the field contaminated soil than the artificially contaminated one. Overall results indicated that the species of Cd not the total content govern the Cd availability and EDTA could be used as the soil treatment agent enhancing phytoextraction of Cd from the contaminated soil.

Key words: Phytoextraction, Cd, availability, EDTA, *Artemisia princeps*

INTRODUCTION

Phytoextraction is one of the cost-effective and aesthetic phytoremediation processes compared to other remedial techniques such as chemical treatment (Salt, 1995). Goals of phytoextraction to reduce concentrations of toxic metal in contaminated soils within a reasonable time depend on two important factors: one is a plant factor in which the selected plants should accumulate a significant amount of metal in their harvestable parts and the other is a soil factor in which the phytoavailability of metals should be increased by controlling the physical and chemical conditions of soils (Blaylock and Huang, 2000; Ok *et al*., 2005).

Initial phytoextraction studies focused on finding hyperaccumulating species which could grow in the extremely polluted soil and could remove up to several percents of metals in their shoot (natural phytoextraction). Recently, different metal tolerant plants such as *Thlaspi caerulescens*, *Thlaspi ochroleucum*, *Brassica juncea*, *Hordeum vulgare*, *Avena sativa* and others have been used for phytoextraction of Cd, Cu, Ni, Pb and Zn from the contaminated soil (Chen *et al*., 2003; Ok *et al*., 2004b). However, due to their slow growth and limited biomass production, researchers estimated that they could not meet the requirements for the design of an economically realistic biotechnology (Ok *et al*., 2004a).

For this reason, more recent research on phytoextraction have focused on the crop species that display significant heavy metal tolerance combined with a high biomass productivity (Hamon *et al*., 1998). To compensate for the relatively low metal accumulation capabilities of these crop species, some researchers proposed to favor the soil-root transfer of metals by adding chelates which favor metal desorption from minerals (chemical assisted phytoextraction) (Sarret *et al*., 2000; Jiang *et al*., 2003; Ok *et al*., 2005). We conducted hydroponic experiments determine Cd availability by native plant (*Artemisia princeps* var. *orientalis*) as influenced by various ligands.

MATERIALS AND METHODS

Hydroponic experiment: Rhizomes of *A. princeps* were field-collected and cultivated for 30 days in a sand bed irrigated with the modified Epstein hydroponic solution. Plant seedlings were transplanted into a hydroponic solution in a reaction vessel and therein equilibrated for 10 days. The reaction vessels that contained the dialysis tube and hydroponic solution were prepared. The dialysis tubes were filled with 25 mL of hydroponic solution and Cd-contaminated soils (both field collected and artificially contaminated with Cd nitrate, refer to soil sample section). Total Cd concentrations in both soils

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Table 1: Physical and chemical properties of the soil used in the hydroponic experiment

Texture	pH_{H2O}	pH_{KCl}	EC (dS m^{-1})	OM[1] (%)	CEC ($cmol_c$ kg^{-1})	P_2O_5 (mg kg^{-1})	TKN (%)	FC[2] (%)
SL	5.12	4.15	0.18	0.49	4.05	165.9	0.08	39.5

[1]Organic matter; [2]Field moisture holding capacity

were 20 mg kg^{-1} (Ok *et al*., 2004a). One mM of EDTA, oxalate or sulfate were added separately into the reaction vessels, followed by transplanting the seedlings into the reaction vessels. After growing the seedlings for 48 h, phytoavailability of Cd by *Artemisia princeps* was determined by analysing Cd contents in roots and shoots. The harvested plant seedlings were rinsed carefully in distilled water, dried at 80°C and weighed. The Cd concentration in the plant was then determined using ICP-AES (JY 138 Ultrace, JOBIN YVON) after a digestion with the H_2SO_4-HNO_3-H_2O_2 solution. The calibration standards were prepared using the standard solution which was certified by the supplier. Five calibration standards and blank solution were used to calibrate the ICP-AES. A linear calibration curve was obtained after calibration. If the correlation coefficient R^2 was less than 0.999, the equipment was re-calibrated to ensure the accuracy of results. All the instrumental conditions were optimized for the maximum sensitivity as indicated by the manufacturer's manual.

Soil sample: Soil samples used in hydroponic experiments were artificially contaminated with the $CdCl_2$ solution to a final concentration of 20 mg kg^{-1} of Cd and were spiked as follows: 400 mg L^{-1} of Cd with the $CdCl_2$ stock solution was prepared and exactly 2.0 kg of soil was placed in a rectangular vessel made of low density polyethylene (LDPE). Fifty milliliters of prepared stock solution was uniformly sprayed on the soil, thoroughly mixed with a plastic rod after 5 min of contact time and air-dried. These steps were repeated until the resulting Cd concentration of spiked soil was 20 mg kg^{-1}. The soil used in this study showed very similar characteristics with typical Korean soils (Table 1): a relatively low pH was found in the soil with a value of 5.12 as compared to the average Korean soil pH of 5.7. According to the USDA classification, the soil was classified as sandy soil. The low organic matter content in the soil was attributed to lack of vegetation resulting from poor soil condition for plant growth (low soil pH, unbalanced nutrition and a high sand fraction). This condition also contributed to low cation exchange capacity (CEC) (4.05 $cmol_c$ kg^{-1}).

RESULTS AND DISCUSSION

The *Artemisia* is one of the largest genera in the family, *Compositae*, found in Korea and it is a common weed but an important medicinal plant used as a styptic in the central part of Korea (Yun and Choi, 2003). Some species are aromatic and used as herbs, spices and folk medicines. The bioconcentration factors for the Cd accumulating plants reported in the Republic of Korea are summarized in Table 2. Among the plants, the bioaccumulation factor was high in the *Artemisia princeps* var. *orientalis* with 5.72 for Cd in its shoot. In a previous research, Ok *et al*. (2003) reported that *Artemisia princeps* var. *orientalis* could be used as a possible phytoremediator to clean up the Cd contaminated soils. The authors showed that in a sequential extraction of the Cd contaminated soil, where *Artemisia princeps* was grown, 2.4 mg kg^{-1} (below 7%) of Cd existed as an exchangeable fraction. But, *A. princeps* took up over 50 mg kg^{-1} of Cd in its harvestable part and was also grown vigorously in the metal contaminated areas. It is also known to have a strong resistance to water stress and grows well under various pH regimes and soil textural conditions (Kim, 2003). Thus, further experiments were performed to evaluate *Artemisia princeps* for phytoextraction of Cd contaminated soils.

Table 2: The bioaccumulation factors for Cd accumulating plants reported in the literature in the Republic of Korea

Plants	Bioaccumulation factor
Abutilon avicennae	2.87
Helianthus annus	2.21
Ambrosia artemisifolia. var *elatior*	5.91
Ambrosia trifida	5.73
Artemisia princeps. var *orientalis*	5.72

Complexes of toxic metals due to organic acids or EDTA facilitate metal accumulation in the plant. This fact is very important in developing the strategies for phytoextraction since chelation can maximize metal accumulation in the shoot as well as in the root. For this reason, chelating agents were added as chemical ameliorants in another experiment to increase the Cd desorption from the soil and to facilitate Cd accumulation in the plant. Oxalic acid was selected among organic acids as the previous research has shown that *Artemisia princeps* released oxalic acid as a major source of organic acids. The contrasting samples of soil for the Cd availability were prepared to compare the Cd accumulation between two treatments: One is the soil artificially contaminated with Cd and the other is the soil naturally contaminated with Cd. The physical and chemical properties of soils (texture, organic matter, CEC and fertility status of soil) were not significantly different (Ok *et al*., 2004b). Table 3 showed that the EDTA (1 mM) was more effective than oxalate or sulfate in mobilizing Cd in the contaminated soil into labile forms, resulting also in enhanced translocation of Cd from roots into shoots. There was no significant difference in Cd contents in

Table 3: Cadmium concentrations in *Artemisia princeps* var. *orientalis* after growing for 48 h in a hydroponic culture (Means with the same letters are not significantly different by Tukey's studentized range test; SE: Standard Error)

Treatments	Dialysis tubes	Shoot (mg kg^{-1}) Mean±SE	Root treatment (mg kg^{-1}) Mean±SE
Nutrient Solution (NS)	Soil-Cd+NS	3.14±1.03b	48.7±4.81b
NS	Solution-Cd+NS	36.1±13.1a	157.0±74.5a
1mM EDTA+NS	Soil-Cd+NS	38.3±6.92a	3.79±0.84b
1mM Oxalic acid+NS	Soil-Cd+NS	1.93±0.80b	22.4±2.22b
1mM Sulfate+NS	Soil-Cd+NS	4.36±4.36b	40.9±8.01b

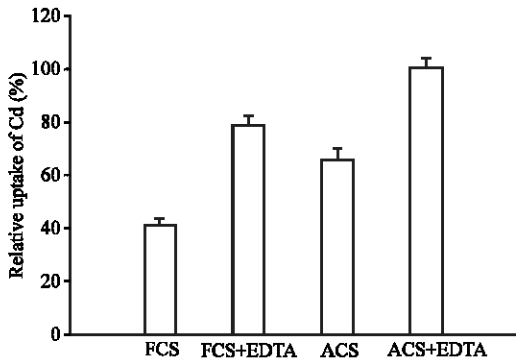

Fig. 1: Rate of Cd uptake from the Cd-contaminated soils by mugwort (*Artemisia princeps* var. *orientalis*) after growing for 48 h in a hydroponic culture (FCS: Field Contaminated Soil; FCS + EDTA: FCS treated with 1 mM EDTA; ACS: artificially contaminated soil: and ACS + EDTA: ACS treated with 1 mM MEDTA)

shoots between the treatments of Cd contaminated soils and the artificial Cd solution. These results were supported by the Cd contents in the roots, as revealed the lower Cd in the root in the EDTA treatment. Oxalate and sulfate had little effect on the Cd availability by *Artemisia princeps*. Figure 1 showed that the availability of Cd was lower in the field contaminated soils than the artificially contaminated soils (Ok *et al*., 2004b). The EDTA increased the Cd availability in both soils with similar increments. Wallace *et al*. (1974) reported that the metal-EDTA complexes formed in the soil could increase metal solubility and promote diffusion and thus increase the potential for plant uptake. Blaylock *et al*. (1997) also showed that EDTA was the most efficient increasing water-soluble Pb concentration. However, Halvorson and Lindsay (1977) found that chelates in the soil lowered metal toxicity and uptake. Moreover, application of EDTA to the soil has been observed to lead to decreases in the uptake of cadmium by plants (Wolterbeek *et al*., 1988). This apparent contradiction in the results of the studies reported by different researchers may be attributable to phytotoxicity of metals or EDTA. These results indicate that the species of Cd not the total content govern the Cd availability and EDTA could be used as the soil treatment agent enhancing phytoextraction of cadmium from the contaminated soil (Blaylock *et al*., 1997; Chen *et al*., 2000).

There was no significant difference of the Cd content in the shoot among the treatments of the Cd contaminated soil and the artificial Cd solution. These results were supported by the Cd contents in the root, as revealed by the lower Cd in the root for the EDTA treatment. Oxalate had little effect of *Artemisia princeps* on Cd availability. Recently, several similar studies on Pb accumulation in the plant in the presence of ^{14}C-labeled EDTA revealed that both Pb and EDTA were present in the shoot, suggesting that Pb was absorbed and transferred as a Pb-EDTA complex (Sarret *et al*., 2000). A physiological basis of uptake of the Pb-EDTA complex and particularly possibility for this negatively charged molecule to cross the membrane is still not clear. However, Vassil *et al*. (2003) suggested that EDTA could damage the membrane of the root cells by chelating Zn and Ca cations that stabilize this membrane, thus allowing a free equilibrium between the soil solution and the xylem sap. Until now, no evidence has been found that favors the hypothesis of uptake of the Pb-$EDTA^{2-}$ complex or of Pb^{2+} and $EDTA^{4-}$.

REFERENCES

Blaylock, M.J., D.E. Salt, S. Dushenkov, O. Zakharova, C. Gussman, Y. Kapulnik, B.D. Ensley and I. Raskin, 1997. Enhanced accumulation of Pb in Indian mustard by soil-applied chelating agents. Environ. Sci. Technol., 31: 860-865.

Blaylock, M.J. and J.W. Huang, 2000. Phytoextraction of Metals. In: Phytoremediation of Toxic Metals: Using Plants to Clean up the Environment. Raskin, I. and B.D. Ensley (Eds.), John Wiley and Sons, New York, pp: 53-70.

Chen, H.M., C.R. Zheng, C. Tu and Z.G. Shen, 2000. Chemical methods and phytoremediation of soil contaminated with heavy metals. Chemosphere, 41: 229-234.

Chen, Y.X., Q. Lin, Y.M. Luo, Y.F. He, S.J. Zhen, Y.L. Tu, G.M. Tian and M.H. Wong, 2003. The role of citric acid on the phytoremediation of heavy metal contaminated soil. Chemosphere, 50: 807-811.

Halvorson, A.D. and W.L. Lindsay, 1977. The critical Zn^{2+} concentration for corn and the nonabsorption of chelated zinc. Soil Sci. Soc. Am. J., 41: 531-534.

Hamon, R.E., M.J. Mclaughlin, R. Naidu and R. Correll, 1998. Long-term changes in cadmium bioavailability in soil. Environ. Sci. Technol., 32: 3699-3703.

Jiang, X.J., Y.M. Luo, Q.G. Zhao, A.M.J. Baker, P. Christie and M.H. Wong, 2003. Soil Cd availability to Indian mustard and environmental risk following EDTA addition to Cd-contaminated soil. Chemosphere, 50: 813-818.

Kim, J.G., 2003. Phytoremediation for abandoned zinc mining area. In: Proceedings of International Symposium on Life and Ecosystem in a Changing Environment. Korea University, Seoul, Korea, pp: 138-159.

Ok, Y.S., S.H. Kim, D.Y. Kim, H.N. Lee, S.K. Lim and J.G. Kim, 2003. Feasibility of phytoremediation for metal-contaminated mining area. Korean J. Soil Sci. Fert., 36: 323-332.

Ok, Y.S., J. Jung, H. Lee, H. Song, N. Jung, S. Lim and J.G. Kim, 2004a. Chemical characterization and bioavailability of cadmium in artificially and naturally contaminated soils. Agric. Chem. Biotechnol., 47: 143-146.

Ok, Y.S., J.G. Kim, J.E. Yang, H.J. Kim, K.Y. Yoo, C.J. Park and D.Y. Chung, 2004b. Phytoremediation of heavy metal contaminated soils using transgenic plants. Korean J. Soil Sci. Fertil., 37: 396-406.

Ok, Y.S., J.E. Yang, K.Y. Yoo, J. Jung and J.G. Kim, 2005. Enhanced uptake of Cd by *Artemisia princeps* using EDTA. Proceedings of the 8th International Conference on the Biogeochemistry of Trace Element, Adelaide, Australia, pp: 246-247.

Salt, D.E., 1995. Phytoremediation: A novel strategy for the removal of toxic metals from the environment using plants. Biotechnology, 13: 468-474.

Sarret, G., J. Vangronsveld, A. Manceau, M. Musso, J.D. Haen, J.J. Menthonnex and J.L. Hazemann, 2000. Accumulation forms of Zn and Pb in *Phaselous vulgaris* in the presence and absence of EDTA. Environ. Sci. Technol., 35: 2854-2859.

Vassil, A.D., R. Clemente, A. Roig and M.P. Bernal, 2003. The role of EDTA in lead transport and accumulation by Indian mustard. Plant Physiol., 117: 447-453.

Wallace, A., R.T. Mueller, J.W. Cha and G.V. Alexander, 1974. Soil pH, excess lime and chelating agent on micronutrients in soybeans and bush beans. Agron. J., 66: 698-700.

Yun, K.W. and S. Choi, 2003. Seasonal variation in allelopathic potential of *Artemisia princeps* var. *orientalis* on plants and microbes. J. Plant Biol., 46: 105-110.