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Enhancement of Cadmium Phytoextraction from Contaminated Soils with *Artemisia princeps* var. *orientalis*

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Abstract: Phytoextraction using plants to remove toxic metals from the environment is an emerging technology for contaminated land remediation. The maximum efficiency of phytoextraction is controlled by the availability of metals in the soil. Plant availability of soil metals are often manipulated by additions of chelating agents. We conducted a greenhouse experiment to evaluate the effects of chelator and ligands on phytoextraction of Cd from contaminated soils with an endemic plant, *Artemisia princeps* var. *orientalis*. Cadmium content in the plant was highest in $(NH_4)_2SO_4$ treatment, but sulfur powder had little effect on Cd accumulation in the plant due to low buffering capacity of the soil and slow turnover rate of S° to SO_4^{2-}. Cadmium content in the plant was slightly increased in oxalic acid and EDTA treatments by accompanying pH decrease in the soil. Phytoremediation Index (PI) increased in the order of control<sulfur powder (S°)<oxalic acid<$(NH_4)_2SO_4$< EDTA treatments. In addition, Cd content in the plant showed the same trend with PI except for EDTA treatment. It could be postulated that EDTA addition should be avoided for the soil with high Cd availability as it might accelerate a continuous leaching of Cd-EDTA complexes from surface to subsoil during the phytoextraction. Overall results indicated that $(NH_4)_2SO_4$ can be used to enhance Cd accumulation in the *Artemisia princeps* var. *orientalis* during phytoextraction.

Key words: Phytoextraction, Cd, availability, sequential extraction, EDTA, $(NH_4)_2SO_4$

INTRODUCTION

In South Korea, over 1,000 metal mines have been closed due to the depression of mining industry since the late 1980's. Most of mine tailings have been abandoned on hillsides and discharged by rain directly to stream water and adjacent agricultural lands (Jung and Thornton, 1997; Chen *et al*., 2000a). The heavy metal run off caused detrimental effects on surrounding soil and water bodies threatening human health with outbreaks of such diseases as itai-itai due to Cd accumulation in rice. Recently, the Korean Soil Environmental Conservation Act (KSECA) announced that soils containing 12 mg kg^{-1} or a greater amount of 0.1N HCl extractable Cd should be monitored continuously and not to be used for agricultural purpose. However, Cd concentration in some Korean paddy soils reported to be > 20 mg kg^{-1} and Cd content in rice grain exceeded 0.2 mg kg^{-1}, the standard guideline of the Korea Food and Drug Administration (KFDA). Thus, proper remediation strategies for contaminated Korean agricultural lands are needed especially for rice paddy adjacent to closed metal mines.

Current remediation methods based on civil engineering techniques are usually expensive, environmentally unsound and labor-intensive. Low cost and nondestructive alternatives that do not generate any by-products to the environment should be developed to remediate the agricultural land. Phytoremediation has been rising as an environmentally safe and inexpensive technique to clean up metal contaminated lands. The baseline of phytoremediation is to remove toxic materials from contaminated soils using plants by immobilizing or detoxifying the relevant matters. Compared to other physical and chemical methods, the cost for phytoremediation is generally up to 1/10 lower (Cunningham *et al*., 1995; Ok *et al*., 2005). However, phytoremediation has certain limitations in that it is time-consuming and not very efficient in extremely contaminated soils as it uses living plants as tool materials (Lee *et al*., 2004). The degree of metal availability, heterogeneity of the contaminated soil and complexity of a pollutant often restrict the efficiency of phytoremediation (Salt, 1995). Recently, various researches have been conducted to overcome these disadvantages. A method using enhancer such as Ethylene Diaminete Traacetic Acid (EDTA) with plant vegetation is one example. Recent studies demonstrated that the accumulation of Cu, Ni, Pb and Zn from the soil could dramatically increase with the presence of synthetic chelates (Blaylock *et al*., 1997; Blaylock and Huang, 2000).

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Blaylock *et al.* (1997) showed that EDTA was the most efficient reagent to increase the water-soluble metal concentration in soil including the enhanced Pb accumulation at *Brassica juncea* shoots. Organic compounds exudated from plant roots also influence the capability of metal solubility and plant uptake by different modes: having an indirect effect on the microbial activity, changing rhizosphere physical properties and root growth dynamics, changing chemical properties of soils such as direct acidification, chelation, precipitation and oxidation/reduction reaction in the rhizosphere. Huang *et al.* (1998) reported that the addition of organic acid such as citric acid selectively increased uranium mobility in soils and subsequently increased plant uptake. They suggested that the strong mobilization of uranium by citric acid was possibly due to formation of citrate-uranyl complexes rather than decreased pH. Phytochelatin, an analogue of metallothionine, affects metal accumulation in certain plants, since it reduces metal toxicity towards plants and enhances metal accumulation in plant biomass. Noctor *et al.* (1997) showed that phytochelatin played a key role in Cd sequestration from soils and minimized Cd toxicity on plants. Biosynthesis of phytochelatin was presumed to be related to sequestration of Cd to plant tissue. Shemidi and Jager (1992) and Noctor *et al.* (1997) reported phytochelatin content was increased by phytochelatin synthesis from glutathion when plants were exposed to Cd (Grill *et al.*, 1985).

This study was based on the hypothesis that ligand or chelate treatments in the Cd contaminated soil might increase the soil Cd availability and in the end increase Cd accumulation in the plant. A greenhouse experiment was performed to evaluate the role of various chelator and ligands on Cd accumulation by *Artemisia princeps* var. *orientalis* grown in the Cd contaminated soil. Sulfur powder and ammonium sulfate were selected to enhance phytochelatin synthesis.

MATERIALS AND METHODS

Soil analysis: Soil samples were collected from upland soil located in Gyeonggi Province, South Korea and were air dried and passed through a 2 mm sieve for analysis. Physical and chemical properties of the soil were determined according to the methods described as follows: texture by pipette method, pH in 1:5 water or KCl extract, electrical conductivity in 1:5 water extract, organic matter by Tyurin method, cation exchange capacity using 1 N NH_4OAc (pH 7.0), available phosphate by Langcaster method and nitrogen by total Kjeldahl nitrogen method (Ok *et al.*, 2004a).

Greenhouse experiment: Soil samples 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}. After an aging period of 30 days, Cd contaminated soil was used for the greenhouse experiment. Organic and inorganic agents, including sulfur powder, ammonium sulfate, oxalic acid and EDTA was treated later on (Table 1). All the experiments were conducted with at least five replicates. Rhizomes of mugwort (*Artemisia princeps* var. *orientalis*) were collected near rice paddy located in Gyeonggi Province in South Korea. Collected rhizomes were incubated in a sand box for 50 days under controlled conditions, after which 30 seedlings were selected and transplanted in Cd contaminated soils. Irrigation was done by direct-watering to fulfill the field capacity. After cultivation, the plant samples were harvested and remaining soils were colleted for further analysis. Geochemical forms of Cd in soils were determined by using a sequential extraction method recommended by Emmerich *et al.* (1982) (Table 2). 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

Table 1: Ratio of applied chemical amendments in Cd contaminated soils

Treatments	Chemical form	Application rate ($kg\ ha^{-1}$)	N-P-K ($kg\ ha^{-1}$)
Control	-	-	32-7.8-19.8
Sulfur powder	S	300	32-7.8-19.8
Ammonium sulfate	$(NH_4)_2SO_4$	300 as S	32-7.8-19.8
Oxalic acid	HO_2CCO_2H	1,200	32-7.8-19.8
EDTA	$N_2H_2(CO_2)_2(CO_2H)_2$	1,200	32-7.8-19.8

Table 2: Selective extraction procedure and designated chemical forms of Cd in contaminated soils

Extractant	Concentration ($mol\ L^{-1}$)	Equilibrium time (h)	Geochmical form
KNO_3	0.50	16	Exchangeable
H_2O	55.5	2 (×3)	Water-soluble
NaOH	0.50	16	Organically bound
Na_2-EDTA	0.05	6	Oxide, carbonated
HNO_3	4.00	16[1]	Sulfide, residual

[1] Extracted in water bath, not on shaker, at 80°C, modified method of Emmerich *et al.* (1982)

Table 3: Physical and chemical properties of the soil used in the greenhouse experiment

Texture	pH_{H2O}	pH_{KCl}	EC	OM[1]	CEC	P_2O_5	TKN	FC[2]	Cd[3]
			$dS\ m^{-1}$	%	$cmol_c\ kg^{-1}$	$mg\ kg^{-1}$	%	%	$mg\ kg^{-1}$
SL	5.12	4.15	0.18	0.49	4.05	165.9	0.08	39.5	0.05

[1]Organic matter, [2]Field moisture holding capacity, [3]Extracted by 0.1 N Hcl

ensure the accuracy of results. All the instrumental conditions were optimized for the maximum sensitivity as indicated by the manufacturer's manual.

RESULTS AND DISCUSSION

The metal accumulation by plant species is influenced by various factors such as availability metals in the soil, translocation of metals from root to shoot and tolerance of metals. This study focused on increase of metal availability in the soil and therefore other factors were controlled in a similar condition. Soil condition is essential element transforming immobile metals into mobile ones (Li and Shuman, 1996; Ok *et al*., 2003). The physical and chemical properties of the soil used in a greenhouse experiment were summarized in Table 3. It is well-known that typical soil type in South Korea is sandy with low organic matter content, Cation Exchange Capacity (CEC) and pH condition (Kim, 1985; Jung *et al*., 2002). The soil used in this study showed very similar characteristics with typical Korean soils: 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 (Kim, 1985). According to the USDA classification, the soil was classified as sandy soil. The 0.1N HCl extractable Cd concentration in the soil was 0.05 mg kg^{-1} which was negligible as compared to that in artificially contaminated soil, 20 mg kg^{-1}. 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) (Jung *et al*., 2002). This condition also contributed to low CEC as 4.05 $cmol_c\ kg^{-1}$.

Cadmium concentration in the soil decreased after the cultivation. However, the plant accumulated different amounts of Cd in their shoots corresponding to each treatment. The highest Cd content was found in the $(NH_4)_2SO_4$ treatment. In previous study, we found that Cd content in *Artemisia princeps* increased up to over 400 mg Cd kg^{-1} in their harvestable parts with the rise of sulfate ion concentrations in nutrient solution (Kim *et al*., 2001). Moreover, the plants accumulated about three times more Cd in their shoots when exposed to a sufficient level of sulfur even if the Cd level was equal among the treatments (Fig. 2). Phytochelatin affects Cd accumulation in plants as it can minimize physiological toxic effects of Cd in plants and thus enhances Cd accumulation. During phytochelatin synthesis, glutathione (γ-Glu-Cys-Gly) and cysteine are used as precursors triggered by inorganic sulfate ion. Thus, sulfate treatment in the soil might reduce Cd toxicity and enhance Cd accumulation in the *Artemisia princeps* (Fig. 1). Kim *et al.* (2001) conducted hydroponic experiments to determine a critical sulfur concentration in Cd uptake by *A. princeps* var. *orientalis*. They found that Cd treatment altered the optimal sulfur concentration in the biomass of *Artemisia princeps* indicating the plant required more sulfur upon Cd stress. When sulfur concentration was low, absorption and translocation processes lagged revealing phytochelatin synthesis was inhibited because of sulfur deficiency. Cadmium content in *Artemisia princeps* increased as sulfur concentration in the nutrient solution increased. The relationship between Cd accumulation in plants and sulfur concentration in solution was $Cd = -10^{-5} S^2+0.15 S-129.91$ ($r = 0.63^{**}$). It can be postulated that sulfate addition to optimal level in Cd contaminated soils increased Cd content in *Artemisia princeps* by maximizing tolerance of Cd toxicity. Sulfur powder treatment, however, did not exert any increase in Cd accumulation by *Artemisia princeps* as sufficient transformation of sulfur to sulfate was not occurred under the experimental condition. The turnover rate of sulfur powder into sulfate

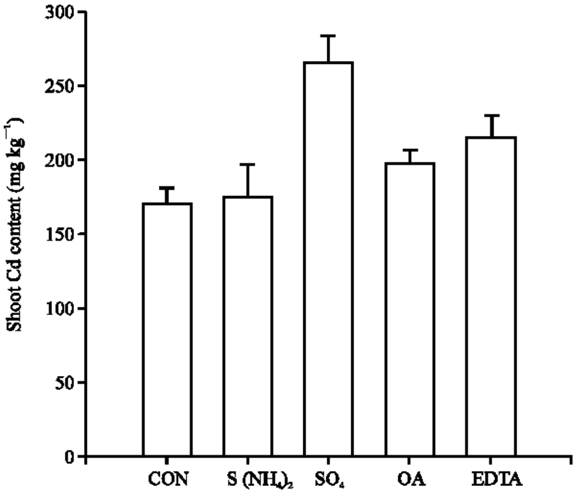

Fig. 1: Cadmium contents of *Artemisia princeps* var. *orientalis* after growing for 60 days in the Cd contaminated soil. (CON: Control, OA: Oxalic Acid, DW: Dry Weight of plantlet; Each error bar represents the standard deviation of the five independent experiments)

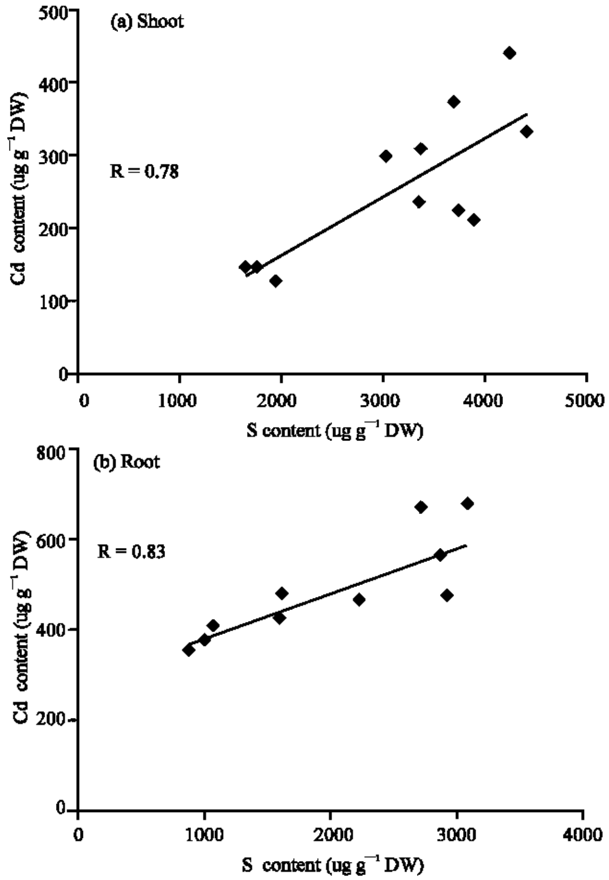

Fig. 2: Simple linear correlation between the S and Cd contents of the shoot and the root of *A. princeps* var. *orientalis* after growing for 42 days in a hydroponic solution containing 100 μM of Cd. (DW: dry weight of plantlet. **: significant at 95% level. Each diamond in the Fig. represents the mean value of the five independent experiments. Modified from Kim *et al.* (2001)

was considerably different according to soil conditions such as temperature, soil texture and organic matter content. An incubation experiment showed that the transformation of sulfur into sulfate took at least several months in the experimental soil (data not shown). The oxalic acid and EDTA treatments slightly increased Cd accumulation in *Artemisia princeps*. The increase in the rate was mainly due to the decrease of pH in the presence of oxalic acid or EDTA. Similarly, Nigam *et al.* (2001) showed that when organic acids, which are commonly exudated from roots of corn plants, were added to soils, heavy metal extraction by plants was enhanced because of pH decrease in the soil. However, Huang *et al.* (1998) suggested that formation of element and organic acid complexes, rather than decrease pH selectively increased uranium mobility in soils (Kim, 2003).

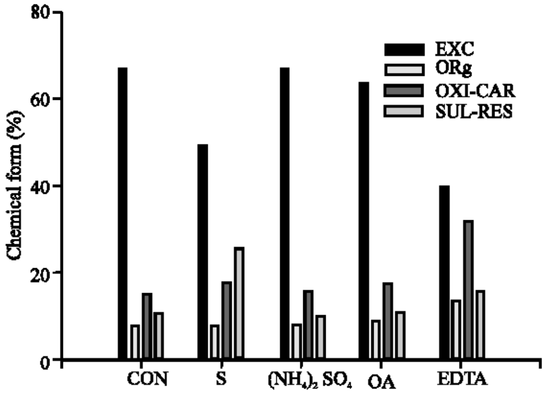

Fig. 3: Changes in chemical forms of Cd in the contaminated soil after plant cultivation. (CON: Control, EXC: Exchangeable, ORG: Organically bound, OXI-CAR: Oxide, Carbonated, SUL-RES: Sulfide, Residual)

Figure 3 showed the geochemical forms of Cd after the *A. princeps* cultivation. The control and EDTA treatment showed the highest and lowest exchangeable Cd concentrations. The levels of exchangeable Cd fraction decreased in the order of EDTA<sulfur powder <oxalic acid<ammonium sulfate <control. Ethylenediaminetetraacetic acid (EDTA) is a synthetic chelating agent that meets this requirement and has been used in recent studies as a model compound for stable soluble organics in soil solution. Therefore, an introduction of EDTA or its chelates in soils could result in the formation of a stable complex with Cd, most of which would be water-soluble and could be transported along with the soil water. Thus, direct application of EDTA into soils can result in the continuous leaching of Cd from surface to subsoils. In addition, the chelating agent, EDTA, not only could form a soluble complex with metals, but may also influence the distribution of metals in the fraction by transforming metals from a less water-soluble fraction into a more soluble fraction (Chen *et al.*, 2000b; Jung *et al.*, 2004; Ok *et al.*, 2004b). Table 4 shows the Phytoremediation Index (PI), based on the amount of Cd accumulated by plants and divided by exchangeable Cd content in the soil after cultivation in each treatment (Kim, 2003). The PI of *Artemisia princeps* increased as the plant Cd uptake increased in the order of control<sulfur powder<oxalic acid<ammonium sulfate<EDTA treatments. The trend conformed to the results of plant Cd accumulation except for the EDTA treatment. According to the results, EDTA treatment would be useful for further field application in phytoremediation. However, speciation] and resulting availability of Cd in the soil needed to be determined in advance to calculate the proper amount of EDTA in phytoremediation for the field application.

Table 4: Phytoremediation index[1] of plants in freshly Cd contaminated soils

Treatments	Shoot	Root	Total
Control	16.40	17.63	17.02
Powder S	21.02	22.07	21.55
$(NH_4)_2SO_4$	23.77	25.64	24.71
Oxalic acid	18.93	26.96	22.95
EDTA	47.53	50.23	48.88

[1] Relative cadmium uptake values were determined by dividing metal concentration in plants by exchangeable metal concentrations in soils as determined through a sequential extraction proposed by Emmerich *et al.* (1982)

CONCLUSIONS

The normal Cd content in plants is very low compared to other metals, as 0.1~0.3 mg kg^{-1} in dried leaves, that a plant which is capable of concentrating Cd up to 100 mg kg^{-1} is considered a hyperaccumualtor with high remediation potential. The *Artemisia*, a common weed, is one of the largest genera in the family, *Compositae*, found in the central part of Korea. This study showed that mugwort (*Artemisia princeps* var. *orientalis*) concentrated Cd over 150 mg kg^{-1} in its harvestable part and also grew vigorously in Cd contaminated areas. Greenhouse experiments demonstrated that $(NH_4)_2SO_4$ and EDTA treatments enhanced Cd accumulation by *Artemisia princeps* grown in Cd contaminated soil. The result also conformed to the tendency of Cd content in *Artemisia princeps* except for EDTA treatment revealing that EDTA application to soil with a high available Cd content might result in continuous leaching of Cd-EDTA complexes from surface to subsoil. The EDTA treatment would be environmentally safe and useful only in phytoremediation for Cd contaminated soil with a low available Cd content. The overall result implies that Cd availability in the soil should be determined in advance for the proper use of EDTA in phytoextraction. Ammonium sulfate can be used to enhance Cd accumulation in the *Artemisia princeps* var. *orientalis* during phytoextraction.

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