Bull Environ Contam Toxicol (2010) 84:255–259
DOI 10.1007/s00128-009-9888-0

Comparison of the Ability of Organic Acids and EDTA to Enhance the Phytoextraction of Metals from a Multi-Metal Contaminated Soil

Sung-Hyun Kim · In-Sook Lee

Received: 7 May 2009 / Accepted: 24 September 2009 / Published online: 6 October 2009

Abstract Chelates have been shown to enhance the phytoextraction of metal from contaminated soil. In this study, we evaluated the ability of chelates to enhance the phytoextraction of metals by barnyard grass (*Echinochloa crus-galli*) from soils contaminated with multiple metals. The results revealed that EDTA increased the ability of barnyard grass to take up Cd, Cu and Pb, but that it resulted in increased soil leaching. Conversely, citric acid induced the removal of Cd, Cu and Pb from soil without increasing the risk of leaching. Furthermore, *E.crus-galli* showed no signs of phytotoxicity in response to treatment with citric acid, whereas its shoot growth decreased in response to treatment with EDTA ($p < 0.05$). Taken together, these results demonstrate that citric acid is a good agent for the enhancement of the phytoextraction of metals.

Keywords *Echinochloa crus-galli* · EDTA · Metal · Organic acids · Phytoextraction

Phytoextraction by metal hyperaccumulator plants is known to be an effective method of removing heavy metals from soils (Baker et al. 1994; Yang et al. 2004). Generally, the success of phytoextraction depends upon the availability of metals for uptake by the hyperaccumulator; therefore, chelating agents are often used to increase the bioavailability of heavy metals in soils undergoing remediation (Huang et al. 1997; Blaylock et al. 1997). Among chelates used to increase metal uptake, EDTA (ethylenediaminetetraacetic acid) has been the most widely used (Blaylock et al. 1997). Although EDTA has been shown to enhance phytoextraction, it has disadvantages including poor biodegradability and decreased plant growth (Chen and Cutright 2001). Recently, low molecular weight organic acids (LMWOA) have been used to enhance the phytoextraction of metals (Krishnamurti et al. 1997). LMWOA are more easily biodegraded in soil when compared to EDTA (Nigam et al. 2001). However, previous studies have shown inconsistent results when evaluating the effects of EDTA and LMWOA on phytoextraction by different plant species. For example, some studies have shown that EDTA enhances the accumulation of soil heavy metals in hyperaccumulator species (Blaylock et al. 1997; Huang et al. 1997). Conversely, some studies have shown that LMWOA can be used to increase metal solubility and plant uptake efficiency (Krishnamurti et al. 1997; Jones et al. 1996). Thus, the effectiveness of chelates is dependent on the species of plant and suitable additives for specific plant-soil combinations may be needed (Long et al. 2002).

Echinochloa crus-galli is highly resistant to a wide range of heavy metal concentrations (Kim et al. 2009). This study was conducted to identify the chelating agent that most effectively enhanced the phytoextraction of Cd, Cu and Pb from a multi metal contaminated soil by *E. crus-galli*. Specifically, we compared the ability of LMWOA (citric, oxalic, malic, and succinic acids) and EDTA to enhance the phytoextraction of Cd, Cu and Pb from heavy metal contaminated soil by *E. crus-galli* .

S.-H. Kim · I.-S. Lee (✉)
Division of Eco Science, Ewha Womans University,
Seoul 120-750, Korea
e-mail: islee@ewha.ac.kr

Materials and Methods

Natural soil was collected from the campus of Ewha Womans University, Seoul, Korea. The physicochemical properties of the soil were as follows (Table 1): soil

Table 1 Physical and chemical characteristics of the soils

Parameters	Content
pH (H_2O)	5.5 ± 0.2
Organic matter (%)	3.8 ± 0.2
Moisture content (%)	13 ± 0.3
Soil texture	
Sand (%)	54.1
Clay (%)	15.0
Silt (%)	30.9
CEC (meq100 g^{-1})	9.6 ± 0.1
DHA (μg g^{-1})	14 ± 3

texture-loamy sand (54.1% sand, 30.9% silt, and 15.0% clay); total organic matter 3.8%; total moisture content 13%; cation exchange capacity (CEC) 9.6 mmol kg^{-1}; Dehydrogenase activity 14 μg g^{-1}; pH 5.5. The soil was contaminated with 40 mg kg^{-1} Cd, 100 mg kg^{-1} Cu, and 600 mg kg^{-1} Pb and then allowed to equilibrate for 1 week. Next, 1 kg of soil was transferred to a column (20 cm height, 8 cm diameter) and planted with *E. crus-galli* seedlings (5 plants per column). The plants were then grown in the growth room. Four weeks after transplantation, EDTA and organic acid (citric, malic, oxalic, succinic acid) were added (10 mmol kg^{-1} soil per column). The treatments included one control (metal contaminated soil with no chelate addition) and all treatments were replicated three times. Fourteen days after the chelating agent was applied the plants were carefully harvested by cutting the shoots at the soil surface, after which the total biomass was determined for each treatment. The tolerant index (TI) was then calculated using the following equation:

$$\text{Tolerance index} = \frac{\text{Biomass(F.W)}_{\text{treated plant}}}{\text{Biomass(F.W)}_{\text{control}}}$$

.

The soil was then broken up and the roots were harvested by hand. Next, the harvested plants were washed with tap water for several minutes. The roots and shoots of these plants were then dried at 70°C in a drying oven until a constant weight was attained to determine the dry weight and then ground prior to metal analysis. Samples of the plants were then digested in concentrated HNO_3 in a micro-wave digester (MDS-2000, CEM). The heavy metals contents were determined using an atomic absorption spectrophotometer (AAS; analysis 100, Perkin Elmer). The system was calibrated using certified reference materials, SRM 1573a (tomato leaves), from the National Institute of Standards and Technology (NIST), USA and analyzed to support quality assurance and control (QA/QC). The recoveries of three elements ranged from 93 to 101% (data not shown). To evaluate the phytoextraction potential of the *E. crus-galli*, the following root to shoot ratio parameter was calculated: the translocation factor C shoots/C roots, where C represents the metal concentration.

Soil leaching solutions were collected by suction pressure using a syringe at 14 days after the chelate was applied (Nascimento et al. 2006). Total soluble heavy metals were analyzed directly by AAS (analysis 100, Perkin Elmer).

Data were analyzed by Tukey's test after one-way ANOVA using SPSS 9.0. One-way ANOVA was used to determine the significance of differences in the metal concentration of the roots and shoots of *E. crus-galli* that were treated with different chelates.

Results and Discussion

The goal of successful phytoextraction is to reduce the levels of heavy metals in contaminated soil to acceptable levels within a reasonable time frame. To accomplish this, plants must accumulate high levels of heavy metals and produce high amounts of biomass. In addition, successful phytoextraction must include the mobilization of heavy metals into soil solution that is in direct contact with the plant roots.

The effects of EDTA and organic acids on the tolerant index (TI) of the biomass of *E. crus-galli* are shown in Table 2. The addition of citric and maleic acid had no effect on biomass when compared to the controls. Conversely, the application of EDTA, oxalic, and succinic acid led to significant decreases in biomass, with the greatest inhibition occurring in response to treatment with EDTA. The reduction in plant growth following EDTA treatment was likely due to the toxicity of the EDTA as well as the toxicity of the EDTA-metal complexes (Chen and Cutright 2001).

Many studies have demonstrated decreases in plant biomass in response to the addition of EDTA (Grčman et al. 2001; Blaylock et al. 1997). For example, Chen and Cutright (2001) reported a decrease in plant growth in response to treatment with EDTA at a concentration of

Table 2 Effects of EDTA and organic acids on the tolerance index (TI) of biomass of *E. crus-galli*

Treatment	Biomass (F.W.g)	TI
Control	1.39 ± 0.19[a]	1
EDTA	0.3 ± 0.03[b]	0.16
Citric	1.38 ± 0.11[a]	0.97
Malic	1.0 ± 0.15[a]	0.67
Oxalic	0.35 ± 0.09[b]	0.2
Succinic	0.5 ± 0.04[b]	0.23

Means with different letters are significantly different from each other ($p < 0.01$) according to the Tukey's test ($n = 15$)

1.25 mM kg^{-1} of soil. Furthermore, Wallace et al. (1977) reported that high concentrations of chelates damaged the plasma membranes of plants, which disrupted the mechanism by which heavy metals were transported to the roots and consequently increased the amounts of heavy metals and chelates absorbed by the plant. Vassil et al. (1998) reported that higher concentrations of NLMWOA also resulted in decreased biomass, probably in response to the destruction of the physiological barrier by NLMWOA in roots that control the uptake of solutes. However, adequate NLMWOA concentrations also have the ability to detoxify intracellular heavy metals via binding (Lee et al. 1977). Similarly, Evangelou et al. (2006) found that the application of NLMWOA at 62.5 mM kg^{-1} showed no adverse effects on the production of dry matter by the shoots.

The results of our study demonstrated that the accumulation of Cd, Cu and Pb in the roots and shoots of *E. crusgalli* increased in response to the addition of chelate (Table 3). The greatest increase in shoot uptake was observed in response to treatment with EDTA. Specifically, the shoot of Cd, Cu, and Pb accumulation by *E. crusgalli* that had been treated with EDTA increased by 2, 10, and 20 fold when compared to the control. The root uptake rate of *E. crusgalli* also increased in response to the addition of citric acid; however, the root to shoot translocation efficiency of EDTA was higher than that of citric acid.

Table 3 Shoot and root metal concentration and shoot-to-root ratio of metal concentration in barnyard grass in soil treated with 10 mmol kg^{-1} of chelates

Treatment	Metal		
	Cd	Cu (μg g^{-1})	Pb
Shoot			
Control	24.9[a]	32.1[a]	34.1[a]
EDTA	43.9[b]	340.8[b]	672.4[b]
Citric	40.2[b]	161.2[d]	777.5[b]
Malic	26.7[a]	101.2[d]	22.6[a]
Oxalic	29.4[c]	286.1[c]	106.6[c]
Succinic	30.0[c]	181.3[d]	50.4[a]
Root			
Control	141.9[a]	102.2[a]	172.1[a]
EDTA	215.6[b]	612.7[b]	1067.2[b]
Citric	416.1[b]	406.8[c]	1143.4[b]
Malic	215.6[a]	242.2[d]	174.1[a]
Oxalic	295.5[c]	384.5[c]	409.9[c]
Succinic	313.6[d]	444[e]	314.9[c]
Root-to-shoot ratio			
Control	0.10[a]	0.31[a]	0.2[a]
EDTA	0.20[b]	0.56[b]	0.63[b]
Citric	0.10[c]	0.40[a]	0.68[c]
Malic	0.12[c]	0.42[a]	0.13[d]
Oxalic	0.10[a]	0.74[c]	0.26[a]
Succinic	0.10[d]	0.41[a]	0.16[a]

Means with different letters are significantly different from each other ($p < 0.01$) according to Tukey's test ($n = 15$)

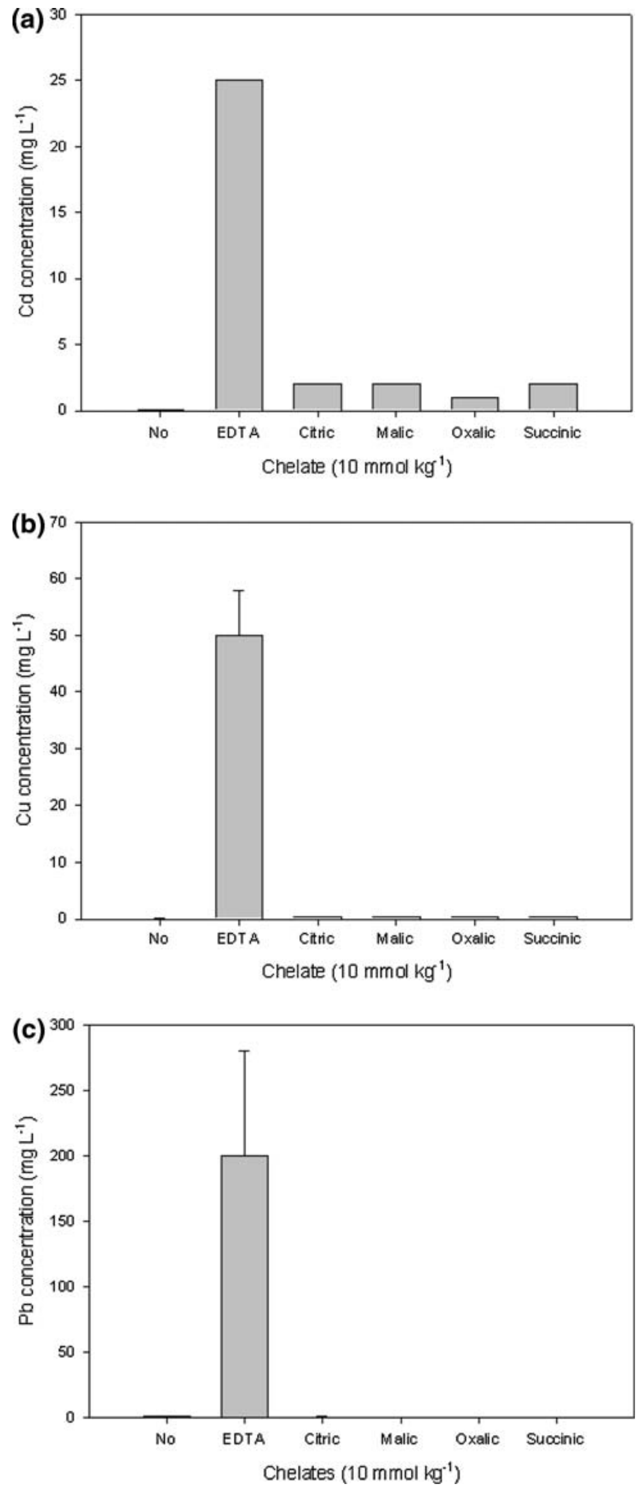

Fig. 1 Effects of chelating agents on the concentration of **a** Cd, **b** Cu, and **c** Pb in soil solutions after the application of EDTA and organic acids. *Error bars* represent the standard deviation ($n = 3$)

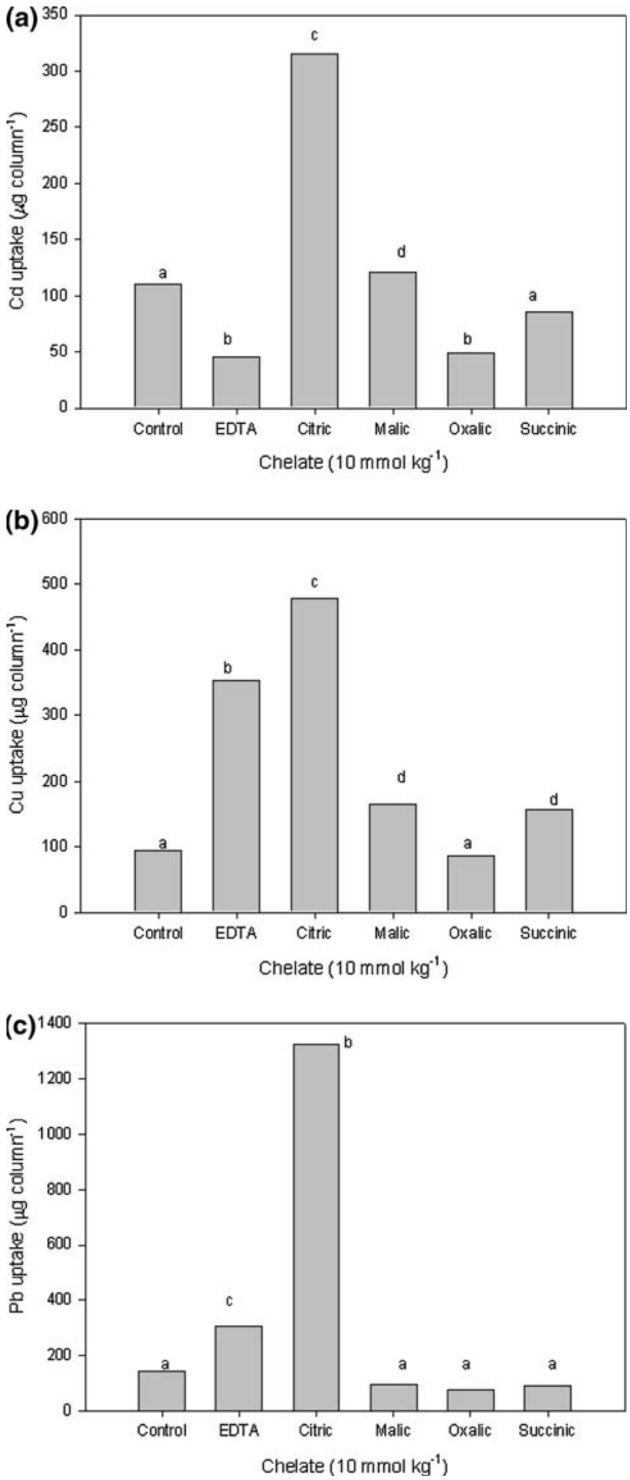

Fig. 2 Removal of **a** Cd, **b** Cu, and **c** Pb by *E. crus-galli* (μg $column^{-1}$) grown in soil treated with 10 mmol kg^{-1} of chelates. Values followed by the same letter do not differ significantly ($p < 0.01$)

The effects of natural and synthetic chelates on the solubility of soil-bound metals are shown in Fig. 1. Among the chelates evaluated in this study, EDTA appears to most effectively solubilize soil-bound metal and maintain a high soluble metal concentration. Soluble metals are potentially bioavailable and can either be taken up by plants, leached into ground water, or desorbed by the soil exchange sites. After the application of EDTA, the metal content remained weakly adsorbed to the soil components (more easily leacheable). Taken together, these results indicate that the addition of EDTA facilitated the uptake of metal by *E. crus-galli*. However, the enhanced metal leaching ability also requires careful control because plants will not be able to assimilate highly mobilized metals easily, which could result in the increased mobility leading to ground water contamination. This result is affected by the biodegradability rates of chelates, with natural chelates being rapidly mineralized by microorganisms and synthetic chelates not being easily degraded (Satroutdinov et al. 2000).

Grčman et al. (2001) reported that application of 10 mmol EDTA kg^{-1} soil resulted in Pb, Zn and Cd concentrations that were 104.6, 3.2 and 2.3-times higher in the shoots of *Brasica rapa* when compared to controls. However, 40% of the total applied EDTA was leached through the soil profile in columns that contained 10 mmol kg^{-1} of EDTA. Therefore, the use of EDTA for phytoextraction in situ has the potential to cause groundwater pollution by leaching.

Evaluation of the net removal of these metals by *E. crus-galli* demonstrated that citric acid can be as efficient as EDTA for use in phytoextraction. This is due to the lower phytotoxicity of citric acid toward *E. crus-galli* when compared to EDTA (Fig. 2).

In this study, we compared the performance of EDTA and natural low molecular weight organic acids (LMWOA; citric, malic, oxalic, succinic acid) during the phytoextraction of metals by *E. crus-galli*. The results of this study support the use of natural, easily biodegradable organic acids for the phytoextraction of metals from multi-metal contaminated soils. Citric acid was able to induce the removal of substantial amounts of Cd, Cu, and Pb from soil without increasing the risk of leaching for these metals.

Acknowledgments This study was conducted with the support of the twenty-first Century Frontier R&D program (R0120050001026802007) and the 16th Winter Institute Program provided by the Japan International Science and Technology Exchange Center.

References

Baker AJM, McGrath SP, Sidoli CM (1994) The possibility of in situ heavy metal decontamination of polluted soils using crops of metal-accumulating plants. J Resour Conserv Recycl 11:10–41

Blaylock M, Salt DE, Dushenkov S, Zakharova O, Gussman Y, Bd Kapulnik, Raskin E (1997) Enhaced accumulation of Pb in Indian mustard by soil-applied chelating agents. Environ Sci Technol 31:860–865

Chen H, Cutright T (2001) EDTA and HEDTA effects on Cd, Cr, and Ni uptake by *Helianthus annus*. Chemosphere 45:21–28

Evangelou MWH, Ebel M, Schaeffer A (2006) Evaluation of the effect of small organic acids on phytoextraction of Cu and Pb from soil with tobacco *Nicotiana tabacum*. Chemosphere 63:996–1004

Grčman HS, Velikonja-Bolta D, Vodnik B, Kos DL (2001) EDTA enhaced heavy metal phytoextraction: Metal accumulation, leaching and toxicity. Plant Soil 235:105–114

Huang JW, Chen J, Berti WR, Cunningham SD (1997) Phytoremediation of lead-contaminated soil: role of synthetic chelates in lead phytoextraction. Environ Sci Technol 3:800–805

Jones DL, Darrah PR, Kochain LV (1996) Critical evaluation of organic acid mediated iron dissolution in the rhizosphere and its potential role in root iron uptake. Plant soil 180:57–66

Kim SH, Park JS, Lee IS (2009) Characterization of Cadmium binding ligands from roots of *Echinochloa crusgalli* var. frumentacea. J Plant Biol 52:167–170

Krishnamurti GSR, Huang PM, Van Rees KCJ (1997) Kinetics of cadmium release from soils as influenced by organic: implications in cadmium availability. J Environ Qual 26:271–277

Lee J, Reeves RD, Brooks RP, Jaffre T (1977) Isolation and identification of a citrate-complex of nickel from nickel-accumulating plants. Phytochemistry 16:1502–1505

Long XX, Ni WZ, Ye ZQ (2002) Effect of organic acids application on Zn uptake and accumulation by two ecotypes of *sedum alfredii Hance*. J Plant Nutri Fertil 8:467–472

Nascimento CWA, Amarasiriwardena D, Xing B (2006) Comparison of natural organic acids and synthetic chelates at enhancing phytoextraction of metals from a multi-metal contaminated soil. Environ Pollut 140:114–123

Nigam R, Srivastava S, Prakash S, Srivastava MM (2001) Cadmium mobilization and plant availability—the impact of organic acids commonly exuded from roots. Plant Soil 230:107–113

Satroutdinov AD, Dedyukhina EG, Chistyakova TI, Witschel M, Minkevich IG (2000) Degradation of metal-EDTA complexs by resing cells of the bacterial strain DSM 9103. Environ Sci Technol 34:1715–1720

Vassil AD, Kapulnik Y, Raskin I, Salt DE (1998) The role of EDTA in lead transport and accumulation by Indian mustard. Plant Physiol. 117:447–453

Wallace A, Romney EM, Cha JW, Soufi SM, Chaudhry FM (1977) Nickel phytotoxicity in relationship to soil pH manipulation and chelating agents. Commum Soil Sci Plant Anal 8:757–764

Yang XE, Long XX, Ye HB (2004) Cd tolerance and hyperaccumulation in a new-Zn hyperaccumulating plant species (*Sedum alfredii* Hance). Plant Soil 258:181–189