

Effects of chelators on chromium and nickel uptake by *Brassica juncea* on serpentine-mine tailings for phytoextraction

Kai-Hsun Hsiao, Po-Hsu Kao, Zeng-Yei Hseu*

Department of Environmental Science and Engineering, National Pingtung University of Science and Technology, Pingtung 91201, Taiwan

Received 20 September 2006; received in revised form 16 February 2007; accepted 19 February 2007

Available online 23 February 2007

Abstract

This study compares the effect of synthetic aminopolycarboxylic acids ethylenediamine tetraacetate (EDTA) and diethylenetriamine pentaacetate (DTPA) with natural low-molecular-weight organic acids (LMWOAs) oxalic acid and citric acid as chelators for enhancing phytoextraction of Cr and Ni by *Brassica juncea* on serpentine-mine tailings. Chelator treatments were applied at doses of 0.05 and 0.10 mmol kg⁻¹ dry soils after seedlings were grown in pots for 56 days. Experimental results indicate that EDTA and DTPA were the most efficient chelators of increasing the levels of Cr and Ni in the soil solutions over time. Additionally, the reduction of plant shoot biomass caused by the two synthetic chelators exceeds that caused by the LMWOAs. The total uptake (mass removal from soil) of metals by plants was enhanced via the chelators. Experimental results supported the use of *B. juncea* for Cr and Ni phytoextraction: *B. juncea* improved the removal of Cr and Ni from serpentine-mine tailings. However, low plant biomass did not assist phytoextraction by using EDTA and DTPA, both of which carry environmental risk. Therefore, adding LMWOAs during phytoextraction can provide an environmentally compatible alternative, which may decrease the use of synthetic chelators.

© 2007 Elsevier B.V. All rights reserved.

Keywords: Chelator; *Brassica juncea*; Heavy metals; Phytoextraction; Serpentine-mine

1. Introduction

Phytoextraction of heavy metals typically persisting in the environment is a low-cost and environmentally compatible alternative to chemical techniques and, therefore, has attracted increased interest since last decade [1–3]. Furthermore, phytoextraction has significant benefits in that it causes minimal environmental disturbance, and does not adversely affect soil matrices. Thus, after successful phytoextraction, soil can be used directly in agriculture. However, phytoextraction generally removes only a very small percentage of heavy metals from contaminated soil, and can only be applied in situation with low-level contamination. For extremely contaminated sites, other approaches must be applied [3]. All plants can extract metals from soil; however, some plants have demonstrated ability to extract, accumulate and tolerate high levels of heavy metals that are toxic to other organisms. These plants are so-called hyperaccumulators that, as vegetation, control soil erosion at

contaminated sites [4–6]. Successful phytoextraction requires plants with high metal uptake capacity and high biomass production. Since most known hyperaccumulators have a low annual biomass production, considerable research is currently investigating methods that enhance the availability of heavy metals in soils and increase phytoextraction efficiency of potential accumulators [2]. Another shortcoming of hyperaccumulators during phytoextraction is not able to accumulate some metals in multi-metal contaminated soils [7,8].

To overcome these limitations, using plant with high biomass yields, such as Indian mustard (*Brassica juncea*), with a chemically enhanced method of phytoextraction has been proposed as a viable strategy for removing heavy metals from soils over a reasonable time frame [1,7,9,10]. To reach such a goal of enhanced phytoextraction, amendments must increase root uptake of metals via metal solubilization and substantially increase the speed of transfer of metals to shoots. Chelating agents, such as LMWOAs and synthetic chelators, are the most common amendments utilized in chemically assisted phytoextraction of metals from soils [8,11–14]. Chelating agents have been used in agriculture as additives in micronutrient fertilizers since the 1950s [15]. Such substances are capable of forming complexes with metal

* Corresponding author. Tel.: +886 8 7740253; fax: +886 8 7740320.
E-mail address: zyhseu@mail.npust.edu.tw (Z.-Y. Hseu).

ions, thereby increasing the bioavailability of heavy metals in soils.

Ethylenediamine tetraacetate (EDTA) is probably the chelating agent that is most efficient at increasing the solubility of heavy metals in soil solutions from the soil solid phase, thus increasing the concentration of heavy metals in plant shoot tissues [1,9,16,17]. However, most synthetic chelators form chemically and microbiologically stable complexes with heavy metals that can contaminate groundwater [18,19]. Synthetic chelators such as EDTA and diethylenetriamine pentaacetate (DTPA), typically form metal complexes with high stability constants that are degraded slowly and have been demonstrated as relatively biologically stable, even under conditions favorable to biodegradation [20,21]. In metal contaminated soils treated with EDTA, metal–EDTA complexes leached into soil pore water and persisted for several weeks [7]. As an alternative to these synthetic chelators, widespread natural sources, such as LMWOAs, can be utilized. These LMWOAs are easily biodegraded and more environmentally compatible than synthetic chelators application to the public acceptance of phytoextraction technology [12,14,22–24]. Exudation of LMWOAs by plant roots can affect the solubility of essential and toxic ions directly and indirectly: directly, through acidification, chelation, precipitation and oxidation–reduction reactions in the rhizosphere, and indirectly through the effects of LMWOAs on microbial activity, rhizosphere physical properties and root growth dynamics [11,25–27].

Serpentine terrain occupies <1% of the earth's land surface; however, it is locally abundant in ophiolite belts along tectonic plate margins. Moreover, ophiolites are sections of oceanic crust and the subjacent upper mantle that have been uplifted or emplaced and exposed within continental crustal rocks [28]. The ore deposits in serpentine terrain are scarce worldwide. Mining activities frequently generate high amounts of waste. Among these wastes, tailings have the strongest environmental effects due to high concentrations of heavy metals in tailings and increased wind and water erosion. Erosion processes in tailings pose risks by decreasing structural stability of soils and releasing heavy metals via tailing drainage. These pollutant effects can attain local and, in some cases, regional scales and impact urban activities and agricultural production. Consequently, a risk of metal uptake by humans is associated with the tailing disposal [29,30]. Previous studies generated little information regarding amendment effects on enhanced metal phytoextraction by hyperaccumulators at mine-tailing sites, particularly, few studies have explored the unique contributions and significance of LMWOAs and synthetic chelators on Cr and Ni uptake by *B. juncea* in serpentine-mine tailings. Kidd and Monterroso [31] examined the phytoextraction efficiency of a species of *Brassicaceae* (*Alyssum serpyllifolium*) for two acid mine-spoil sites in Spain. They identified that increased uptake of Cr and Ni can be attained by increasing plant biomass via fertilized with lime, nitrogen, phosphorus and potassium. Robinson et al. [32] indicated that uptake of Co and Ni by *Berkheya coddii* (*Brassicaceae*) was enhanced by sulfur fertilizer in a serpentine-spoil in New Zealand.

Studies investigating phytoextraction by *B. juncea* largely focused on issues associated with uptake of toxic metals enhanced by different chelators in contaminated sites where heavy metals were introduced into soils from wastewater discharge, hazardous waste dumping and sewage sludge application. These principal metals of concern at such sites for chelator-enhanced phytoextraction by *B. juncea* are Cd, Cu, Ni, Pb and Zn [2,3,13]. Phytoextraction must remove toxic metals from serpentine-mine tailings, and provide vegetation to control soil erosion [29,30]. Conversely, phytoextraction efficiency is related to both plant metal concentration and dry matter yield, thus the ideal plant species to remediate a contaminated site should be a high-yield crop that tolerates and accumulates target contaminants [2]. Phytomining, a common use for such plants, is fundamentally similar to phytoremediation; however, the aim of phytomining operation is to mine metals from low-grade ore bodies [4,32]. To date most phytoremediation work has focused on the accumulation of moderate-to-high levels of Cd, Cu, Pb and Zn by *B. juncea* grown on spiked or contaminated soils [1,6,14,16]; however, few studies have investigated removal of Cr and Ni for serpentine-mine tailings [32,33]. The present study has the following three objectives: (i) investigate biomass production of *B. juncea* in serpentine-mine tailings under pot experiments; (ii) explore the effects of natural and synthetic chelators on Cr and Ni mobilization in tailings and uptake of metals by *B. juncea*; and (iii) compare the efficiencies of different chelators in technology-enhanced phytoextraction by *B. juncea*.

2. Materials and methods

2.1. Serpentine-mine tailing characterization

Serpentine minerals are locally found in the eastern section of the Central Ridge and Costal Range in eastern Taiwan, adjacent to the convergent boundary of the Eurasia Plate and Philippine Sea Plate [34]. Serpentine minerals are formed by exotic blocks of ophiolite from the Philippine Sea Plate. Study tailings were obtained from a spoiled site with histories of serpentine-mining activities in eastern Taiwan for 20 years (1960–1980). The mine was exploited for serpentine and talc. The mine tailings were spread over approximately 0.4 ha of land at the mine surface that was devoid of plant growth. Five duplicated soil samples (0–5 cm) were obtained from the 0.4 ha land area and mixed well into a composite sample. The soil sample was air-dried, ground, and passed through a 2-mm sieve for pot experiments in a greenhouse and for physiochemical analysis in a laboratory.

The soil pH was measured in a mixture of soil and deionized water (1:1, w/v) with a glass electrode [35]. Total organic carbon (OC) content was determined using the Walkley-Black wet oxidation approach [36]. Total N was determined using the Kjeldhal method [37]. Total P was measured using the sodium carbonate fusion method [38]. Cation exchange capacity (CEC) and amounts of exchangeable Ca and Mg were determined using the ammonium acetate method OR (pH 7.0) [39]. The total amount of heavy metals was digested by a mixture of HF–HNO₃–HClO₄–H₂SO₄ [40]. Metal contents in

Table 1
Characteristics of the serpentine-mine tailing

pH	Texture (%)			OC ^a (%)	Total (%)		CEC ^b (cmol kg ⁻¹)	Total (mg kg ⁻¹)						
	Sand	Silt	Clay		Nitrogen	Phosphorus		Cd	Cr	Cu	Ni	Pb	Zn	Ca/Mg
6.5	5.0	31	64	0.39	0.03	0.005	69.3	0.02	3100	63.0	3400	9.12	124	0.2

^a Organic carbon.

^b Cation exchange capacity.

all solutions were determined with a flame atomic absorption spectrophotometer (FAAS) (Hitachi Z-8100, Japan). Finally, soil particle-size distribution was determined using the pipette method [41]. Table 1 shows the selected soil properties.

2.2. Pot experiments

Air-dried soil (2 kg) was weighed and transferred into a plastic pot 10 cm in diameter. Seeds of Indian mustard (*B. juncea* L.) were incubated at 25 °C on filter papers in Petri dishes containing 10 ml distilled water for 5 days. The seeds (accession no. PI426308; origin, Pakistan; plant name, K-100) were obtained from the USDA-ARS Plant Introduction Center at Iowa State University. Five germinated seeds were planted at a depth of 0.5 cm in the soil in each pot, which was fertilized with 50 mg N kg⁻¹ (ammonium sulfate), 100 mg P kg⁻¹ (calcium phosphate), and 40 mg K kg⁻¹ (potassium chloride), in a greenhouse. Greenhouse relative humidity was 70–90%, air temperature was 23–27 °C, and day length was approximate 12 h. After 1 week, the seedlings were thinned to 1 per pot. The soil moisture content was adjusted daily to 75% of its water holding capacity by weighing the pots and adding deionized water to compensate for weight loss [42]. The pot bottoms were sealed to eliminate leaching of mobilized heavy metals. One week after the seedlings were thinned, different chelators were applied to the soil. Treatments included one control (soil with no chelator) and four chelators – oxalic acid (O), citric acid (C), EDTA (E), and DTPA (D) – applied to soil surface as solutions at doses of 0.05 and 0.10 mmol kg⁻¹ dry soil for each chelator. These designed chelator levels exceeded the total moles of metals in the pore water of the native soil, and, thus, met the threshold proposed by Nowack et al. [22] for chelator-enhanced phytoextraction. Treatments were performed in triplicate in a random block design; thus, 27 pots were used.

The plant shoots, cut at the soil surface, were harvested at 56 days following chelator application. The soil was then broken up and roots were harvested by hand. The roots were washed in tap water until free of soil particles. The shoots and roots were further washed with deionized water, oven-dried at 70 °C for 24 h, weighed, and then ground and passed through a 1.0 mm sieve. Aliquots of plant powder (0.5 g dried weight) were digested overnight in 14 M HNO₃ (5 ml) and 30% H₂O₂ (v/v; 10 ml) and heated at 120 °C for 2 h [43]. The digested solutions were filtered using Whatman No. 42 filter paper and diluted to 50 ml with deionized water. The concentrations of Cr and Ni in the digested solution were determined by FAAS. To evaluate the ability of chelators to promote the transfer of Cr and Ni from roots to shoots, the metal transfer factor (TF) was

determined from compartment concentrations of Cr and Ni as $TF = C_{shoot}/C_{root}$. The bioaccumulation factor (BF) for evaluating plant hyperaccumulation ability was also determined by the ratio of metal concentrations in plant shoot to that in soil solutions at day 56.

2.3. Soil solution collection and analysis

To examine the impacts of chelators on the solubilization of Cr and Ni in the serpentine-spoiled soil, one soil moisture sampler (2-mm in diameter) with no ion exchange capacity (RSMS: Rhizosphere Research Product, Eijkelkamp, Giesbeek, The Netherlands) was inserted vertically into the center of each pot. Soil solution samples were collected by suction using a syringe at regular intervals in the soil with chelators on days 7, 14, 28, 42 and 56. All soluble Cr and Ni in these solutions were directly analyzed by FAAS.

2.4. Quality assurance, quality control and statistical analysis

A standard reference material, SRM 1573a (tomato leaves) obtained from the National Institute of Standards and Technology (NIST), USA, was digested in triplicate and analyzed using the plant digestion method described for quality assurance and control purposes. Recovery of Cr and Ni was 80 and 111%, respectively. A blank was run for each digestion procedure to correct the measurements. For sets of every 10 samples, a procedure blank and spiked sample, involving all reagents, was performed to check for interference and cross-contamination.

Significant differences between concentrations of heavy metals in soil solutions and plants, and plant yields following different treatments were analyzed using Tukey's multiple range tests [44]. Statistical significance was $p < 0.05$.

3. Results and discussion

3.1. Effect of chelators on pH change in the tailings

The amount of Ca obtained from soils was significantly higher than for Mg regarding higher plant growth [15]. However, the soils derived from serpentine, displayed strong chemical fertility limitations due to a very low Ca/Mg ratio and limited available phosphorus [45–47]. Therefore, the application rate for calcium phosphate amended in the study soil was 2-fold the general recommended rate for agricultural soils used in vegetable growth in Taiwan. The study soil was weakly acidic (Table 1). The high clay content and CEC value generated a

strong adsorption capacity of nutrients and heavy metals on the soil surface; however, the organic carbon content and level of N were very low, as were P and the Ca/Mg ratio. The fertilization and potential acidification was offset by adding chelators to the soil.

The solubility of heavy metal increases as acidity increases when organic acids are added to the soil [25]; however, lowering the pH for solubilization of heavy metals is unfavorable for metal–chelator stability [21]. Meers et al. [48] defined the ligand effect as the mmol concentration of heavy metals mobilized in the soil solution per mmol chelator added to the soil. The benefit of fertilization is increased plant biomass, and promotion of the ligand effect by buffering soil pH. At the initial period of plant growth, the pH values for all treated soils were higher than the native soil pH (Table 2). This increased pH likely resulted from fertilizer application. Moreover, application of chelators caused decreased the pH at day 56. The pH fluctuations in most cases over time were significant ($p < 0.05$), particularly for the EDTA and DTPA treatments, whereas the pH value for EDTA- and DTPA-treated soils were lower than those for oxalic and citric acid treatments.

3.2. Effect of chelators on the mobility of chromium and nickel in the tailings

The total contents of Cr and Ni in the tailings were considerably higher than the control values for soils according to Taiwan's Soil and Groundwater Pollution Remediation Act (250 mg Cr kg⁻¹ and 200 mg Ni kg⁻¹). The total level of Cr was similar to that of Ni in the study tailing soil (Table 1); however, the bioavailability and processes of solubilization differed markedly between Cr and Ni [47]. Table 3 presents the mobilization of Cr assessed by its level in soil solution during the pot experiments. Over time, Cr concentrations following all treatments increased irregularly after 56 days. However, Cr was gradually released from the solid phase and was detectable in the control soil solution and soil treated with oxalic and citric acid on day 28. The synthetic chelators EDTA and DTPA were the most efficient amendments for increasing concentrations of Cr in soil solutions. The concentrations of Cr in soil solutions with treated with synthetic chelators were always significantly higher those with treated with natural LMWOAs. In soils treated with oxalic and citric acid, the pattern of Cr concentrations was characterized by an initial build-up phase for the first 42 days, followed by a final reduction phase at day 56, likely because the natural organic acids were easily biodegraded, thereby reducing the complex capacity of the metal. Additionally, differences in Cr concentrations between oxalic acid and citric acid treatments were negligible at day 56.

In serpentine soils, Ni is not strongly held by clay and Fe-oxyhydroxide surfaces relative to other transition elements; consequently, Ni was more mobile than Cr in the serpentinitic landscape surrounding the study area [47]. Thus, Ni in the soil solutions for all treatments during the experimental period was always at detectable levels (Table 4). The concentrations of Ni in most cases were much higher than those of Cr, particularly in soils to which synthetic chelators EDTA and DTPA were added.

Table 2
The pH values of the serpentine-mine tailing treated with chelators during 56-day plant growth

Day	Control	Oxalic acid (mmol kg ⁻¹)		Citric acid (mmol kg ⁻¹)		EDTA (mmol kg ⁻¹)		DTPA (mmol kg ⁻¹)	
		0.05	0.10	0.05	0.10	0.05	0.10	0.05	0.10
7	7.11 ± 0.02 A b ^a	7.12 ± 0.03 A b	7.35 ± 0.02 A a	7.41 ± 0.05 A a	7.33 ± 0.06 A a	7.02 ± 0.08 A b	7.08 ± 0.08 A b	7.23 ± 0.07 A a	7.28 ± 0.07 A a
14	7.22 ± 0.08 A b	7.04 ± 0.07 A bc	7.14 ± 0.06 A b	7.35 ± 0.12 A a	7.21 ± 0.09 A b	7.00 ± 0.07 A c	6.84 ± 0.12 A d	7.10 ± 0.07 A b	7.04 ± 0.07 B bc
28	7.21 ± 0.04 A b	7.02 ± 0.08 A c	7.15 ± 0.07 A b	7.30 ± 0.04 A a	7.15 ± 0.06 A b	6.91 ± 0.08 A c	6.80 ± 0.02 A d	7.04 ± 0.07 B c	7.04 ± 0.06 B c
42	7.30 ± 0.11 A a	7.03 ± 0.08 A b	7.03 ± 0.07 B b	7.05 ± 0.07 B b	7.05 ± 0.09 B b	6.72 ± 0.10 B c	6.25 ± 0.12 B d	7.00 ± 0.09 B b	6.84 ± 0.11 C c
56	7.24 ± 0.08 A a	6.89 ± 0.04 A b	6.89 ± 0.12 B b	6.92 ± 0.08 B b	6.92 ± 0.05 B b	6.54 ± 0.14 B c	6.24 ± 0.09 B d	6.50 ± 0.13 C c	6.45 ± 0.07 D c

^a Values followed by different uppercase letters (A–D) within the same column and by different lowercase letters (a–c) within the same row are different significantly at 5% level according to the Tukey's multiple range test.

Table 3
Chromium concentration (mg l^{-1}) in the soil solution of the serpentine-mine tailing treated with chelators during 56-day plant growth

Day	Control	Oxalic acid (mmol kg^{-1})		Citric acid (mmol kg^{-1})		EDTA (mmol kg^{-1})		DTPA (mmol kg^{-1})	
		0.05	0.10	0.05	0.10	0.05	0.10	0.05	0.10
7	ND ^a	ND	ND	ND	ND	0.15 ± 0.08 C b ^b	0.21 ± 0.07 C a	0.11 ± 0.08 C b	0.19 ± 0.05 C ab
14	ND	ND	ND	ND	ND	0.29 ± 0.09 C b	0.48 ± 0.08 C a	0.18 ± 0.09 C c	0.25 ± 0.07 C b
28	0.21 ± 0.03 B c	0.28 ± 0.08 B c	0.28 ± 0.07 C c	0.32 ± 0.07 A c	0.45 ± 0.07 A bc	0.66 ± 0.08 B ab	0.75 ± 0.15 B a	0.52 ± 0.12 B b	0.52 ± 0.13 B b
42	0.18 ± 0.04 B f	0.51 ± 0.14 A d	0.56 ± 0.08 A cd	0.21 ± 0.04 B f	0.33 ± 0.03 B e	1.21 ± 0.10 A b	1.42 ± 0.42 B a	0.71 ± 0.07 B c	0.73 ± 0.09 B c
56	0.36 ± 0.07 A c	0.26 ± 0.09 B c	0.40 ± 0.09 B c	0.18 ± 0.05 B cd	0.49 ± 0.04 A c	1.48 ± 0.21 A b	3.05 ± 0.21 A a	1.67 ± 0.16 A b	1.46 ± 0.18 A b

^a ND: not detectable ($<0.08 \text{ mg l}^{-1}$).

^b Values followed by different uppercase letters (A–C) within the same column and by different lowercase letters (a–f) within the same row are different significantly at 5% level according to the Tukey's multiple range test.

Table 4
Nickel concentration (mg l^{-1}) in the soil solution of the serpentine-mine tailing treated with chelators during 56-day plant growth

Day	Control	Oxalic acid (mmol kg^{-1})		Citric acid (mmol kg^{-1})		EDTA (mmol kg^{-1})		DTPA (mmol kg^{-1})	
		0.05	0.10	0.05	0.10	0.05	0.10	0.05	0.10
7	0.04 ± 0.01 A f ^a	0.07 ± 0.02 A f	0.09 ± 0.01 B ef	0.14 ± 0.02 A e	0.24 ± 0.04 A d	8.15 ± 0.24 B b	11.9 ± 1.01 A a	2.45 ± 0.63 C c	9.50 ± 0.97 C b
14	0.03 ± 0.01 AB c	0.05 ± 0.01 AB c	0.03 ± 0.01 C c	0.08 ± 0.01 C b	0.08 ± 0.01 CD b	10.6 ± 1.07 A a	12.1 ± 1.11 A a	11.7 ± 1.54 A a	12.5 ± 1.03 B a
28	0.05 ± 0.02 A e	0.06 ± 0.02 A e	0.13 ± 0.02 A d	0.14 ± 0.03 A d	0.15 ± 0.03 B d	10.9 ± 0.81 A c	12.6 ± 0.89 A b	10.8 ± 1.04 A c	17.8 ± 2.26 A a
24	0.02 ± 0.01 B d	0.04 ± 0.01 B d	0.04 ± 0.01 C d	0.08 ± 0.01 C c	0.09 ± 0.01 C c	8.52 ± 0.28 B a	7.65 ± 1.21 B a	5.65 ± 0.97 B b	5.30 ± 1.07 D b
56	0.03 ± 0.01 AB d	0.06 ± 0.02 A cd	0.08 ± 0.03 B c	0.10 ± 0.01 B c	0.12 ± 0.04 C c	11.4 ± 0.67 A a	10.4 ± 0.99 A a	4.50 ± 1.00 B b	5.15 ± 0.88 D b

^a Values followed by different uppercase letters (A–D) within the same column and by different lowercase letters (a–f) within the same row are different significantly at 5% level according to the Tukey's multiple range test.

Furthermore, the Ni concentrations in the soil solutions treated with EDTA and DTPA were markedly higher than those in the control soil and those treated with oxalic and citric acid. Similar to Cr, the mobilization of Ni in soils treated with oxalic and citric acid was largely dose independent.

The increase in the application rate for EDTA and DTPA generally increased the concentrations of Cr and Ni in most soil solutions, implying that no saturation of metals complexation was noted within the range of EDTA and DTPA applied. Increases in the doses of EDTA and DTPA applied increased the mobilization of heavy metals; however, environmental risk associated with synthetic chelators is consequently increased regardless of the EDTA and DTPA application rate under such saturation [1,16]. The relationships between metal mobilization and application rate for oxalic and citric acid are similar to those for EDTA and DTPA, but not significant in all cases for oxalic and citric acid. This experimental finding suggests that the stability of metal–chelator complexes was impacted by competitor ions, particularly by abundant base cations in serpentine soils, such those in the serpentine-mine tailings in this study. Chelators form weak complexes with base cations Ca and Mg than with Cr and Ni; however, the reduced stability is compensated by the relatively higher concentrations of base cations in the tailings. This competition effect was high for oxalic and citric acid, resulting in increased mobilization of Cr and Ni by the two LMWOAs in this study. More resistance in biodegradation was generally identified in the chelators complexed with metals than those without metals; however, contradictory results have been obtained concerning the biodegradation of EDTA and DTPA in soils and sediments [19,24]. Some studies identified no biological breakdown, whereas other studies observed a slow microbial decomposition under aerobic conditions. No mineralization was identified under anaerobic conditions for aminopolycarboxylic acids [21]. Hence, the question regarding the importance of biodegradation for these chelators remains unanswered.

3.3. Dry matter production of plant

At harvest, the plants were in the nutrient growth stage without flowering. Consequently, no seeds were generated. Mean plant height for all treatments was 18–63 cm, and only DTPA treatment significantly reduced plant height. Additionally, the mean number of leaves per plant in the DTPA treatment was significantly lower than those for other treatments (Table 5). Wu et al. [13] demonstrated that the leaves of *B. juncea* developed numerous brown dots at 2–4 days after adding 3.0 mmol kg⁻¹ EDTA to the soil; the whole leaves yellowed and died slowly,

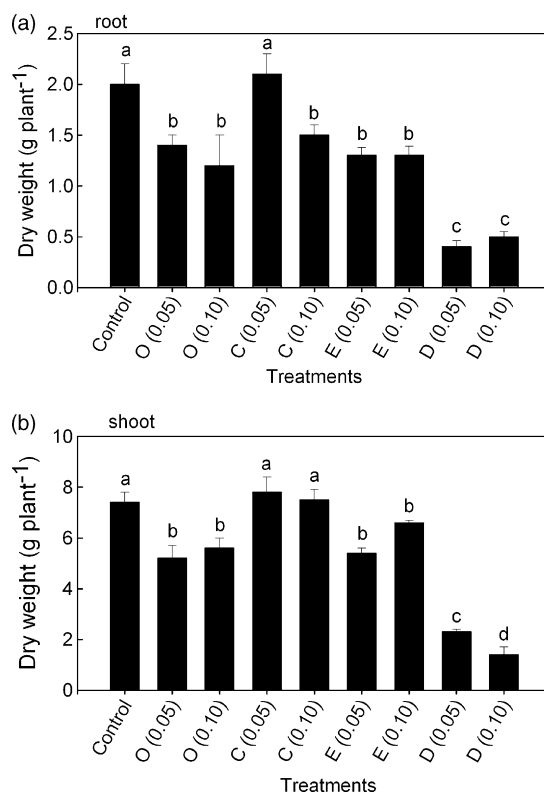


Fig. 1. Dry matter yield (g plant⁻¹) in the root (a) and shoot (b) of *B. juncea* grown on the tailings treated with different chelators. Different letters following the vertical bars are different significantly between treatments at 5% level according to the Tukey's multiple range test. O, oxalic acid; C, citric acid; E, EDTA; D, DTPA. Numbers in parenthesis of treatment are the doses of the chelating agent (mmol kg⁻¹).

indicating phytotoxicity of the EDTA–metal complex. In this study, some chlorosis of leaves was found for the EDTA and DTPA treatments under experimental conditions at day 56, particularly in old leaves. Compared to the control treatment, dry weights of plant roots were significantly ($p < 0.05$) reduced for all chelator treatments, with the exception of 0.05 mmol kg⁻¹ citric acid (Fig. 1a). The dry matter yields for roots decreased as the application rate of oxalic and citric acids increased; however, these yields were only significantly different for 0.05 and 0.10 mmol kg⁻¹ citric acid treatments. The yields were not dose-dependent for the EDTA and DTPA treatments. For each chelator treatment, the dry matter yields of shoots were much higher than those of roots. Compared to the dry weight of control shoots, the addition of any chelator significantly reduced the dry matter production of shoots, except for the addition of citric acid (Fig. 1b). However, the largest reduction in dry matter

Table 5

Shoot height and leaf number per plant of Indian mustard grown on the serpentine-mine tailing treated with different chelators

	Control	Oxalic acid		Citric acid		EDTA		DTPA	
		0.05	0.10	0.05	0.10	0.05	0.10	0.05	0.10
Shoot height (cm)	60 ± 10 a ^a	42 ± 10 a	45 ± 8 a	55 ± 11 a	63 ± 6 a	40 ± 10 a	46 ± 6 a	20 ± 4 b	18 ± 5 b
Number of leaves	24 ± 4 a	18 ± 5 a	15 ± 5 a	22 ± 3 a	20 ± 5 a	14 ± 4 ab	13 ± 5 ab	5 ± 2 b	6 ± 4 b

^a Values followed by different letters (a and b) within the same row are different significantly at 5% level according to the Tukey's multiple range test.

was for the addition of DTPA, suggesting that the phytotoxicity of DTPA is higher than that of EDTA and LMWOAs [21]. Under a dose of $0.10 \text{ mmol kg}^{-1}$ DTPA, shoot dry weight was lower than that for $0.05 \text{ mmol kg}^{-1}$. However, the shoot yields were not dose-dependent for oxalic and citric acids and EDTA treatments.

3.4. Plant uptake of chromium and nickel

The Cr concentrations in the roots of *B. juncea* under all chelator treatments were significantly ($p < 0.05$) higher than those for the control soil (Fig. 2a). The uptake of Cr in roots was markedly enhanced with EDTA. The efficiencies of Cr uptake in roots was much higher than those reported by Gupta and Sinha [10], who found that Cr was not detectable in the *B. juncea* grown on a fly-ash amended soil in India. Furthermore, the Cr concentrations in the roots of *B. juncea* in this work were slightly higher than those in the roots of *A. serpyllifolium* on mine-spoil soils in Spain [31]. Although oxalic and citric acid had a positive effect on Cr concentrations in plant roots (Fig. 2a), the Cr concentrations in soil solutions for most cases were not clearly influenced by addition of oxalic and citric acid, as compared to the control treatment (Table 3). Fig. 2b shows the Cr concentrations in *B. juncea* shoots for all treatments. Compared with control soil, no chelator, except for $0.05 \text{ mmol kg}^{-1}$ citric acid and $0.10 \text{ mmol kg}^{-1}$ EDTA, increased Cr levels in shoots. Therefore, EDTA had the highest efficiency in uptake of

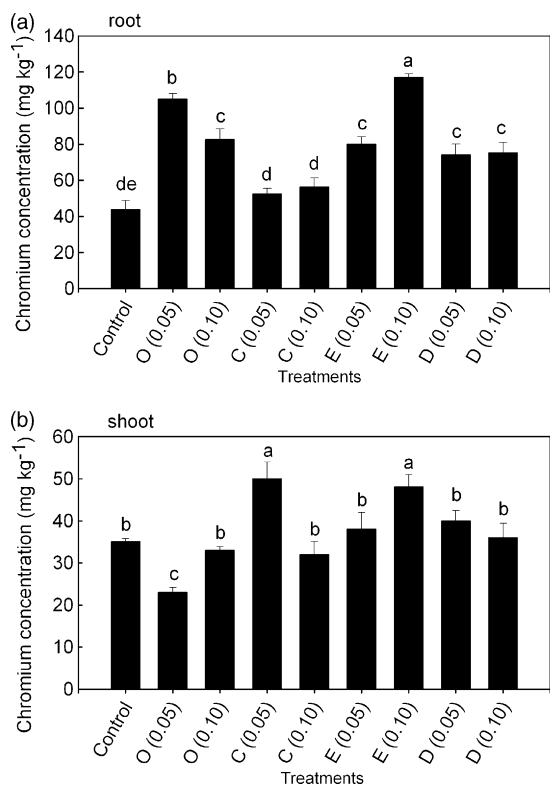


Fig. 2. Chromium concentration (mg kg^{-1}) in the root (a) and shoot (b) of *B. juncea* grown on the tailings treated with different chelators. The meanings of letters following the vertical bars and abbreviations of treatments are explained in Fig. 1.

Table 6

The metal transfer factor (TF) and bioaccumulation factor (BF) of Indian mustard grown on the serpentine-mine tailing treated with different chelators

Treatment	Cr	Ni
Transfer factor ^a		
Control	$0.80 \pm 0.17 \text{ A}^b$	$0.50 \pm 0.17 \text{ C}$
$0.05 \text{ mmol kg}^{-1}$ oxalic acid	$0.22 \pm 0.10 \text{ C}$	$1.15 \pm 0.28 \text{ A}$
$0.10 \text{ mmol kg}^{-1}$ oxalic acid	$0.40 \pm 0.12 \text{ B}$	$0.89 \pm 0.16 \text{ A}$
$0.05 \text{ mmol kg}^{-1}$ citric acid	$0.95 \pm 0.35 \text{ A}$	$1.43 \pm 0.34 \text{ A}$
$0.10 \text{ mmol kg}^{-1}$ citric acid	$0.57 \pm 0.21 \text{ B}$	$1.23 \pm 0.23 \text{ A}$
$0.05 \text{ mmol kg}^{-1}$ EDTA	$0.48 \pm 0.27 \text{ B}$	$0.93 \pm 0.14 \text{ A}$
$0.10 \text{ mmol kg}^{-1}$ EDTA	$0.41 \pm 0.12 \text{ B}$	$0.87 \pm 0.14 \text{ A}$
$0.05 \text{ mmol kg}^{-1}$ DTPA	$0.54 \pm 0.11 \text{ B}$	$0.70 \pm 0.11 \text{ B}$
$0.10 \text{ mmol kg}^{-1}$ DTPA	$0.48 \pm 0.14 \text{ B}$	$0.58 \pm 0.10 \text{ BC}$
Bioaccumulation factor ^a		
Control	$120 \pm 27 \text{ B}$	$27 \pm 10 \text{ B}$
$0.05 \text{ mmol kg}^{-1}$ oxalic acid	$92 \pm 32 \text{ B}$	$58 \pm 12 \text{ B}$
$0.10 \text{ mmol kg}^{-1}$ oxalic acid	$114 \pm 20 \text{ B}$	$38 \pm 21 \text{ B}$
$0.05 \text{ mmol kg}^{-1}$ citric acid	$250 \pm 56 \text{ A}$	$312 \pm 84 \text{ A}$
$0.10 \text{ mmol kg}^{-1}$ citric acid	$130 \pm 25 \text{ B}$	$267 \pm 70 \text{ A}$
$0.05 \text{ mmol kg}^{-1}$ EDTA	$48 \pm 12 \text{ C}$	$14 \pm 10 \text{ BC}$
$0.10 \text{ mmol kg}^{-1}$ EDTA	$32 \pm 16 \text{ C}$	$8 \pm 6.2 \text{ C}$
$0.05 \text{ mmol kg}^{-1}$ DTPA	$44 \pm 21 \text{ C}$	$11 \pm 5.2 \text{ BC}$
$0.10 \text{ mmol kg}^{-1}$ DTPA	$37 \pm 10 \text{ C}$	$6 \pm 3.7 \text{ C}$

^a TF is defined as the ratio of metal concentration in plant shoot to that in plant root, and BF as the ratio of metal concentration in plant shoot to that in soil solution at the 56 day.

^b Values followed by different letters (A–C) within the same column are different significantly at 5% level according to the Tukey's multiple range test.

Cr by *B. juncea* roots and shoots. All TF values of Cr for the plant were < 1.0 (Table 6); thus, the transport of Cr from root to shoot is restricted despite the addition of chelators. The Cr concentrations in the *B. juncea* shoots for all treatments were $21\text{--}50 \text{ mg kg}^{-1}$, lower than those in a contaminated soil inoculated with bacteria to enhance *B. juncea* growth [26]. However, the Cr concentrations in the *B. juncea* shoots in this work are much higher than those ($< 15 \text{ mg kg}^{-1}$) in the shoots of *B. juncea* in a Cr-spiked soil treated with 2.0 mmol kg^{-1} EDTA [49].

Chromium(III) is typically considered more stable than Cr(VI) in soils; however, Cr(VI) forms chromates and dichromates, which are soluble over a wide pH range and mobile in soil and groundwater. Hexavalent Cr is a primary toxic and mutagenic contaminant. Chromium levels in the foliar portions of plants are normally $< 1 \text{ mg kg}^{-1}$ over a wide range of soil Cr concentrations [50]. These low levels of Cr in plants are associated with the low phytoavailability of trivalent Cr. However, some plants, such as *B. juncea*, can accumulate appreciable quantities of Cr [49]. Additionally, Cr concentrations in roots were higher than those in shoots, implying that Cr is not easily translocated within *B. juncea*. At low Cr soil concentrations, Cr has high transfer mobility from roots to shoots, and when roots take up additional Cr from soils, transfer efficiency from roots to shoots declines. This may be a mechanism for the plant uses to survive in environments with high Cr levels (Table 3). Hexavalent Cr is easily absorbed by an active mechanism in the roots of plant; however, trivalent Cr is absorbed by plants to a lesser degree through a passive mechanism and retained by cation-exchange sites on cell walls. Moreover, precipitation of Cr in

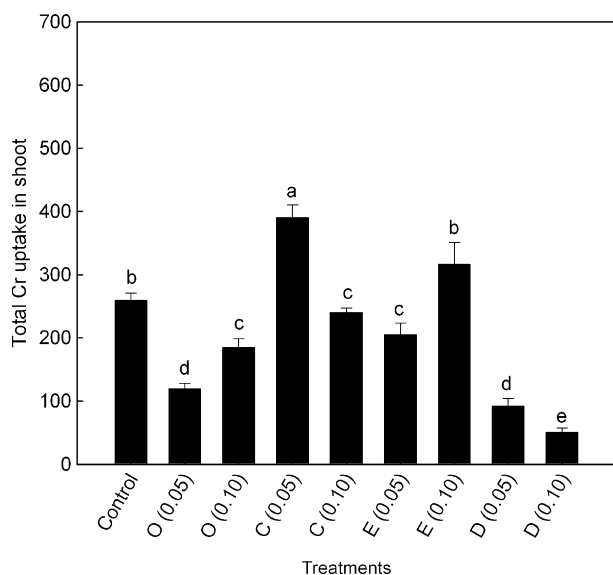


Fig. 3. Total Cr uptake in shoot ($\mu\text{g plant}^{-1}$) from the tailings treated with different chelators. The abbreviations of treatments are explained in Fig. 1. Different letters followed the vertical bars are different significantly at 5% level according to the Tukey's multiple range test.

root cell vacuoles results in low translocation of Cr from roots into shoots with low injury [51]. Han et al. [49] determined that Cr concentrations in lower leaves (old leaves) of *B. juncea* in Cr-contaminated soils are 2.5 times higher than those in upper leaves (new leaves). This finding indicates that Cr is less mobile in the shoots of *B. juncea* grown in soils, particularly on soils with high concentrations of trivalent Cr.

Total uptake of Cr in shoots grown in LMWOA-treated soil is higher than that from the EDTA and DTPA treatments (Fig. 3), indicating that the efficiency in cleaning up Cr by phytoremediation with LMWOAs is comparable to that achieved with synthetic chelators from serpentine-mine tailings. The total uptake of Cr in the control soil is higher than those achieved with oxalic acid, EDTA and DTPA treatments, and $<0.05 \text{ mmol kg}^{-1}$ for citric acid treatment, suggesting that *B. juncea* may show discernable phytoextraction efficiency without any chelator. Nevertheless, many studies have identified lower effectiveness of LMWOAs, such as citric and oxalic acid, in inducing accumulation of metals in plants compared with synthetic chelators [7,13,14]. The additions of DTPA in this study clearly decreased total uptake of Cr and Ni compared with the control treatment, causing increased concentrations of Cr and Ni in the soil solution. This reduction in the metal uptake was attributed to competition with the plant's own metal-binding agents, thereby causing the Cr and Ni to diffuse downward to the root system of the plant [32]. Bioaccumulation factors for Cr in all treatments were 32–250, significantly higher than values found for *A. serpyllifolium* (up to 28) grown on two mine-spoiled soils [31]. Furthermore, the BF values in this study decreased with increasing Cr concentrations in the soil solution. Notably, the BF value for the control treatment was higher than those for all chelator treatments, except that for citric acid. Adding EDTA to soil did not increase total uptake of Cr in *B. juncea* shoots, also indicated by the low TF and BF values compared with

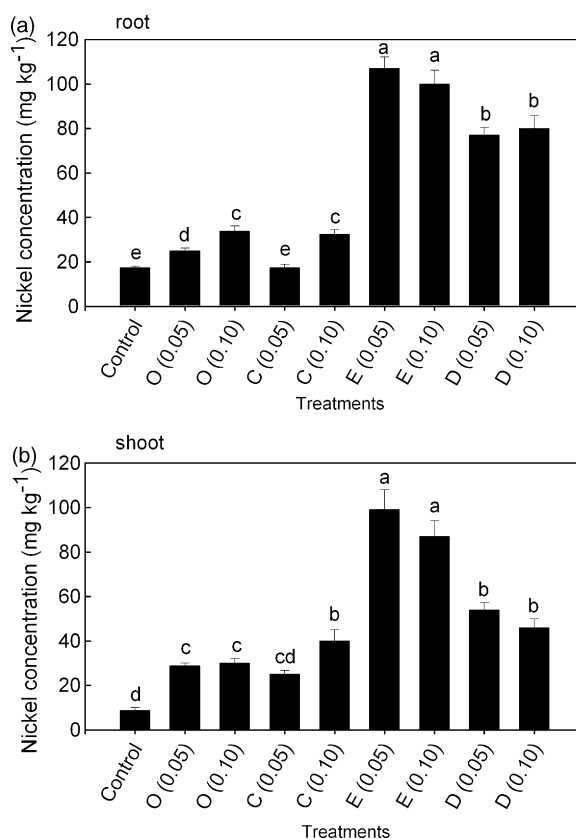


Fig. 4. Nickel concentration (mg kg^{-1}) in the root (a) and shoot (b) of *B. juncea* grown on the tailings treated with different chelators. The meanings of letters following the vertical bars and abbreviations of treatments are explained in Fig. 1.

those of the control soil. However, EDTA stimulated translocation of Cr from soils to roots. The low bioaccumulation of Cr is not surprising as Cr is considered an immobile element in soils, particularly when abundant trivalent Cr exists in chromite and Fe oxides from serpentinites [47]. Nevertheless, Fernandez et al. [45] determined that foliage of some agricultural crops (i.e., sugarbeet, cabbage, pasture) grown on serpentine soils in northwestern Spain can accumulate considerable amounts of Cr, despite the low EDTA-extractable amounts in the soils. Some of this stimulated translocation may have been due to non-specific stripping of Cr from root cell walls. Application of both EDTA and DTPA resulted in significant increases in soluble Cr in soil. Thus, synthetic chelator treatments did not substantially increase Cr removal by the plants, but rendered a larger soil Cr fraction vulnerable to loss processes with consequent potential environmental risk.

Large variations in Ni concentrations in the plant roots were measured for all treatments (Fig. 4a). The concentrations of Ni in the roots were lower than those of Cr in the roots for each treatment. In the control and oxalic and citric acid treatments, concentrations of Cr were much higher than those of Ni in the soil solutions during days 28–56, and concentrations of Cr in roots were still higher than those of Ni. Conversely, for the EDTA and DTPA treatments, Ni concentrations in soil solutions were markedly higher than those of Cr; however, the Ni concentrations in roots were lower than those of Cr. The Ni levels in roots

in this study were lower than those measured by Gupta and Sinha [10], which ranged from 79.4 to 196 mg kg⁻¹ for a soil amended with fly ash, and those obtained by Nascimento et al. [8], ranging from 143 to 212 mg kg⁻¹ for a Ni-spiked soil. However, the phytoextracted concentrations of Ni in *B. juncea* roots in this study were much higher than those measured for *A. serpyllifolium* in mine-spoiled soils from Spain [31]. Fig. 4b shows the concentrations of Ni in *B. juncea* shoots. In the control soil, the Ni concentration in shoots was only 8.81 mg kg⁻¹, much lower than that of Cr, indicating that *B. juncea* absorbed more Cr than Ni in the native serpentine-spoil. However, the uptake of Ni in shoots was significantly enhanced with all chelators, particularly with EDTA treatments. Therefore, EDTA was the most effective chelator not only for plant uptake of Ni, but also for Cr. The two EDTA treatments produced a significantly higher concentration of Ni in shoots, as compared with the control and the other LMWOAs and DTPA applications. Furthermore, the levels of Ni in shoots were similar to those for Cr for all chelator treatments except EDTA. The concentrations of Ni in *B. juncea* shoots with EDTA treatments in this study were considerably lower than those in a Ni-spiked soil obtained by Nascimento et al. [8]. Nevertheless, the dose of EDTA was as high as 10 mmol kg⁻¹ dry soil; thus, the dry matter weights of plant shoots reported by Nascimento et al. [8] were much lower than those measured in this study. This difference is attributable to the phytotoxicity effect of large amounts of EDTA on *B. juncea* growth. However, the concentrations of Ni in *B. juncea* shoots for all chelators was markedly higher than those obtained for *B. rapa* with 1.0 mmol kg⁻¹ EDTA and for *Helianthus annuus* with 0.8 mmol kg⁻¹ EDTA in dredged sediments [24,48]. Nevertheless, the TF values for Ni in oxalic and citric acid treatments exceeded significantly that for the control treatment; however, the TF values for EDTA and DTPA did not exceed markedly that for the control treatment (Table 6), suggesting that long distance translocation of metal–synthetic chelator complexes is poorer than that of metal–LMWOA complexes in *B. juncea*. This result is in agreement with those obtained by Senden et al. [52] and Evangelou et al. [6].

Unlike Cr, Ni is easily mobilized during weathering, is relatively stable in aqueous solutions, and can migrate over long distances. Additionally, Ni formed stronger complexes than Cr with organic acids [50]. The Ni concentration in a hyperaccumulating plant is strongly correlated with the soluble Ni concentration in a serpentine soil [33], and, thus, Pearson's correlations for concentrations of Ni (mg kg⁻¹) in roots and shoots of *B. juncea*, total uptake in shoots and Ni in soil solution collected on day 56 day are 0.77, 0.64 and 0.37 ($p < 0.05$); however, those of Cr are all insignificant in this study. Maximum Ni concentrations up to 98 mg kg⁻¹ in *B. juncea* shoots in this study (Fig. 4b), was markedly lower than the 1000 mg kg⁻¹ value utilized by Brooks et al. [4] to define Ni hyperaccumulation. High clay content and CEC value in this study (Table 1) is probably another reason accounting for the low recovery of metals from soil by *B. juncea* because of the high Ni adsorption capacity of the soil. The Ni concentrations in plants grown on soils other than those with excessive Ni levels are generally <10 mg kg⁻¹, and those <50 mg kg⁻¹ can be considered excessive [15]. The

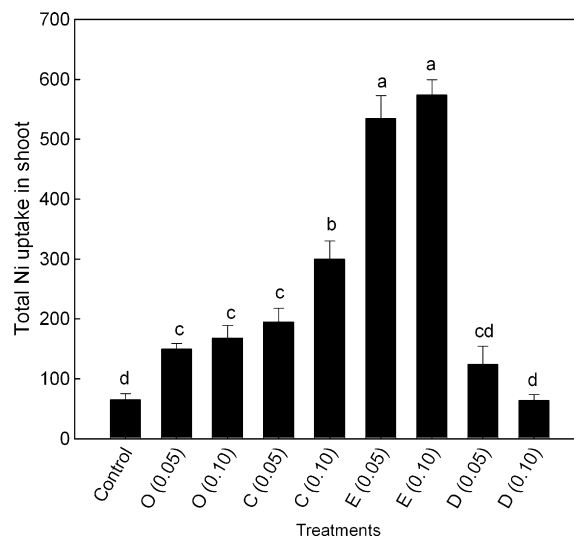


Fig. 5. Total Ni uptake in shoot ($\mu\text{g plant}^{-1}$) from the tailings treated with different chelators. The abbreviations of treatments are explained in Fig. 1. Different letters followed the vertical bars are different significantly at 5% level according to the Tukey's multiple range test.

concentration and total uptake of Ni in shoots grown in soil treated with LMWOAs was lower than those treated with EDTA (Figs. 4b and 5). This experimental result for the difference in Ni hyperaccumulation between synthetic chelators and LMWOAs agreed with findings obtained by Wu et al. [13] and Quartacci et al. [14].

3.5. Implications for phytoextraction potential

The damage caused by EDTA and DTPA to the biomass production of *B. juncea* suggests that the two synthetic chelators are non-selective when extracting metals; consequently, these chelators extract various metals, including various non-essential and/or toxic ions, that adversely affect plant growth [53]. However, long distance translocation from plant roots to shoots is unfavorable for metals complexed with EDTA and DTPA. Additionally, microbial activity in the soil was reduced by EDTA and DTPA [21], such that soil fertility was reduced. All chelators in this study increased root uptake of Cr and Ni by metal solubilization; however, only oxalic and citric acids increased the speed of the transfer of Cr and Ni from roots to shoots. Thus, BF values with oxalic and citric acid treatments were much higher than those with EDTA and DTPA, despite the BF value for Cr in the control treatment being higher than those for most chelators (Table 6). The mass of Cr and Ni in each pot soil was 6200 and 6800 mg, respectively, but total Cr and Ni uptake per plant with any treatment was much lower than 1.0 mg during the growth period. This low total uptake demonstrates that this study requires more than 6000 times of *B. juncea* growth to remove Cr and Ni from the study soil and to meet the limits in Taiwan's Soil and Groundwater Pollution Remediation Act.

Current theories regarding the translocation of metals from plant roots to shoots propose that the responsible chelators are phytochelatin and organic acids, such as oxalic acid and citric acid, the latter translocating via the xylem [52]. All carrier

molecules, including chelators, regardless of their task and origin, have limited binding capacity. Thus, depending on the number of binding sites, these molecules can carry only a restricted number of molecules or ions. Assuming that LMWOAs have a positive effect on the mobility of heavy metals in the serpentine-mine tailings, increased amounts of Cr and Ni are absorbed by roots and translocated to shoots via carriers. The advantage of LMWOA-treated plants is that natural organic acid is significantly more environmentally compatible than EDTA and DTPA over a short time period. Plants must be selected that can tolerate high toxic metal concentrations, i.e., that have hyperaccumulator properties. Evangelou et al. [6] suggested using a combination of natural chelators and a plant with a high biomass and adequate metal tolerance to increase phytoextraction efficiency. In addition to high metal uptake capacity and high biomass production for a successful hyperaccumulator in phytoremediation, as recommended by Baker et al. [2], a high total uptake and good plant growth (no toxicity symptoms) can be improved with different amendments. This work reached the aims of removing Cr and Ni from serpentine-mine tailings, and of vegetation maintain and enhanced groundwater transpiration by *B. juncea*.

4. Conclusion

The concentrations of Cr and Ni in the soil solutions of soil treated with EDTA and DTPA were higher than those for the control soil and soils treated with oxalic acid and citric acid during the experiment; however, the two synthetic chelators produced a greater reduction in plant biomass than did LMWOAs, despite relatively high levels of Cr and Ni in *B. juncea* with the two synthetic chelators. Although both EDTA and DTPA increase the concentrations of Cr and Ni in *B. juncea*, they also result in low plant biomass and are hazardous to environment. Therefore, other alternative amendments should be explored. Based on experimental results, LMWOAs can enhance the tolerance of *B. juncea* grown on the serpentine-mine tailings to accumulated Cr and Ni. Adding can provide an environmentally compatible alternative that may decrease the use of synthetic chelators in phytoremediation for Cr- and Ni-contaminated sites. Therefore, further research should investigate other naturally occurring organic acids to replace synthetic chelators.

Acknowledgements

The authors would like to thank the National Science Council of the Republic of China, Taiwan for financially supporting this research under Contracts No. NSC 95-2221-E-020-019. Miss Chang, I.S. is appreciated for her assistance in the field sampling and laboratory analysis.

References

- [1] M.J. Blaylock, D.E. Salt, S. Dushenkov, O. Zakharova, C. Gussman, Y. Kapulnik, B.D. Ensley, I. Raskin, Enhanced accumulation of Pb in Indian mustard by soil-applied chelating agents, *Environ. Sci. Technol.* 31 (1997) 860–865.
- [2] A.J.M. Baker, S.P. McGrath, R.S. Reeves, J.A.C. Smith, Metal hyperaccumulator plant: a review of the ecology and physiology of a biological

- resource for phytoremediation of metal-polluted soils, in: N. Terry, G.N. Bannelos (Eds.), *Phytoremediation of Contaminated Soil and Water*, Lewis Publ., FL, USA, 1998, pp. 85–107.
- [3] M.M. Lasat, Phytoextraction of toxic metals: a review of biological mechanisms, *J. Environ. Qual.* 31 (2002) 109–120.
- [4] R.R. Brooks, J. Lee, R.D. Reeves, T. Jaffré, Detection of nickeliferous rocks by analysis of berbarium specimens of indicator plants, *J. Geochem. Explor.* 7 (1977) 49–57.
- [5] S.D. Cunningham, D.W. Ow, Promises and prospects of phytoremediation, *Plant Physiol.* 110 (1996) 715–719.
- [6] M.W.H. Evangelou, H. Daghan, A. Schaeffer, The influence of humic acids on the phytoextraction of cadmium from soil, *Chemosphere* 57 (2004) 207–213.
- [7] E. Lombi, F.J. Zhao, S.J. Dunham, S.P. McGrath, Phytoremediation of heavy metal-contaminated soils: natural hyperaccumulation vs. chemically enhanced phytoextraction, *J. Environ. Qual.* 30 (2001) 1919–1926.
- [8] C.W.A. do Nascimento, A. Amarasiriwardena, B. Xing, Comparison of natural organic acids and synthetic chelates at enhancing phytoextraction of metals from a multi-metal contaminated soil, *Environ. Pollut.* 140 (2006) 114–123.
- [9] J.W. Huang, J. Chen, W.R. Berti, S.D. Cunningham, Phytoremediation of lead-contaminated soils: role of synthetic chelates in lead phytoextraction, *Environ. Sci. Technol.* 31 (1997) 800–805.
- [10] A.K. Gupta, S. Sinha, Role of *Brassica juncea* (L.) Czern. (var. Vaibhav) in the phytoextraction of Ni from soil amended with fly ash: selection of extractant for metal bioavailability, *J. Harzard. Mater.* B136 (2006) 371–378.
- [11] S.A. Wasay, S. Barrington, S. Tokunaga, Remediation of soils polluted by heavy metals using salts of organic acids and chelating agents, *J. Environ. Technol.* 19 (1998) 369–380.
- [12] E. Meers, M. Hopgood, E. Lesage, P. Vervaeke, F.M.G. Tack, M.G. Verloo, Enhanced phytoextraction: in search of EDTA alternatives, *Intern. J. Phytoremed.* 6 (2004) 95–109.
- [13] L.H. Wu, Y.M. Luo, X.R. Xing, P. Christie, EDTA-enhanced phytoremediation of heavy metal contaminated soil with Indian mustard and associated potential leaching risk, *Agric. Ecosyst. Environ.* 102 (2004) 307–318.
- [14] M.F. Quartacci, A.J.M. Baker, F. Navari-Izzo, Nitrotriacetate- and citric acid-assisted phytoextraction of cadmium by Indian mustard (*Brassica juncea* (L.) Czernj, *Brassicaceae*), *Chemosphere* 59 (2005) 1249–1255.
- [15] H.D. Foth, B.G. Ellis, *Soil Fertility*, John Wiley & Sons, New York, USA, 1988, 212 p.
- [16] S.D. Ebbs, M.M. Lasat, D.J. Brady, J. Cornish, R. Gordon, L.V. Kochian, Phytoextraction of cadmium and zinc from a contaminated soil, *J. Environ. Qual.* 26 (1997) 1424–1430.
- [17] J. Wu, F.C. Hsu, S.D. Cunningham, Chelate-assisted Pb phytoextraction: Pb availability, uptake, and translocation constraints, *Environ. Sci. Technol.* 33 (1999) 1898–1904.
- [18] A.C. Alder, H. Siegrist, W. Gujer, Behaviour of NTA and EDTA in biological wastewater treatment, *Water Res.* 24 (1990) 733–742.
- [19] A.D. Satroudinov, E.G. Deedyukhina, T.I. Chistyakova, M. Witschel, I.G. Minkevich, V.K. Eroshin, T. Egli, Degradation of metal-EDTA complexes by resting cells of the bacterial strain DSM 9103, *Environ. Sci. Technol.* 34 (2000) 1715–1720.
- [20] P.K.A. Hong, S.K. Banerji, T. Regmi, Extraction, recovery and biostability of EDTA for remediation of heavy metal contaminated soil, *J. Soil Sediment Contam.* 8 (1999) 81–103.
- [21] M. Bucheli-Witschel, T. Egli, Environmental fate and microbial degradation of aminopolycarboxylic acids, *FEMS Microbiol. Rev.* 25 (2001) 69–106.
- [22] B. Nowack, R. Schulin, B.H. Robinson, Critical assessment of chelant-enhanced metal phytoextraction, *Environ. Sci. Technol.* 40 (2006) 5225–5232.
- [23] K. Wenger, S.K. Gupta, G. Furrer, R. Schulin, The role of nitrotriacetate in copper uptake by tobacco, *J. Environ. Qual.* 32 (2003) 1669–1676.
- [24] E. Meers, A. Ruttens, M. Hopgood, D. Samson, F.M.G. Tack, Comparison of EDTA and EDDS as potential soil amendments for enhanced phytoextraction of heavy metals, *Chemosphere* 58 (2005) 1011–1022.

- [25] D.L. Jones, Organic acids in the rhizosphere: a critical review, *Plant Soil* 205 (1998) 25–44.
- [26] M. Rajkumar, R. Nagendran, K.J. Lee, W.H. Lee, S.Z. Kim, Influence of plant growth promoting bacteria and Cr^{6+} on the growth of Indian mustard, *Chemosphere* 62 (2006) 741–748.
- [27] S. Zaidi, S. Usmani, B.R. Singh, J. Musarrat, Significance of *Bacillus subtilis* strain SJ-101 as a bioinoculant for concurrent plant growth promotion and nickel accumulation in *Brassica juncea*, *Chemosphere* 64 (2006) 991–997.
- [28] R.G. Coleman, C. Jove, Geological origin of serpentinites, in: A.J.M. Baker (Ed.), *The Vegetation of Ultramafic (Serpentine) Soils: Proceedings of the First International Conference on Serpentine Ecology*, Intercept, Andover, Hampshire, UK, 1991, pp. 1–17.
- [29] M.H. Wong, Ecological restoration of mine degraded soils, with emphasis on metal contaminated soils, *Chemosphere* 50 (2003) 775–780.
- [30] W.H.O. Ernst, Phytoremediation of mine wastes—options and impossibilities, *Chem. der Erde* 65 (2005) 29–42.
- [31] P.S.C. Kidd, C. Monterroso, Metal extraction by *Alyssum serpyllifolium* ssp. *Lusitanicum* on mine-spoil soils from Spain, *Sci. Total Environ.* 336 (2005) 1–11.
- [32] B.H. Robinson, R.R. Brooks, B.E. Clothier, Soil amendments affecting nickel and cobalt uptake by *Berkheya coddii*: potential use for phytomining and phytoremediation, *Ann. Bot.* 84 (1999) 689–694.
- [33] B.H. Robinson, A. Chiarucci, R.R. Brooks, D. Petit, J.H. Kirkman, P.E.H. Gregg, V. de Dominics, The nickel hyperaccumulator plant *Alyssum bertolonii* as a potential agent for phytoremediation and phytomining of nickel, *J. Geochem. Explor.* 59 (1997) 75–86.
- [34] C.S. Ho, *An Introduction to the Geology of Taiwan: Explanatory Text of the Geologic Map of Taiwan*, 2nd ed., Central Geological Survey, Taipei, Taiwan, 1988, 164 pp.
- [35] E.O. McLean, Soil pH and lime requirement, in: A.L. Page, R.H. Miller, D.R. Keeney (Eds.), *Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties. Agronomy Monograph 9*, 2nd ed., Agronomy Society of America and Soil Science Society of America, Madison, WI, USA, 1982, pp. 199–224.
- [36] D.W. Nelson, L.E. Sommers, Total carbon, organic carbon, and organic matter, in: A.L. Page, R.H. Miller, D.R. Keeney (Eds.), *Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties. Agronomy Monograph 9*, 2nd ed., Agronomy Society of America and Soil Science Society of America, Madison, WI, USA, 1982, pp. 539–577.
- [37] J.M. Bremner, C.S. Mulvaney, Nitrogen: total content, in: A.L. Page, R.H. Miller, D.R. Keeney (Eds.), *Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties. Agronomy Monograph 9*, 2nd ed., Agronomy Society of America and Soil Science Society of America, Madison, WI, USA, 1982, pp. 595–624.
- [38] S. Kuo, Phosphorus, in: D.L. Sparks (Ed.), *Methods of Soil Analysis. Part 3. Soil Science Society of America Book Series No. 5*, Agronomy Society of America and Soil Science Society of America, Madison, WI, USA, 1996, pp. 869–919.
- [39] J.D. Rhoades, Cation exchange capacity, in: A.L. Page, R.H. Miller, D.R. Keeney (Eds.), *Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties. Agronomy Monograph 9*, 2nd ed., Agronomy Society of America and Soil Science Society of America, Madison, WI, USA, 1982, pp. 149–157.
- [40] D.E. Baker, M.C. Amacher, Nickel, copper, zinc and cadmium, in: A.L. Page, R.H. Miller, D.R. Keeney (Eds.), *Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties. Agronomy Monograph 9*, 2nd ed., Agronomy Society of America and Soil Science Society of America, Madison, WI, USA, 1982, pp. 323–336.
- [41] G.W. Gee, J.W. Bauder, Particle-size analysis, in: A. Klute (Ed.), *Methods of Soil Analysis. Part 1. Physical and Mineralogical Properties. Agronomy Monograph 9*, 2nd ed., Agronomy Society of America and Soil Science Society of America, Madison, WI, USA, 1986, pp. 383–411.
- [42] R.L. Cook, C.E. Millar, Some techniques which help to make greenhouse investigation comparable with field plot experiments, *Soil Sci. Soc. Am. Proc.* 11 (1946) 298–304.
- [43] M.J. Mench, V.L. Didier, M. Loffler, A. Gomez, P. Masson, A mimicked in situ remediation study of metal-contaminated soils with emphasis on cadmium and lead, *J. Environ. Qual.* 23 (1994) 58–63.
- [44] S.A.S. Institute, SAS Statistical Package Version 6.12, SAS Institute Inc., Cary, NC, USA, 1989.
- [45] S. Fernandez, S. Seoane, A. Merino, Plant heavy metal concentrations and soil biological properties in agricultural serpentine soils, *Commun. Soil Sci. Plant Anal.* 30 (1999) 1867–1884.
- [46] R. Burt, M. Fillmore, M.A. Wilson, E.R. Gross, R.W. Langridge, D.A. Lammers, Soil properties of selected pedons on ultramafic rocks in Klamath Mountains, Oregon, *Commun. Soil Sci. Plant Anal.* 32 (2001) 2145–2175.
- [47] Z.Y. Hseu, Concentration and distribution of chromium and nickel fractions along a serpentinitic toposequence, *Soil Sci.* 171 (2006) 341–353.
- [48] E. Meers, A. Ruttens, M. Hopgood, E. Lesage, F.M.G. Tack, Potential of *Brassica rapa*, *Cannabis sativa*, *Helianthus annuus* and *Zea mays* for phytoremediation of heavy metals from calcareous dredged sediment derived soils, *Chemosphere* 61 (2005) 561–572.
- [49] F.X. Han, B.B. Maruthi Sridhar, D.L. Monts, Y. Su, Phytoavailability and toxicity of trivalent and hexavalent chromium to *Brassica juncea*, *New Phytol.* 162 (2004) 489–499.
- [50] B.J. Alloway, *Heavy Metals in Soils*, Blackie and Son Ltd., London, UK, 1991, 339 pp.
- [51] M.D. Vazquez, C.H. Poschenrieder, J. Barcelo, Chromium VI induced structural and ultrastructural changes in bush bean plants (*Phaseolus vulgaris* L.), *Ann. Bot.* 59 (1987) 427–438.
- [52] M.H.M.N. Senden, F.J.M. van Paassen, A.J.G.M. van der Meer, H.T.H. Wolterbeek, Cadmium-citric acid–xylem cell wall interactions in tomato plants, *Plant Cell Environ.* 15 (1990) 71–79.
- [53] H. Chen, T. Cutright, EDTA and HEDTA effects on Cd, Cr, and Ni uptake by *Helianthus annuus*, *Chemosphere* 45 (2001) 21–28.