The EDTA effect on phytoextraction of single and combined metals-contaminated soils using rainbow pink (*Dianthus chinensis*)

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Abstract

Rainbow pink (*Dianthus chinensis*), a potential phytoextraction plant, can accumulate high concentrations of Cd from metal-contaminated soils. The soils used in this study were artificially added with different metals including (1) CK: original soil, (2) Cd-treated soil: 10 mg Cd kg⁻¹, (3) Zn-treated soil: 100 mg Zn kg⁻¹, (4) Pb-treated soil: 1000 mg Pb kg⁻¹, (5) Cd–Zn-treated soil: 10 mg Cd kg⁻¹ and 100 mg Zn kg⁻¹, (6) Cd–Pb-treated soil: 10 mg Cd kg⁻¹ and 1000 mg Pb kg⁻¹, (7) Zn–Pb-treated soil: 100 mg Zn kg⁻¹ and 1000 mg Pb kg⁻¹, and (8) Cd–Zn–Pb-treated soil: 10 mg Cd kg⁻¹, 100 mg Zn kg⁻¹, and 1000 mg Pb kg⁻¹. Three concentrations of 2Na-EDTA solutions (0 (control), 2, and 5 mmol kg⁻¹ soil) were added to the different metals-treated soils to study the influence of applied EDTA on single and combined metals-contaminated soils phytoextraction using rainbow pink. The results showed that the Cd, Zn, Pb, Fe, or Mn concentrations in different metals-treated soil solutions significantly increased after applying 5 mmol EDTA kg⁻¹ ($p < 0.05$). The metal concentrations in different metals-treated soils extracted by deionized water also significantly increased after applying 5 mmol EDTA kg⁻¹ ($p < 0.05$). Because of the high extraction capacity of both 0.005 M DTPA (pH 5.3) and 0.05 M EDTA (pH 7.0), applying EDTA did not significantly increase the Cd, Zn, or Pb concentration in both extracts for most of the treatments. Applying EDTA solutions can significantly increase the Cd and Pb concentrations in the shoots of rainbow pink ($p < 0.05$). However, this was not statistically significant for Zn because of the low Zn concentration added into the contaminated soils. The results from this study indicate that applying 5 mmol EDTA kg⁻¹ can significantly increase the Cd, Zn, or Pb concentrations both in the soil solution or extracted using deionized water in single or combined metals-contaminated soils, thus increasing the accumulated metals concentrations in rainbow pink shoots. The proposed method worked especially well for Pb ($p < 0.05$). The application of 2 mmol EDTA kg⁻¹ might too low to enhance the phytoextraction effect when used in silty clay soils.

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1. Introduction

Phytoremediation is the use of plants to remediate organic, inorganic, or nuclear pollutants in contaminated sites. This method includes phytoextraction,

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phytostabilization, phytovolatilization, phytodegradation, and phytofiltration techniques (Salt et al., 1995; Chaney et al., 1997; Lombi et al., 1998; Wenzel et al., 1999; Rodrigues and Duarte, 2003). Of these techniques, phytoextraction accumulates heavy metals from contaminated soil in plant roots with subsequent transfer into the shoots. These plants are then harvested, incinerated and buried (Kumar et al., 1995; Chaney et al., 1997; Wenzel et al., 1999; Garbisu and Alkorta, 2001).

Over 400 hyperaccumulator plant species from all over the world can accumulate high concentrations of metals from contaminated sites (Baker et al., 2000). However, many of these plants have a low growth rate and very low biomass. Therefore much time is needed to remove contaminants from soils. Two strategies, one involving synthesized chelating agents and the other involving genetic engineering, are being developed to increase the phytoextraction effect. Synthesized chelating agents, such as EDTA (ethylenedinitrilotetraacetic acid), DTPA (diethylenetrinitriropentaacetic acid), HEDTA (hydroxyethylenediaminetriacetic acid), CDTA (trans-1,2-cyclohexylenedinitrilotetraacetic acid), and EGTA (ethylenebis(oxyethylenetrinitrilo)tetraacetic acid), were applied to metal-contaminated soil to increase the mobility and bioavailability of the metals in the soils, thereby increasing the amount of heavy metals accumulated in the upper parts of phytoextracting plants (Huang and Cunningham, 1996; Blaylock et al., 1997; Huang et al., 1997; Ebbs and Kochian, 1998; Cooper et al., 1999; Lombi et al., 2001; Lai and Chen, 2004). These results revealed that adding synthetic chelating agents can increase both the solubility of the metal in the soil solution and the metal concentration in the plant shoots in single metal-contaminated soils. However, in soils contaminated with multiple metals, the application of synthetic chelating agents can reduce both the plant biomass and the total amount of metals removed because the high concentrations of other metals in the soil solution are toxic to the plant (Chen and Cutright, 2001; Lombi et al., 2001). Synthetic chelating agents at high concentrations can also be toxic to plants (Cooper et al., 1999). Applying synthetic chelating agents to soils contaminated by multiple metals represents another challenge for chemical phytoextraction enhancement by plants grown in contaminated sites.

Using commercial or garden plants to remove metals from contaminated soils is a popular and satisfactory strategy, because it returns the contaminated sites to their natural condition and generates economic value if appropriate flower species are cultivated. Chen and Lee (1997) reported that when rainbow pink was grown in a Cd-contaminated site in Northern Taiwan for five weeks, the Cd concentration in the plant shoots increased from 1.56 (under the control treatment) to 115 mg kg⁻¹ (73.7-fold) and the total Cd uptake was about 100 g ha⁻¹ y⁻¹. These findings show that this

plant can accumulate high concentrations of Cd. Moreover, the accumulated Cd concentration can reach a threshold (100 mg Cd kg⁻¹) of a Cd hyperaccumulator (Baker et al., 2000). Lai and Chen (2004) reported that applying 5 or 10 mmol EDTA kg⁻¹ can significantly increase the Cd, Zn, and Pb concentration in the soil solution and significantly increase the accumulated Pb concentration in the shoots of rainbow pink when growing in Cd, Zn, and Pb-contaminated soils. However, the Cd and Zn concentration in rainbow pink shoots did not increase significantly after applying EDTA solutions. This result revealed that the EDTA effect on phytoextraction by rainbow pink is not identical in single and combined metal-contaminated soils.

The objectives of this study are (1) to identify the EDTA effect on the heavy metal concentrations in the soil solution or different soil extracts when rainbow pink is grown in artificial single or combined metal-treated soils, and (2) to evaluate the EDTA effect on the phytoextraction of single and combined metal-treated soils using rainbow pink.

2. Materials and methods

2.1. Soil characteristics

A rural soil contaminated by discharged wastewater from manufacturing plants and artificially added metals was used in pot experiments. The soil pH value was determined using glass electrodes in a soil to water ratio of 1:1 (McLean, 1982). The soil particle size distribution was analyzed using the pipette method (Gee and Bauder, 1986). The organic carbon content was determined using the Walkley–Black wet combustion method (Nelson and Sommers, 1982). The cation exchange capacity (CEC) was determined using the ammonium acetate method. The total Cd, Zn, and Pb concentrations in contaminated soils were digested by aqua regia and determined using atomic absorption spectrometer (Hitachi 180-30 type) (EPA/ROC, 2002).

2.2. Soil pre-treatment

The soil used in this study was collected from a Cd-contaminated site in Northern Taiwan. The surface soil (0–20 cm) of the contaminated site was sampled, air-dried, and ground to pass through a 2 mm sieve. The soil was stored in large plastic bottles for laboratory analysis and pot experiments. Three single or combination solutions, Cd(NO₃)₂ · 4H₂O, ZnO, or Pb(NO₃)₂, were added to the air-dried soil to control the total concentrations (as individual element concentration) of three metals used in the following treatments: (1) CK: original soil, (2) Cd-treated soil: 10 mg Cd kg⁻¹, (3) Zn-treated soil: 100 mg Zn kg⁻¹, (4) Pb-treated soils: 1000 mg Pb kg⁻¹,

(5) Cd–Zn-treated soil: combined polluted using 10 mg Cd kg⁻¹ and 100 mg Zn kg⁻¹, (6) Cd–Pb-treated soil: combined polluted using 10 mg Cd and 1000 mg Pb kg⁻¹, (7) Zn–Pb-treated soil: combined polluted using 100 mg Zn kg⁻¹ and 1000 mg Pb kg⁻¹, and (8) Cd–Zn–Pb-treated soil: combined polluted using 10 mg Cd kg⁻¹, 100 mg Zn kg⁻¹, and 1000 mg Pb kg⁻¹. The metal-treated soils were wetted for two weeks by adding deionized water to maintain a water content of 60% of the water-holding capacity. The soils were then dried at room temperature for approximately four weeks. The artificially contaminated soil was subjected to three cycles of wet and dry processes before pot experiments were conducted to enable the added heavy metals to reach a steady state (Blaylock et al., 1997).

2.3. Pot experiments and laboratory analysis

Each pot (16 cm diameter and 19 cm height) contained 3.0 kg (dry weight) of artificially metals-treated soils. Two rhizon soil moisture samplers (RSMS) were placed into each pot at a depth of about 10 cm below the soil surface. One rainbow pink seedling (*Dianthus chinensis*) was planted in each pot. The pot experiments were conducted in a phytotron using a RCBD (randomized complete block design) with a number of treatments and three replicates. The soil moisture content was maintained at 60% of the water-holding capacity, by weight with deionized water added every two days.

After growing for 21 d, three 2Na-EDTA solution concentrations (0 (control), 2, and 5 mmol kg⁻¹ soil) were added to the different metal-treated soils. The growth conditions and observed toxicity symptoms displayed by the plants were also recorded. The rainbow pink shoots were harvested on the seventh day after the EDTA solution was first added to the pot. The harvested plants were rinsed with tap-water to remove soil adhered to the stem and leave surfaces and then oven-dried at 60 °C for 72 h, weighed, and ground to 0.5 mm for analysis. Then 0.2 g of the plants were digested using the H₂SO₄/H₂O₂ digestion method (Harmon and Lajtha, 1999). The digested solutions were filtered through Whatman No. 42 filter paper, and then diluted with deionized water to a volume of 50 ml in a flask. The Cd, Zn, and Pb concentrations were determined using inductively coupled plasma optical emission spectrometer (ICP-OES) (Perkin Elmer 2000 DV).

Soil solutions were also collected directly using RSMS before adding EDTA solutions and at 7 d after adding EDTA solutions. The Cd, Zn, and Pb concentrations in the tested soil after harvesting were extracted using deionized water (Mench et al., 1994), 0.05 M EDTA (pH 7.0) (Mench et al., 1994), and 0.005 M DTPA (pH 5.3) (Lindsay and Norvell, 1978). The Cd, Zn, and Pb concentrations in the soil solutions or in different extracts were determined using ICP-OES (Perkin

Elmer 2000 DV). To understand the concentration change in other elements after applying the EDTA solutions, the Ca, Mg, Fe, and Mn concentrations in the soil solutions were determined using ICP-OES.

2.4. Statistics

The variance and significance of the differences among the element concentrations in the soil solutions, extracted using different agents, and in the plants were analyzed by ANOVA. Statistical significance was defined as $p = 0.05$.

3. Results and discussion

3.1. Soil characteristics

Table 1 shows the physical and chemical characteristics of the studied soil. The studied soil texture was silty clay with a moderate cation exchange capacity of 13.9 cmol₍₊₎ kg⁻¹ soil. The original total metal concentrations in the contaminated soil, digested by aqua regia, were 2.58 ± 0.08 mg Cd kg⁻¹, 80.0 ± 5.32 mg Zn kg⁻¹, and 31.3 ± 2.09 mg Pb kg⁻¹, respectively.

3.2. The change in element concentration in the soil solutions after applying EDTA

Adding Cd(NO₃)₂ · 4H₂O can increase the Cd concentration in a soil solution from not detectable (ND, below detection limit) in original soil (CK) to 0.14 ± 0.07 mg l⁻¹ in Cd-treated soil, 0.03 ± 0.01 mg l⁻¹ in Cd–Zn-treated soil, 1.34 ± 0.47 mg l⁻¹ in Cd–Pb-treated soil, and 1.38 ± 0.43 mg l⁻¹ in Cd–Zn–Pb-treated soil, respectively. Adding ZnO or Pb(NO₃)₂ can also increase the Zn or Pb concentration in soil solutions (data not shown).

In the different heavy metals-treated soils, applying two concentrations of EDTA solutions (2 and 5 mmol kg⁻¹) can significantly increase the concentration of Cd, Zn, or Pb in soil solution ($p < 0.05$), except for applying 2 mmol EDTA kg⁻¹ in both Zn-treated soil and Cd–Pb-treated soil (Table 2). The soil used in this study was contaminated by Cd from wastewater discharged by manufacturing plants. The total Cd, Zn, and Pb concentration in the original contaminated soil analyzed by aqua regia was 2.58 ± 0.08 , 80.0 ± 5.32 , and 31.3 ± 2.09 mg kg⁻¹, respectively. In the original soil (CK) without artificially added metals, 2 or 5 mmol applied EDTA kg⁻¹ solutions could significantly increase the Cd, Zn, or Pb concentrations in the soil solutions ($p < 0.05$). However, the amount of increase was slight because of the low Cd, Zn, and Pb concentrations in the tested soil. Because the tested soil was a silty clay soil and the low EDTA application rate, no similar

Table 1
The physical and chemical properties of the studied soil

pH (H ₂ O)	OC (g kg ⁻¹) ^a	Texture	CEC (cmol ₍₊₎ kg ⁻¹ soil) ^b	Metal concentration (mg kg ⁻¹) ^c		
				Cd	Zn	Pb
4.9	16.9	Silty clay ^d	13.9	2.58 ± 0.08	80.0 ± 5.32	31.3 ± 2.09

^a OC: organic carbon.

^b CEC: cation exchangeable capacity.

^c The total content of Cd, Zn, and Pb in the original soil was digested by aqua regia.

^d Sand 14.2%, silt 46.2%, and clay 39.6%.

Table 2
The change in element concentrations in the soil solutions before application and at the seventh day after applying 2 and 5 mmol kg⁻¹ EDTA solutions

Treatment	EDTA concentration (mmol kg ⁻¹ soil)	Cd	Zn	Pb	Ca	Mg	Fe	Mn
CK	0	ns	ns	ns	ns	ns	ns	ns
	2	*	*	ns	ns	ns	**	**
	5	**	**	*	ns	ns	**	*
Cd	0	ns	ns	ns	ns	ns	ns	ns
	2	**	***	***	ns	ns	**	***
	5	***	***	***	ns	ns	***	*
Zn	0	ns	ns	ns	ns	ns	ns	ns
	2	ns	ns	ns	ns	ns	*	*
	5	**	***	***	ns	ns	***	*
Pb	0	ns	ns	ns	ns	ns	ns	ns
	2	ns	ns	*	ns	ns	ns	ns
	5	ns	ns	**	ns	ns	*	ns
Cd + Zn	0	ns	ns	ns	ns	ns	ns	ns
	2	***	***	***	ns	ns	***	ns
	5	***	***	***	ns	ns	**	***
Cd + Pb	0	ns	ns	ns	ns	ns	ns	ns
	2	ns	ns	*	ns	ns	**	ns
	5	*	*	**	ns	ns	***	ns
Zn + Pb	0	*	ns	ns	ns	ns	ns	ns
	2	*	**	*	ns	ns	ns	**
	5	***	***	***	ns	ns	***	**
Cd + Zn + Pb	0	ns	ns	ns	ns	ns	ns	ns
	2	*	*	***	ns	ns	*	ns
	5	*	**	**	*	ns	***	***

“ns”: No significantly increased after applying EDTA solutions; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

change in the Cd, Zn, or Pb soil solution concentration occurred after applying 2 mmol EDTA kg⁻¹ compared with 5 mmol applied EDTA kg⁻¹.

Except for the Ca concentration in 5 mmol EDTA kg⁻¹ treatment, no significant increase in the Ca and Mg soil solution concentration occurred after applying two EDTA treatments. However, most of the Fe and Mn soil solution concentrations increased significantly after applying 5 mmol EDTA kg⁻¹ ($p < 0.05$). Consistent with the Cd, Zn, and Pb soil solution concentration results after applying EDTA, no identical

Fe and Mn concentration results occurred after applying 2 mmol EDTA kg⁻¹.

This result reveals that applying EDTA can increase the Cd, Zn, Pb, Fe, and Mn soil solution concentrations for metal-treated soils, but not for Ca and Mg. Because of the low permeability of the tested soil compared with other studies (Huang et al., 1997; Chen and Cutright, 2001; Puschenreiter et al., 2001; Hammer and Keller, 2002) and the low EDTA application rate (2 mmol kg⁻¹ soil) used in this study, the EDTA effect was statistically significant for only 5 mmol EDTA kg⁻¹.

3.3. EDTA effect on the soil solution element concentration

The Cd, Zn, and Pb concentration in the original soil increased significantly after applying 5 mmol EDTA kg⁻¹ ($p < 0.05$) (Fig. 1). When considering the Cd soil solution concentration in different Cd-treated soils (i.e. Cd, Cd-Zn, Cd-Pb, and Cd-Zn-Pb-treated soil), applying 2 or 5 mmol EDTA kg⁻¹ significantly increased the Cd concentration in the soil solution ($p < 0.05$), except for the Cd-Pb-treated soil (Fig. 1a). The Cd concentration in the soil solution of Cd-Pb-treated soil also increased after applying 2 or 5 mmol EDTA kg⁻¹, but was no significant difference. After applying 5 mmol EDTA kg⁻¹, the Cd concentration in the soil solution increased significantly from 0.14 ± 0.07 mg l⁻¹ to 5.90 ± 1.14 mg l⁻¹ (42-fold) in Cd-treated soil, and from 0.03 ± 0.01 mg l⁻¹ to 5.65 ± 0.10 mg l⁻¹ (188-fold) in Cd-Zn-treated soil, respectively ($p < 0.05$). Because of the low 2 mmol EDTA kg⁻¹ application rate, no significant increase occurred in CK, Cd-Pb, and Cd-Zn-Pb-treated soils after applying 2 mmol EDTA kg⁻¹.

The Zn soil solution concentrations result after applying different EDTA solution concentrations was

Fig. 1. The (a) Cd, (b) Zn, and (c) Pb concentrations in the soil solutions sampled at the seventh day after applying 2 and 5 mmol kg⁻¹ EDTA solutions. The probability level of significant difference is at $p = 0.05$. Replicates (n) = 3.

similar to the Cd result (Fig. 1b). Applying 5 mmol EDTA kg⁻¹ could significantly increase the Zn concentration in the soil solutions of different Zn-treated soils (i.e. Zn, Cd-Zn, Zn-Pb, and Cd-Zn-Pb-treated soil) ($p < 0.001$). The Zn concentrations in the soil solutions of different Zn-treated soils were all below 4.0 mg l⁻¹ before adding EDTA treatments. After treating with 5 mmol EDTA kg⁻¹, the Zn concentration increased significantly to 13–22 mg l⁻¹ ($p < 0.001$). In soil treated artificially with Zn, the Zn concentration in the soil solution increased significantly from 3.37 ± 0.66 mg l⁻¹ to 21.3 ± 2.67 mg l⁻¹ (6.3-fold) after applying 5 mmol EDTA kg⁻¹ treatment ($p < 0.001$). This was the highest concentration after applying 5 mmol EDTA kg⁻¹. Because of the low artificially added Zn concentration into the Zn-treated soils, the Zn soil solution concentration after applying EDTA treatments was not as high as that reported in previous study (Lai and Chen, 2004).

Because of the low solubility of Pb in contaminated soil (Alloway, 1995), the Pb concentrations in the soil solutions of different Pb-treated soils were low without adding EDTA (Fig. 1c). They were 2.49 ± 1.38 mg l⁻¹ in Pb-treated soils, 3.87 ± 0.88 mg l⁻¹ in Cd-Pb-treated soil, 5.29 ± 2.23 mg l⁻¹ in Zn-Pb-treated soil, and 4.11 ± 0.99 mg l⁻¹ in Cd-Zn-Pb-treated soil, respectively. Except when applying 2 mmol EDTA kg⁻¹ in artificially Pb-treated soil, applying both EDTA solution concentrations significantly increased the Pb concentration in different Pb-treated soil solutions (i.e. Pb, Cd-Pb, Zn-Pb, and Cd-Zn-Pb-treated soil) ($p < 0.05$), especially for the 5 mmol EDTA kg⁻¹ (Fig. 1c). These results indicated that applying EDTA solutions can effectively to increase the metal concentration, especially for Pb, in the soil solution. The differences in chelating effects between EDTA and metals are reflected by the formation constant ($\log K_f = 19.0$) Pb-ligand complexes, which exceeded those produced by Cd-ligand ($\log K_f = 17.4$) and Zn-ligand complexes ($\log K_f = 17.5$). The maximum Pb soil solution concentration was 498 ± 38.2 mg l⁻¹ in the Zn-Pb-treated soil after applying the 5 mmol EDTA kg⁻¹ solution. After applying 2 mmol EDTA kg⁻¹, the Pb soil solution concentration of artificially Pb-treated soil was higher than that for the 5 mmol EDTA kg⁻¹ treatment. The Pb concentration was significantly increased from 2.49 ± 1.38 mg l⁻¹ (without applying EDTA solutions) to 236 ± 123 mg l⁻¹ ($p < 0.05$). However, there was no significant difference between 2 and 5 mmol EDTA kg⁻¹ in artificially Pb-treated soil. This might be high variation of Pb concentration in the soil solutions after applying 2 mmol EDTA kg⁻¹ and some errors were occurred in the period of pot experiment.

The result in this study showed that applying 5 mmol EDTA kg⁻¹ efficiently increased the Cd, Zn, or Pb soil solution concentration in different metal-treated soils. This finding is consistent with those obtained following the EDTA treatments to contaminated soils in other

studies (Blaylock et al., 1997; Huang et al., 1997; Wu et al., 1999; Luo et al., 2000; Chen and Cutright, 2001; Lombi et al., 2001; Hopgood et al., 2003). Because the soil used in this study was a silty clay soil, with lower permeability compared with sandy soil, the effects of applying 2 mmol EDTA kg⁻¹ on increasing the soil solution metal concentration were not identical. Lai and Chen (2004) reported that applying 5 and 10 mmol EDTA kg⁻¹ can significantly increase the soil solution metal concentration for the same soil used in this study. We thus propose that when applying EDTA to silty clay soil, more than 5 mmol EDTA kg⁻¹ is necessary to efficiently and uniformly increase the soil solution metal concentration.

The Ca, Mg, Fe, and Mn soil solution concentrations were also analyzed to investigate the changes in other element concentrations after applying EDTA solutions. The results showed that the Ca and Mg soil solution concentrations did not exhibit identical concentration increases after applying 2 or 5 mmol EDTA kg⁻¹ (Fig. 2a and b). However, the Fe and Mn soil solution concentrations increased significantly after applying both EDTA solutions ($p < 0.05$), especially for the 5 mmol EDTA kg⁻¹ treatment (Fig. 2c and d). Cooper et al. (1999) reported that applying high DTPA concentrations can significantly increase the Fe and Mn concentration in plants. However, the results were not significant for Ca and Mg. Applying EDTA could significantly increase the Fe and Mn soil solution concentrations in this study, producing easier plant uptake. The result from this study was similar to the result by Cooper et al. (1999). The Fe soil solution concentrations without EDTA treatments were not detected in different metal-treated soils. Seven days after 2 mmol kg⁻¹ EDTA solutions were added to the soils, the Fe soil solution concentration increased significantly to the 70–210 mg l⁻¹ ($p < 0.05$) level. Applying 5 mmol EDTA kg⁻¹ was more efficient than that applying 2 mmol EDTA kg⁻¹, significantly increasing the Fe concentration to the 150–590 mg l⁻¹ ($p < 0.05$) level. The Mn soil solution concentration after applying EDTA showed the same increase as Fe, even though some of the increases were not statistically significant.

3.4. EDTA effect on the element extractable concentration

3.4.1. Deionized water

After applying 5 mmol EDTA kg⁻¹, the deionized water extractable Cd, Zn, or Pb concentration of different metal-treated soils increased significantly ($p < 0.05$) (Fig. 3). The concentrations of metals extracted using deionized water were equal to the water soluble form in the contaminated soil (Gupta and Chen, 1975; Gatehouse et al., 1977; Mathur and Levesque, 1983; Jones et al., 1984; Miller et al., 1986), which were easily lea-

Fig. 2. The (a) Ca, (b) Mg, (c) Fe, and (d) Mn concentrations in the soil solutions sampled at the seventh day after applying 2 and 5 mmol kg⁻¹ EDTA solutions. The probability level of significant difference is at $p = 0.05$. Replicates (n) = 3.

ched into the groundwater along with rainfall. Because the soil used in this study was contaminated by heavy metals, applying 5 mmol EDTA kg⁻¹ could significantly increase the deionized water extractable concentration of Cd, Zn, and Pb from the original contaminated soil. Applying 2 mmol EDTA kg⁻¹ could not significantly increase the amount water soluble metals in most treatments, except for the Cd concentration in Cd-treated soil and the Zn concentration in Zn and Cd–Zn-treated soil ($p < 0.05$). The reason is similar to that after applying 2 mmol EDTA kg⁻¹, which was described in Sections 3.2 and 3.3. Among these three metals, applying 5 mmol EDTA kg⁻¹ was the most efficient in increasing the Pb concentration in soil extracted using deionized water. In the different artificially Pb-treated soils (i.e. Pb, Cd–Pb, Zn–Pb, and Cd–Zn–Pb-treated soil) without EDTA solution treatment, the deionized

Fig. 3. The deionized water extractable concentrations for: (a) Cd, (b) Zn, and (c) Pb in soils sampled at the seventh day after applying 2 and 5 mmol kg⁻¹ of EDTA solutions. The probability level of significant difference is at $p = 0.05$. Replicates (n) = 3.

water extractable Pb concentrations were all below 2 mg kg⁻¹. After applying 5 mmol EDTA kg⁻¹, the Pb concentration significantly increased ($p < 0.05$), and the concentration levels were from 300 to 500 mg kg⁻¹ (Fig. 3c). These results show that applying EDTA into the different metal-treated soils could significantly increase the water soluble metal fraction in these soils ($p < 0.05$).

3.4.2. DTPA and EDTA

Fig. 4 shows the 0.005 M DTPA (pH 5.3) extractable concentrations for different metal-treated soils after applying 2 or 5 mmol EDTA kg⁻¹ solutions. The results show that the concentrations extracted using DTPA were not significantly different for most metal-treated soils after applying EDTA treatments. This might be because (1) The metals in artificially contaminated soils treated with different solutions existed for only a short period (about three months). This made the metals easier to extract than long-term contaminated soils. (2) The extracting agents (0.005 M DTPA) used in this study were strong enough to extract metals from the different metal-treated soils. The bioavailability of metals decreases with the amount of contamination time (Vig

Fig. 4. The 0.005 M DTPA (pH 5.3) and extractable: (a) Cd, (b) Zn, and (c) Pb soil concentrations sampled at the seventh day after applying 2 and 5 mmol kg⁻¹ EDTA solutions. The probability level of significant difference is at $p = 0.05$. Replicates (n) = 3.

et al., 2003). Soil artificially contaminated by heavy metal will have high bioavailability compared with long-term contaminated soils. DTPA can extract higher amounts of metals in artificially contaminated soils than in long-term contaminated soils. Some authors indicated that 0.005 M DTPA was able to extract heavy metals that were water soluble, exchangeable, organic matter bound, and fractions occluded in oxides (Stover et al., 1976; Sposito et al., 1982; Mathur and Levesque, 1983; Jones et al., 1984; Goldberg and Smith, 1984). We consider that most of the metals in the different metal-treated soils in this study existed in water soluble, exchangeable, or organic matter bound fractions. Because of the strong extracting capacity of 0.005 M DTPA, which could extract most of the metal fractions in this tested soil, the Cd, Zn, and Pb concentrations in the extracts after adding different EDTA concentrations did not increase significantly for most of the metal-treated soils. Even through 0.005 M DTPA is not suitable in assessing the EDTA effect on phytoextraction of heavy metals-contaminated soils by rainbow pink in this study. The DTPA extractable soil Cd, Cu, Pb, and Zn concentration is useful to predict that concentration in plants (Parkian et al., 2002).

Fig. 5. The 0.05 M EDTA (pH 7.0) extractable: (a) Cd, (b) Zn, and (c) Pb soil concentrations sampled at the seventh day after applying 2 and 5 mmol kg⁻¹ EDTA solutions. The probability level of significant difference is at $p = 0.05$. Replicates (n) = 3.

The 0.05 M EDTA (pH 7.0) extractable Cd, Zn, and Pb concentration results were similar to the 0.005 M DTPA (pH 5.3) results (Fig. 5). There was not significantly different for most of the EDTA extractable metals in the different metal-treated soils after adding different EDTA concentration treatments. Previous studies indicated that 0.05 M EDTA (pH 7.0) can extract the same fractions with 0.005 M DTPA (pH 5.3) (Jones et al., 1984; Miller et al., 1986). There were no significant increases in extract concentrations after applying 2 or 5 mmol kg⁻¹ EDTA solutions because of the strong extracting capacity of 0.05 M EDTA.

3.5. EDTA effect on Cd, Zn, and Pb uptake by rainbow pink

In the different artificial Cd-treated soils (i.e. Cd, Cd–Zn, Cd–Pb, and Cd–Zn–Pb-treated soil), applying both 2 and 5 mmol EDTA kg⁻¹ significantly increased the Cd concentration in rainbow pink shoots ($p < 0.05$), except for the Cd–Pb-treated soil (Fig. 6a). The accumulated Cd concentrations in rainbow pink shoots when growing in Cd–Pb-treated soils were not statistically significant because of (1) the Cd concentrations in soil solutions were not significantly increased after applying 2 and

Fig. 6. The (a) Cd, (b) Zn, and (c) Pb concentrations in rainbow pink shoots harvested at the seventh day after applying 2 and 5 mmol kg⁻¹ EDTA solutions. Rainbow pink was grown for 21 d before applying the EDTA solutions. The probability level of significant difference is at $p = 0.05$. Replicates (n) = 3.

5 mmol kg⁻¹ EDTA solutions. (2) High variations in the Cd concentration in Cd–Pb-treated soils after applying 2 or 5 mmol EDTA kg⁻¹ solutions. Applying EDTA solutions significantly increased the Cd concentrations in both the soil solutions and Cd, Cd–Zn, and Cd–Zn–Pb-treated soil ($p < 0.05$) extracted using deionized water. The accumulated Cd concentration in the rainbow pink shoots was thus significantly increased ($p < 0.05$). This result is similar to that reported by Lai and Chen (2004).

Although both the Zn concentration in soil solutions and deionized water extractable concentrations significantly increased after applying both EDTA solution concentrations ($p < 0.05$), the Zn concentrations in rainbow pink shoots were not significantly increased except for the Cd–Zn–Pb-treated soil ($p < 0.05$) (Fig. 6b). The Zn concentrations levels in rainbow pink shoots were from 50 to 110 mg kg⁻¹ in different Zn-treated soils. Zinc is an essential element in maintaining plant growth. The mean content in these plants was from 1.2 to 73 mg kg⁻¹ (Kabata-Pendias and Pendias, 2001). We considered that because of the low Zn concentration added into the different artificial metal-treated soils (0.05 M EDTA and 0.005 M DTPA extracted Zn were

all below 30 mg kg^{-1}), the Zn concentration in these shoots was sufficient for rainbow pink growth. Although the Zn concentration in both the soil solution and deionized water extracted increased significantly after applying EDTA solutions ($p < 0.05$), the Zn concentration in rainbow pink shoots was not significantly affected by the EDTA solution application when growing in different metal-treated soils. Some interactions may also contribute to the result described above. The synergism or antagonism effect among these metals is illustrated in this result.

When growing in the different metals-treated soils without added EDTA solutions, all accumulated Pb concentrations in rainbow pink shoots were below 30 mg kg^{-1} (Fig. 6c). This pattern is illustrated by the low Pb concentration in both the soil solution ($< 6 \text{ mg l}^{-1}$) (Fig. 1c) and deionized water extracted samples ($< 2 \text{ mg kg}^{-1}$) (Fig. 3c). After applying 2 and $5 \text{ mmol EDTA kg}^{-1}$, the Pb concentration in rainbow pink shoots was increased significantly ($p < 0.05$).

Among the three metals, EDTA was most efficient in increasing the Pb concentration in rainbow pink shoots. This result is similar to that from other studies when EDTA was applied to metal-contaminated soil (Blaylock et al., 1997; Lai and Chen, 2004).

4. Conclusion

EDTA is considered a chelating agent that promotes the solubility of metals in contaminated sites, especially for Pb. The EDTA solution application can significantly increase the metal concentrations in both soil solutions and deionized water extracted single or combined metal-contaminated soils ($p < 0.05$). Applying EDTA solution at a high concentration (5 mmol kg^{-1}) increases the metal concentration in the soil solution or deionized water extracted concentration greater than that obtained using EDTA at a lower concentration (2 mmol kg^{-1}). However, EDTA solutions may contribute to ground-water pollution. Both extracting agents, 0.005 M DTPA (pH 5.3) and 0.05 M EDTA (pH 7.0), used in this study were too strong to identify the EDTA effect on the phytoextraction of single or combined metal-treated soils. Further study should avoid using these two extraction methods, especially when the metals in the contaminated soil are produced artificial addition or only contaminated for short period of time.

Adding EDTA solutions can efficiently and significantly increase the Cd and Pb concentrations in rainbow pink shoots for most single and combined metal-treated soils ($p < 0.05$), especially for Pb. However, there was no uniform result for Zn because of the low Zn concentration added into the different metal-treated soils or the accumulated Zn in the shoots was sufficient rainbow pink growth. Some interactions among these three met-

als might also exist in combined metal-treated soils and affect the accumulated metal concentrations in rainbow pink shoots. This will be discussed further in a future article.

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