

The influence of EDTA application on the interactions of cadmium, zinc, and lead and their uptake of rainbow pink (*Dianthus chinensis*)

Hung-Yu Lai, Zueng-Sang Chen*

Graduate Institute of Agricultural Chemistry, National Taiwan University, No. 1, Sec. 4, Roosevelt Rd., Taipei 10617, Taiwan

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Abstract

Soil used in this study was artificially contaminated with Cd, Zn, Pb, or applied in combinations (Cd–Zn, Cd–Pb, Zn–Pb, or Cd–Zn–Pb) to study the interactions of metals in soil contaminated with multiple metals. After planting rainbow pink (*Dianthus chinensis*) in these soils for 21 days, three different concentrations of ethylenedinitrilotetraacetic acid (EDTA) solutions were added to study the effect of applying EDTA on the interactions among these metals. The concentrations of Cd, Zn, and Pb in the soil solutions of different metals-treated soils increased significantly after applying 5 mmol EDTA kg⁻¹ soil ($p < 0.05$). The potential of groundwater contamination will increase after applying EDTA and it is not recommended to be in situ used or have to use very carefully. The existence of Pb in the Cd-contaminated soil enhanced the uptake of Cd in rainbow pink in the treatments of control and 2 mmol EDTA kg⁻¹ soil. Cadmium inhibited the concentration of Zn without applying EDTA. However, whether the application of EDTA or not and the applied EDTA concentration had the greatest effect on the uptake of Pb when compared to Cd and Zn. After applying 5 mmol EDTA kg⁻¹ soil, Cd or Zn in the Pb-contaminated soil inhibited the uptake of Pb in rainbow pink, but there were no effect in other treatments.

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1. Introduction

Metals in contaminated soils can be removed by phytoextraction: plant roots uptake metals in soils and then transfer the metals into the plant shoots [1–6]. Over 420 hyperaccumulator plant species are capable of accumulating high concentrations of metals when grown in contaminated sites [7]. Many of these hyperaccumulators, however, have a low growth rates, limited biomass and require long periods of time to remove soil contaminants. Synthesized chelating agents were added to metal-contaminated soils to increase the mobility and bioavailability of metals included EDTA (ethylenedinitrilotetraacetic acid), EDDS ((*S,S*)-*N,N'*-ethylenediamine disuccinic acid), DTPA (diethylenetrinitrilopentaacetic acid), HEDTA (hydroxyethylenediaminetriacetic acid), CDTA (*trans*-1,2-cyclohexylene-dinitrilotetraacetic acid), and EGTA (ethyl-

enebis(oxyethylenetrinitrilo)tetraacetic acid), etc. [6,8–13]. However, some of these synthesized chelating agents (e.g. EDTA) have negative effects on the groundwater quality because of their persistence of biodegradation [6,10–13]. For example, EDTA will increase the solubility of metals and thus to increase their downward movement to the groundwater table. The risk of groundwater contamination was increased during EDTA-assisted phytoextraction, especially for sandy soils. It should be used as a reference compound and is not recommended to be in situ used in the contaminated site. In single metal-contaminated soils, particular for Pb-contaminated soils, applying synthetic chelating agents increased both the solubility of metal in soil and the metal concentration in plant shoots. However, the application of synthetic chelating agents reduced both plant biomass and total metal removal of metals in soils containing more than one metal because of the interactions among metals [11,14]. Effective application of synthetic chelating agents to soils contaminated with more than one metal represents considerable challenge for chemical enhancement of phytoextraction.

Interactions between metals in soils and those in the plants existed when soil was contaminated with metals. The Zn concen-

* Corresponding author. Tel.: +886 2 2369 8349; fax: +886 2 2392 4335.

E-mail addresses: d89623401@ntu.edu.tw (H.-Y. Lai), soilchen@ntu.edu.tw (Z.-S. Chen).

tration in the young spinach leaves increased with an increasing Cd concentration in soil solution, but an increase of Zn concentration in the soil solution reduced the Cd concentration in the young lettuce and spinach leaves [15]. Lead competes with Cd for exchange sites on colloid surfaces. Consequently, the Cd concentration in both soil solutions and plants increased when Pb was existed in the soil simultaneously [16]. Carlson and Rolfe [17] identified that the Cd concentration in rye grass increased with the addition of Pb. Other studies also reported that Pb had synergistic effect on Cd concentration in plants [18–20].

Using garden plants to remove metals from contaminated soils is an acceptable and satisfactory strategy as it returns contaminated sites to their natural conditions and can, if appropriate flower species are cultivated, generate economic value. Chen and Lee [21] identified that when rainbow pink was grown in a Cd-contaminated site in northern Taiwan for 5 weeks, the Cd concentration in plant shoots increased from 1.56 mg kg⁻¹ (before planting) to 115 mg kg⁻¹ (73.7-fold); the total Cd uptake was approximately 100 g ha⁻¹ year⁻¹. The Cd concentration in the shoot of plant can reach the threshold (100 mg Cd kg⁻¹) of a Cd hyperaccumulator [7]. Lai and Chen [6] reported that applying 5 or 10 mmol EDTA kg⁻¹ soil significantly increased the Cd, Zn, and Pb concentrations in soil solution and significantly increased the Pb concentration in the shoot of rainbow pink when grown in a Cd-, Zn-, and Pb-contaminated soil. However, the Cd and Zn concentration in shoots of rainbow pink did not significantly increase after applying EDTA solutions. Lai and Chen's result revealed that the effect of EDTA on phytoextraction by rainbow pink was not identical in single and combined metals-contaminated soils.

The interactions of metals in the soils contaminated with combined metals affected both the uptake of metals in plants and the effect of EDTA on the phytoextraction of combined metals in contaminated soils. The objectives of this study are: (1) to identify the interactions between Cd, Zn, and Pb when rainbow pink was planted to remediate soil contaminated with combined metals and (2) to evaluate the effect of EDTA on the interactions of these three metals.

2. Materials and methods

2.1. Soil characteristics

A rural soil contaminated with Cd and Pb, discharged from continuous effluent cadmium ester or lead ester of industrial wastewater in 1988, was used for this experiment. The surface soil (0–20 cm) of the contaminated site was sampled, air-dried, and passed through a 2 mm (10 mesh) sieve. It was stored in large plastic containers for laboratory analysis and pot experiments. The pH value of the soil was measured by using glass electrodes in a soil-to-water ratio of 1:1 [22]. Soil particle size distribution was measured using a pipette method [23]. Organic carbon content was measured by the Walkley–Black wet combustion method [24]. Cation exchange capacity (CEC) was measured using the ammonium acetate method [25,26]. The total concentration of Cd, Zn, and Pb initially in contaminated soils were digested with aqua regia and measured with

an atomic absorption spectrometer (AAS) (Hitachi 180-30 type) [27].

2.2. The phytoextraction potentiality of rainbow pink

To prepare the contaminated soils, a combination of three solutions, Cd(NO₃)₂·4H₂O, ZnO, or Pb(NO₃)₂, were added to the air-dried soil and in controlled concentrations (as individual element concentration) of the three metals, 20 mg Cd kg⁻¹, 500 mg Zn kg⁻¹, and 1000 mg Pb kg⁻¹, respectively. The soil samples contaminated with combined metals were wetted for 2 weeks by adding deionized water to maintain an average water content of 60% of the water-holding capacity (WHC) to enable the metal to reach an assumed steady state as far as possible when added to the samples. Then, the samples were dried at room temperature for approximately 4 weeks. The artificially contaminated soil was subjected to three cycles of wetting-and-drying processes, about 6 months, before the pot experiment [8]. Because of the short period of incubation of contaminated soil, probably steady state has not been reached and that the results may not be directly transferred to the field soils.

A total of 3.5 kg (dry weight) of artificially contaminated soil was put into plant pots (16 cm in diameter and 19 cm in height). Two rhizon soil moisture samplers (RSMS; Eijkelkam Company, The Netherlands) were put in each pot at a depth of approximately 10 cm below the soil surface. Two rainbow pink (*Dianthus chinensis*) seedlings were planted in each pot. Pot-based experiments were conducted in a phytotron with a randomized complete block design (RCBD) with three replicates. The soil moisture content was maintained at 60% WHC by weighing and adding deionized water every 2 days. The rainbow pink shoots were harvested after planting in the combined metals-contaminated soils for 50 days. The harvested plants were oven-dried at 60 °C for 72 h, weighed, and ground into particles 0.5 mm in size for analysis. Then, 0.2 g of the plants were digested with the H₂SO₄/H₂O₂ method [28]. The digested solutions were filtered through Whatman No. 42 filter papers, and then diluted with deionized water to a volume of 50 mL in a flask. The Cd, Zn, and Pb concentrations were measured with an AAS (Hitachi 180-30 type).

The soils after pot experiments were sampled and the 0.05 M EDTA (pH 7.0) [29] and 0.005 M DTPA (pH 5.3) [30] extractable Cd, Zn, and Pb concentrations and total concentration (aqua regia method) [27] in soils were measured with an AAS (Hitachi 180-30). Soil solutions were also collected with an RSMS prior to harvesting. The bioavailable concentrations of Cd, Zn, and Pb in the contaminated soils were evaluated from the concentrations of metals in the soil solution, which were determined with an inductively coupled plasma optical emission spectrometer (ICP-OES) (Perkin-Elmer 2000 DV).

2.3. Interaction study: pre-experiment and soil treatments

The withered yellow of leaves of rainbow pink caused by soil toxicities affect the accumulated metal concentrations in rainbow pink shoots and, thus, affect the determination of the interactions between metals. To avoid this potential prob-

Table 1
The physical and chemical characteristics of the study soil

pH (H ₂ O)	O.C. ^a (g kg ⁻¹)	Texture	CEC ^b (cmol ₍₊₎ kg ⁻¹ soil)	Total 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 O.C., 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, 142 g kg⁻¹; silt, 462 g kg⁻¹; clay, 396 g kg⁻¹.

lem, rainbow pink was planted in the concentrations of Cd-treated soils (5, 10, and 15 mg kg⁻¹), Zn-treated soils (500, 1000, and 1500 mg kg⁻¹), or Pb-treated soils (500, 1000, and 1500 mg kg⁻¹) that were first incubated with the same process described in Section 2.2, to determine the maximum allowable metal concentrations in soils. After growing rainbow pink for 1 month in the phytotron, the health of plants was recorded and utilized to determine the suitable concentration of artificially added Cd, Zn, or Pb for the pot-based experiment in Section 2.4.

After determining the suitable concentrations of Cd, Zn, or Pb during the pre-experiment stage, single or combined solutions of Cd(NO₃)₂·4H₂O, ZnO, or Pb(NO₃)₂ were added to the air-dried soil to control the three metals as following treatments: (1) CK denotes initially contaminated soil sampled from the Cd-contaminated site; (2) Cd-treated soil denotes soil contaminated with Cd; (3) Zn-treated soil denotes soil contaminated with Zn; (4) Pb-treated soils denotes soils contaminated with Pb; (5) Cd–Zn-treated soil denotes soil contaminated with Cd and Zn; (6) Cd–Pb-treated soil denotes soil contaminated with Cd and Pb; (7) Zn–Pb-treated soil denotes soil contaminated with Zn and Pb; (8) Cd–Zn–Pb-treated soil denotes soil contaminated with Cd, Zn, and Pb. The different metal-treated soils were subjected to the process of incubation as described in Section 2.2.

2.4. Interaction study: pot experiment and laboratory analysis

Each pot (16 cm in diameter and 19 cm in height) contained 3.0 kg (dry weight) of different metal-treated soils. Two RSMS were put in each pot at a depth of around 10 cm below the soil surface. One rainbow pink (*D. chinensis*) seedling was planted in each pot. The pot-based experiment was conducted in the phytotron using an RCBD with three replicates. The soil moisture content was maintained at 60% WHC by weighing and adding deionized water every 2 days.

To make sure the uniform effect of applying EDTA solutions into pot soils, final soil water content will not exceed the WHC. After rainbow pink was planted in the soil for 21 days, 300 mL of three Na₂-EDTA solutions (pH 5.0) (0 (control), 2, and 5 mmol kg⁻¹ soil) were added to the different metal-treated soils. The plants' growth conditions and symptoms of toxicity were observed and recorded. The rainbow pink shoots were harvested on seventh day after the EDTA solution was added to the potted soil. The harvested plants were treated and the concen-

trations of Cd, Zn, and Pb were measured with the same process as described in Section 2.2.

Soil solutions were also collected with an RSMS prior to adding EDTA solutions and on the seventh day after adding EDTA solutions. The concentrations of Cd, Zn, and Pb in the soil solutions were measured with an ICP-OES (Perkin-Elmer 2000 DV).

2.5. Statistics

The variance and significance of the differences among concentrations of elements in the soil solutions, extractants, and in the plants were analyzed by ANOVA. Statistical significance was defined as $p = 0.05$.

3. Results

3.1. Soil characteristics

Table 1 lists the physical and chemical characteristics of the studied soil. The soil texture was silty clay with a moderate cation exchange capacity of 13.9 cmol₍₊₎ kg⁻¹ soil; its pH value was 4.9 and the organic carbon was 16.9 g kg⁻¹. The initial total metal concentrations in the contaminated soil, digested with 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.

3.2. The phytoextraction potentiality of rainbow pink

The Cd, Zn, and Pb concentrations in the soil solutions and in soil digested with aqua regia or extracted with 0.005 M DTPA (pH 5.3) or 0.05 M EDTA (pH 7.0) after planting rainbow pink for 50 days are shown in Table 2. The total concentrations of Cd, Zn, and Pb in the soils contaminated with combination of metals

Table 2
The Cd, Zn, and Pb concentration in soil solution and in soil digested by aqua regia or extracted by 0.005 M DTPA or 0.05 M EDTA after planting rainbow pink for 50 days

	Analytic methods			
	Soil solution (mg L ⁻¹)	Aqua regia (mg kg ⁻¹)	0.05 M EDTA (mg kg ⁻¹)	0.005 M DTPA (mg kg ⁻¹)
Cd	3.72 ± 0.76	17.4 ± 0.86	18.5 ± 0.46	17.1 ± 0.62
Zn	95.7 ± 17.8	458 ± 26.3	360 ± 7.51	336 ± 7.80
Pb	4.73 ± 1.48	938 ± 45.5	822 ± 51.9	671 ± 45.2

were 17.4 ± 0.86 , 458 ± 26.3 , and $938 \pm 45.5 \text{ mg kg}^{-1}$, respectively. These metal concentrations are about 87, 92, and 94% of the target concentrations of 20 mg Cd kg^{-1} , $500 \text{ mg Zn kg}^{-1}$, and $1000 \text{ mg Pb kg}^{-1}$. These metal concentrations are close to the target values of contamination if we consider the variation of concentrations distributed in the contamination site. To take the total Pb concentration of 1000 mg kg^{-1} for interpretation, the Pb concentration in the soil solution was low and revealed that Pb was not soluble in the soil. The 0.005 M DTPA (pH 5.3) and 0.05 M EDTA (pH 7.0) extracted almost the same concentrations of Cd and Zn in the contaminated soils, but not so for Pb. Both extractors, 0.005 M DTPA and 0.05 M EDTA, extracted most of the Cd and about 70–80% of the Zn and Pb from the artificially contaminated soil with combined metals. Previous studies showed that 0.05 M EDTA and 0.005 M DTPA extracted the same fractions metals in soil including water soluble, exchangeable, and organically bounding forms [31–35]. Experimental results of both 0.05 M EDTA and 0.005 M DTPA extractable metals concentrations showed that the metals added to the soil were easy to uptake by plants [20].

After growing rainbow pink in the soils contaminated with multiple metals for 50 days, the Cd, Zn, and Pb concentrations in the shoots of rainbow pink were 82.2 ± 11.0 , 3736 ± 577 , and $216 \pm 166 \text{ mg kg}^{-1}$, respectively. Except for the low Pb concentration in the soil solution, rainbow pink accumulated high concentrations of Cd and Zn in their shoots. Experimental result was similar to that reported by Chen and Lee [21]. The accumulated Cd concentration was also close the threshold value of a Cd hyperaccumulator (100 mg kg^{-1}) [7]. These results revealed that rainbow pink was potentially effective for phytoremediation of soils contaminated with multiple metals and uptake measurements demonstrated high concentration of Cd and Zn in plant shoots.

3.3. Pre-experiment results

Among the three Cd-treated soils (5, 10, and 15 mg Cd kg^{-1}) and the three Pb-treated soils (500 , 1000 , and $1500 \text{ mg Pb kg}^{-1}$), rainbow pink grew well when planted in the 5 or 10 mg Cd kg^{-1} - and 500 or $1000 \text{ mg Pb kg}^{-1}$ -treated soils. However, chlorosis occurred in the tips of leaves when planted in the 15 mg Cd kg^{-1} - or $1500 \text{ mg Pb kg}^{-1}$ -treated soil. Among the three different Zn concentrations (500 , 1000 , and $1500 \text{ mg Zn kg}^{-1}$) applied in the pre-experiment, most leaves were damaged by the toxicity of Zn in soil and showed withering and yellowing. For considering the healthy plants to explain the interactions of metals, the Cd, Zn, and Pb concentrations in the artificially addition to the initial contaminated soil were determined as 10, 100, and 1000 mg kg^{-1} , respectively.

3.4. The concentration changes of elements in soil solutions after applying EDTA

Artificially added Cd, Zn, or Pb to the initial metals-contaminated soil increased the concentrations of specific metals in the soil solution (Fig. 1). For instance, adding

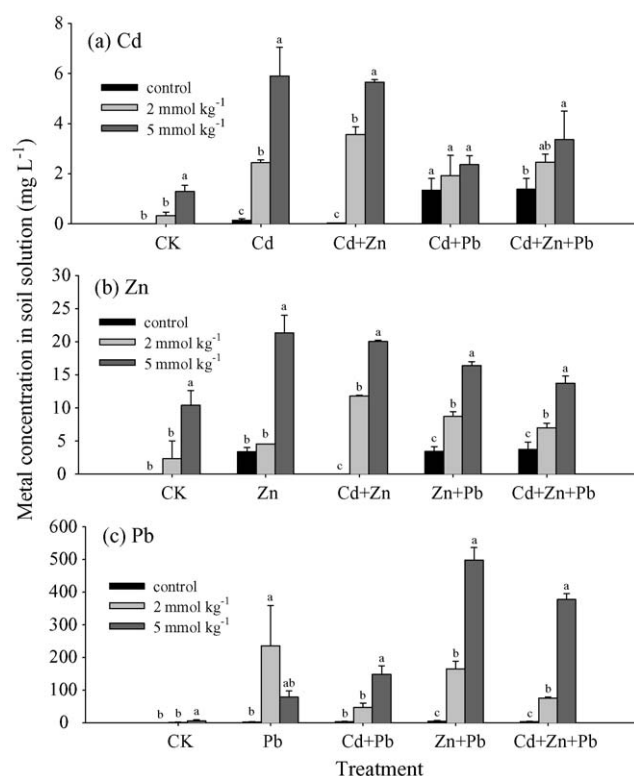


Fig. 1. The effect of applying different concentrations of EDTA on: (a) Cd concentration, (b) Zn concentration, and (c) Pb concentration in soil solution. The probability level of significant difference is at $p = 0.05$. Replicates (n) = 3.

$\text{Cd}(\text{NO}_3)_2$ to the soil raised the level Cd concentration in the soil solution from not detectable (ND, below the detection limit of 0.0027 mg L^{-1}) in initially contaminated soil (CK) to $0.14 \pm 0.07 \text{ mg L}^{-1}$ (Cd-treated soil), $0.03 \pm 0.01 \text{ mg L}^{-1}$ (Cd–Zn-treated soil), $1.34 \pm 0.47 \text{ mg L}^{-1}$ (Cd–Pb-treated soil), and $1.38 \pm 0.43 \text{ mg L}^{-1}$ (Cd–Zn–Pb-treated soil), respectively (Fig. 1a). Possessing the same result with that treated with Cd solution, artificial adding ZnO or $\text{Pb}(\text{NO}_3)_2$ also increased the Zn or Pb concentrations in the soil solutions (Fig. 1b and c).

In most of the single or combined metals-treated soils, applying 5 mmol EDTA kg^{-1} soil significantly increased the concentrations of Cd, Zn, or Pb in soil solutions ($p < 0.05$) (Fig. 1). This result was similar to that in other studies [6,8–11,13,14,36]. EDTA can complex with soil metals, which are in the low available fractions initially, and thus to increase the metal concentrations in the soil solutions. Experimental result also revealed that the potential risk of groundwater contamination will increase after applying EDTA and it should be used very carefully or use biodegradable substitutes, like EDDS [12]. Soil used in this study was contaminated with Cd as a result of wastewater discharged by industrial plants. The total concentrations of Cd, Zn, and Pb in the initially contaminated soil measured with aqua regia were 2.58 ± 0.08 , 80.0 ± 5.32 , and $31.3 \pm 2.09 \text{ mg kg}^{-1}$, respectively. In CK, without artificially added metals, applying 5 mmol EDTA kg^{-1} soil solutions significantly increased the concentrations of Cd, Zn, or Pb in soil solutions ($p < 0.05$). However, the increase was minimal compared to other treatments because of the low concentrations of Cd, Zn, and Pb initial in

the soil. The Cd, Zn, and Pb concentrations in soil solution after applying 2 mmol EDTA kg⁻¹ soil also increased, however, some were not statistically significantly.

Experimental results of this study revealed that applying 2 or 5 mmol EDTA kg⁻¹ soil significantly increased most of the Cd, Zn, and Pb concentrations in soil solutions of different metal-treated soils ($p < 0.05$). However, because of the soils low permeability (silty clay) compared to soils employed in other studies [9,14,37,38] and also because of the low EDTA application rate (2 mmol EDTA kg⁻¹ soil) used in this study, the effect of EDTA was only statistically significant for 5 mmol EDTA kg⁻¹ soil ($p < 0.05$).

3.5. The interactions of Cd, Zn, and Pb

The interactions between the metals in contaminated soil and accumulated in the rainbow pink shoots were identified by utilizing the concentration of metal in the rainbow pink shoots. Enhancement (EH), inhibition (IH), or no effect (NE) was determined by the guidance as shown in Table 3. When soil has X and Y elements at the same time, the concentration of Y in the growing plant significantly increased ($p < 0.05$) compared with that growing in the soil only has high concentration of Y element. Then, we say the X element has enhancement effect on the uptake of Y element.

In the control, without applying EDTA solutions, the Cd concentration in rainbow pink shoots increased significantly in the Cd–Zn-treated soil compared with the Cd-treated soil ($p < 0.05$) (Fig. 2). Without applying EDTA, the addition of Zn to the soil significantly increased the Cd concentration in the plant shoots from ND (<0.68) to 14.0 ± 0.00 mg kg⁻¹ ($p < 0.05$). The addition of Zn in the Cd-contaminated soil enhanced the Cd concentrations in rainbow pink shoots. Result of previous studies showed that Zn could reduce the uptake of Cd by both root and foliar parts. Experimental result of this study was similar to two reports [20,39]. However, the Cd concentrations in the rainbow pink shoots when growing in the Cd–Zn-treated soil significantly decreased ($p < 0.05$) in the treatment of 2 mmol EDTA kg⁻¹ soil or decreased in the treatment of 5 mmol EDTA kg⁻¹ soil compared to growing in the Cd-treated soil. The artificially contaminated soil has higher molecular number of Zn (1.53 mmol kg⁻¹) in relative to Cd (0.089 mmol kg⁻¹). After applying 2 or 5 mmol EDTA kg⁻¹ soil, the soil solution of Cd–Zn-treated soil also has higher Zn concentration compared to control treatment without applying EDTA (Fig. 1b). Besides, Zn is an essential element for the

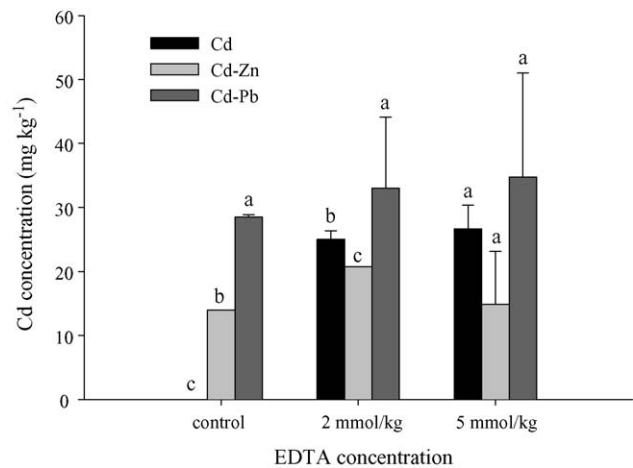


Fig. 2. The concentrations of Cd in the shoots of rainbow pink after planting in the different metals-treated soils, which was harvested at 7th day after applying different concentrations of EDTA. The probability level of significant difference is at $p = 0.05$. Replicates (n) = 3.

growth of plants. Rainbow pink thus accumulated significantly higher Zn concentration in their shoots and thus decreased the Cd uptake when it grown in the Cd–Zn-treated soil ($p < 0.05$) (Fig. 2) compared to Cd-treated soil. The EH effect of the Cd uptake in rainbow pink by adding Zn to soil without adding EDTA disappeared and became to IH or NE after applying 2 or 5 mmol EDTA kg⁻¹ soil, respectively. The addition of Pb (Cd–Pb-treated soil) significantly increased the Cd concentration in the rainbow pink shoots from ND to 28.5 ± 0.35 mg kg⁻¹ ($p < 0.05$) without applying EDTA (Fig. 2). Lead was demonstrated to compete against Cd on the adsorbing sites of soil particles. The Cd concentration in soil solution (Fig. 1a) and in the shoots of rainbow pink (Fig. 2) was thus significantly increased in Cd–Pb-treated soil compared to Cd-treated soil ($p < 0.05$) without applying EDTA. Study results demonstrated that the existence of Pb in Cd-contaminated soil had an EH effect on the Cd concentration in rainbow pink shoots without applying EDTA. Previous studies reported that the addition of Zn or Pb had synergistic effects on the Cd accumulated in plants [16,17,20,39]. The observation results of this study were similar to previous studies. When comparing the result in Cd–Pb-treated soil with that result for Cd-treated soils after application of two EDTA concentrations, the Cd concentration in the plant shoots increased when grown in the Cd–Pb-treated soil; however, the increases were only statistically significant in the treatment of 2 mmol EDTA kg⁻¹ soil ($p < 0.05$). Regardless of whether EDTA applied, the existence of Pb in the Cd-contaminated soils increased the Cd concentration in rainbow pink shoots. Lead had an EH effect on the Cd concentration in the rainbow pink shoots for most treatments. In the treatment of 5 mmol EDTA kg⁻¹ soil, the addition of Pb also increased the Cd concentration in the shoots, but was not statistically significantly because the variation was larger than those for the other treatments.

The existence of Cd in the Zn-contaminated soil decreased the Zn concentration in the rainbow pink shoots with or without applying EDTA, but was only statistically significant without applying EDTA ($p < 0.05$) (Fig. 3). In the con-

Table 3
The guidance used in this study to determine the interactions of metals

Interaction	Code	Metal concentration changed	Significantly different
Enhancement	EH	Trend to increase	$p < 0.05$
Inhibition	IH	Trend to decrease	$p < 0.05$
No effect	NE	Trend to increase	NS ^a
No effect	NE	Trend to decrease	NS
No effect	NE	Have no trend	NS

^a No significant difference.

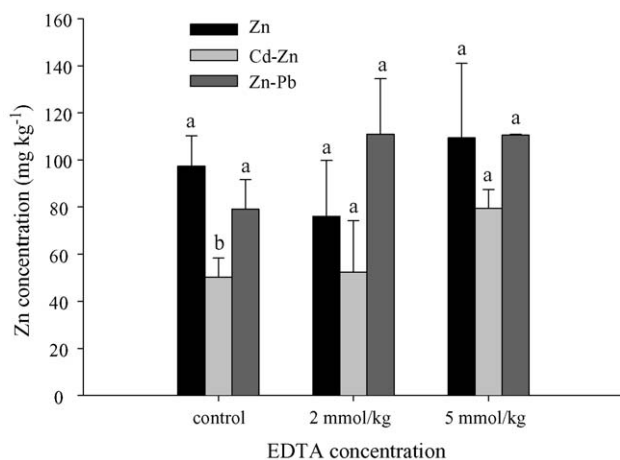


Fig. 3. The concentrations of Zn in the shoots of rainbow pink after planting in the different metals-treated soils, which was harvested at 7th day after applying different concentrations of EDTA. The probability level of significant difference is at $p=0.05$. Replicates (n)=3.

control with applying EDTA, the Zn concentration in the shoots decreased significantly from $97.3 \pm 13.0 \text{ mg kg}^{-1}$ (Zn-treated soil) to $50.3 \pm 8.13 \text{ mg kg}^{-1}$ (Cd-Zn-treated soil) ($p < 0.05$). Cadmium had an IH effect on the Zn concentration in the shoot of rainbow pink without applying EDTA. Without applying EDTA, Zn-treated soil has higher Zn concentration in soil solution compared to Cd-Zn-treated soil (Fig. 1b). Rainbow pink thus accumulated significantly higher Zn concentration in their shoots when grown in the Cd-Zn-treated soil compared to that in the Zn-treated soil (Fig. 3). This experimental result agreed with results in previous studies [20,39]. After applying 2 and 5 mmol EDTA kg^{-1} soil, the Zn concentration in the shoots decreased from $76.0 \pm 23.7 \text{ mg kg}^{-1}$ (Zn-treated soil) to $52.4 \pm 21.7 \text{ mg kg}^{-1}$ (Cd-Zn-treated soil) and from $109 \pm 31.6 \text{ mg kg}^{-1}$ (Zn-treated soil) to $79.4 \pm 7.95 \text{ mg kg}^{-1}$ (Cd-Zn-treated soil). The application of both EDTA concentrations changed the IH effect of Cd without applying EDTA, to that of NE after applying EDTA. Lead had NE on the Zn concentration in the shoots (Fig. 3). In the control without applying EDTA, the Zn concentration in the shoots decreased from $97.3 \pm 13.0 \text{ mg kg}^{-1}$ (Zn-treated soil) to $79.0 \pm 12.6 \text{ mg kg}^{-1}$ (Zn-Pb-treated soil). After applying 2 mmol EDTA kg^{-1} soil, the existence of Pb increased the Zn concentration in the shoots from $76 \pm 23.7 \text{ mg kg}^{-1}$ (Zn-treated soil) to $111 \pm 23.6 \text{ mg kg}^{-1}$ (Zn-Pb-treated soil), however, this increase was not statistically significant. After applying 5 mmol EDTA kg^{-1} soil, the Zn concentrations in the shoots grown in both Zn- and Zn-Pb-treated soils were about 110 mg kg^{-1} . The addition of Pb to soil had NE on the Zn concentration in the shoots after applying 5 mmol EDTA kg^{-1} soil. The added Pb in soil had NE on the Zn concentration in the shoots with or without applying EDTA. The antagonism of Zn on the uptake of Pb by plants was reported by previous study [20]. However, no interaction was found between Zn and Pb with or without applying EDTA although Zn concentrations were significantly increased after applying 2 or 5 mmol EDTA kg^{-1} soil ($p < 0.05$). We supposed that the preferred uptake of rainbow pink on Zn was the possi-

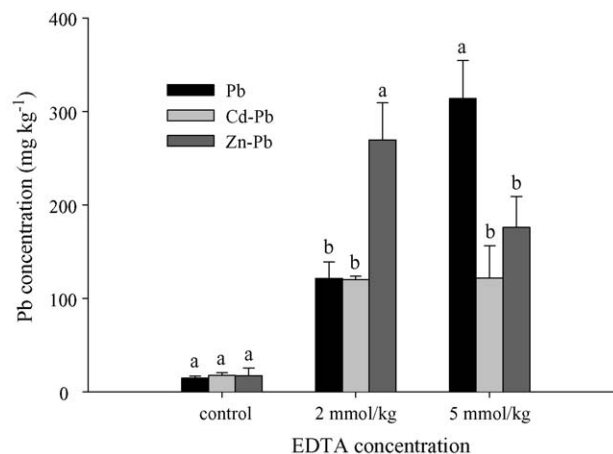


Fig. 4. The concentrations of Pb in the shoots of rainbow pink after planting in the different metals-treated soils, which was harvested at 7th day after applying different concentrations of EDTA. The probability level of significant difference is at $p=0.05$. Replicates (n)=3.

ble reason because Zn is an essential element for the growth of plants under this concentration of Zn.

The Pb concentration in the shoots of rainbow pink planted in the Pb-, Cd-Pb- or Zn-Pb-treated soils without applying EDTA were all about 15 mg kg^{-1} , which was only 1.5% of the total Pb concentration in soil. The addition of Cd or Zn had NE on the Pb concentration in the shoots without applying EDTA solutions (Fig. 4). The low Pb concentration in the shoots of rainbow pink was resulted from the low Pb concentration in soil solution (Fig. 1c). Analyzed result showed that most of the spiked Pb in the study soil was in the non-available fractions for the uptake of rainbow pink. Similar to the results for the control without applying EDTA, the added Cd in Pb-treated soils had NE on the Pb concentrations in the shoots of rainbow pink after applying 2 mmol EDTA kg^{-1} soil (Fig. 4). The Pb concentrations in the shoots after applying 2 mmol EDTA kg^{-1} soil were $121 \pm 17.5 \text{ mg kg}^{-1}$ (Pb-treated soil) and $120 \pm 3.54 \text{ mg kg}^{-1}$ (Cd-Pb-treated soil). The added Cd in Pb-treated soils had NE on the Pb concentration in the shoots after applying 2 mmol EDTA kg^{-1} soil. However, the addition of Cd (Cd-Pb-treated soil) had a drastic effect on the Pb concentrations in the shoots after applying 5 mmol EDTA kg^{-1} soil. The Pb concentration significantly decreased from about $314 \pm 40.7 \text{ mg kg}^{-1}$ in Pb-treated soil to $122 \pm 34.7 \text{ mg kg}^{-1}$ in Cd-Pb-treated soil after applying 5 mmol EDTA kg^{-1} soil ($p < 0.05$). The addition of Cd in Pb-treated soil had an IH effect on the Pb concentration in the shoots with the treatment of 5 mmol EDTA kg^{-1} soil. After applying 2 mmol EDTA kg^{-1} soil to the Zn-Pb-treated soils, the Pb concentrations in the shoots increased significantly compared to that in the Pb-treated soils ($p < 0.05$) (Fig. 4). The concentrations of Pb in the shoots increased significantly from $121 \pm 17.5 \text{ mg kg}^{-1}$ (Pb-treated soil) to $269 \pm 39.8 \text{ mg kg}^{-1}$ (Zn-Pb-treated soil) after applying 2 mmol EDTA kg^{-1} soil ($p < 0.05$). Zinc had an EH effect on the Pb concentration in the shoot of plant after applying 2 mmol EDTA kg^{-1} soil, but changed to an IH effect after applying 5 mmol EDTA kg^{-1} soil. The Pb concentration in the shoots

decreased significantly from 314 ± 40.7 to 176 ± 33.5 mg kg^{-1} after applying 5 mmol EDTA kg^{-1} soil ($p < 0.05$).

4. Discussion

Table 4 shows the interactions between the metal concentration in the soil and in the shoot of rainbow pink when we consider the change of metal concentrations in the shoot of plant. Result demonstrated that without applying EDTA, the addition of Zn or Pb had an EH effect on the Cd uptake in plants and Cd had an IH effect on the Zn uptake in plants. The observed result was identical with those obtained in previous studies [16–20,39]. If no EDTA was applied, the uptake of metals in the shoots of rainbow pink was controlled by the characteristic of metals in soil. Among the three metals used in this study, cadmium was rapidly uptake by plants compared to Pb and Zn [39]. Rainbow pink accumulated significantly higher Cd concentration in their shoots when grown in the Cd–Zn- or Cd–Pb-treated soil in relative to Cd-treated soil. The existence of Cd, thus, has inhibition effect on the uptake of Zn. Whether the addition with EDTA or not and the concentration of added EDTA affected the interactions between the three metals. After applying two concentrations of EDTA solutions, the EH effect of adding Zn on the Cd concentration in the shoots changed to an IH effect in 2 mmol EDTA kg^{-1} soil and NE in the treatment of 5 mmol EDTA kg^{-1} soil. The total concentration of artificially spiked Cd, Zn, and Pb in this study was about 0.089, 1.53, and 4.82 mmol kg^{-1} , respectively. When we use an assumed chelating ratio (EDTA:metal) of 1:1, application of 2 mmol EDTA kg^{-1} soil was sufficiently to increase the Zn concentration in soil solution of Cd–Zn-treated soil except for Pb. Furthermore, Zn is also an essential element for the growth of plants. Rainbow pink thus accumulated more Zn when grown in the Cd–Zn-treated soil after applying 2 mmol EDTA kg^{-1} soil. Zn thus has inhibition effect on the uptake of Pb in the treatment of 2 mmol EDTA kg^{-1} soil. In the treatment of 5 mmol EDTA kg^{-1} soil, the added EDTA was sufficiently to form complexes with the whole Pb in soil because of the higher formation constant of EDTA–Pb complex ($\log K_f = 19.0$) compared to EDTA–Cd complex ($\log K_f = 17.4$) and EDTA–Zn complex ($\log K_f = 17.5$). After applying 5 mmol EDTA kg^{-1} soil, rainbow pink accumulated more Pb concentration when grown in the Pb-treated soil in relative to Cd–Pb- or Zn–Pb-treated soil.

The no effect of addition of Cd or Zn to soil on the Pb concentration in the shoots without applying EDTA solutions changed to an IH effect after applying 5 mmol EDTA kg^{-1} soil.

There was a linear relationship between the Cu and Zn concentration in soil solution and their uptake of sunflowers in the presence of EDDS [40]. In the absence of chelating agents, experimental result of previous study indicated that there was a logarithmic relationship between the Cd concentration in the leaf and stem or grain part of wheat and that in soil solution or in soil extracted with 0.05 M EDTA or 0.005 M DTPA [41]. In the end of EDTA-enhanced phytoextraction experiment, there was also a logarithmic relationship between the Zn or Pb concentration in the shoot of rainbow pink and that in soil solution [6]. However, the final Zn and Pb concentration of shoot was not same as that uptake before the addition of EDTA. During chelant-assisted phytoextraction, plants might have different uptake behaviors (or linear uptake model) in the presence of chelating agents [40].

In this study, we assumed that rainbow pink uptake metals evenly during whole 28 days experiment and their uptake in the last 7 days after applying EDTA was assumed about 25% of the total uptake. For single or combined metals-contaminated soils, there are logarithmic relationships between the metal concentration in soil solution and that in the shoots of rainbow pink in the presence of 2 or 5 mmol EDTA kg^{-1} soil (Fig. 5). Because of enhancement or inhibition interactions between metals (Table 4), the correlation coefficients (R^2) between metals concentration in the shoot of rainbow pink and that in soil solution in the presence of EDTA are only 0.12 (Cd), 0.33 (Zn), and 0.42 (Pb), respectively (Fig. 5). There are some drawbacks when using above assumption to estimate the uptake of metals by rainbow pink in the presence of EDTA. Because the objective of this study is to assess the interaction between Cd, Zn, and Pb, it is difficult to assess the uptake behavior because we do not have enough data in the presence of EDTA. The uptake of metals by rainbow pink might not evenly during the whole 28 days and the interactions of metals will also affect the metal concentration in the shoots of plants. For further study to investigate the uptake behavior of metals during chelant-assisted phytoextraction, the experimental design should separate into two parts (i.e. with or without applying chelating agents). The metal uptake of plants in the absence or presence of chelating agents will help us for further understanding the uptake behavior during chelant-assisted phytoextraction.

Table 4
The interactions between the metal in soil and concentration in the shoot of rainbow pink (mg kg^{-1}) when considering the concentration change of metal in the shoot of plant

	EDTA treatments								
	Control (0 mmol EDTA kg^{-1})			2 mmol EDTA kg^{-1}			5 mmol EDTA kg^{-1}		
	Cd	Zn	Pb	Cd	Zn	Pb	Cd	Zn	Pb
Added metal									
Cd	–	IH	NE	–	NE	NE	–	NE	IH
Zn	EH	–	NE	IH	–	EH	NE	–	IH
Pb	EH	NE	–	EH	NE	–	NE	NE	–

EH, enhancement; IH, inhibition; NE, no effect; –, no effect for the same element.

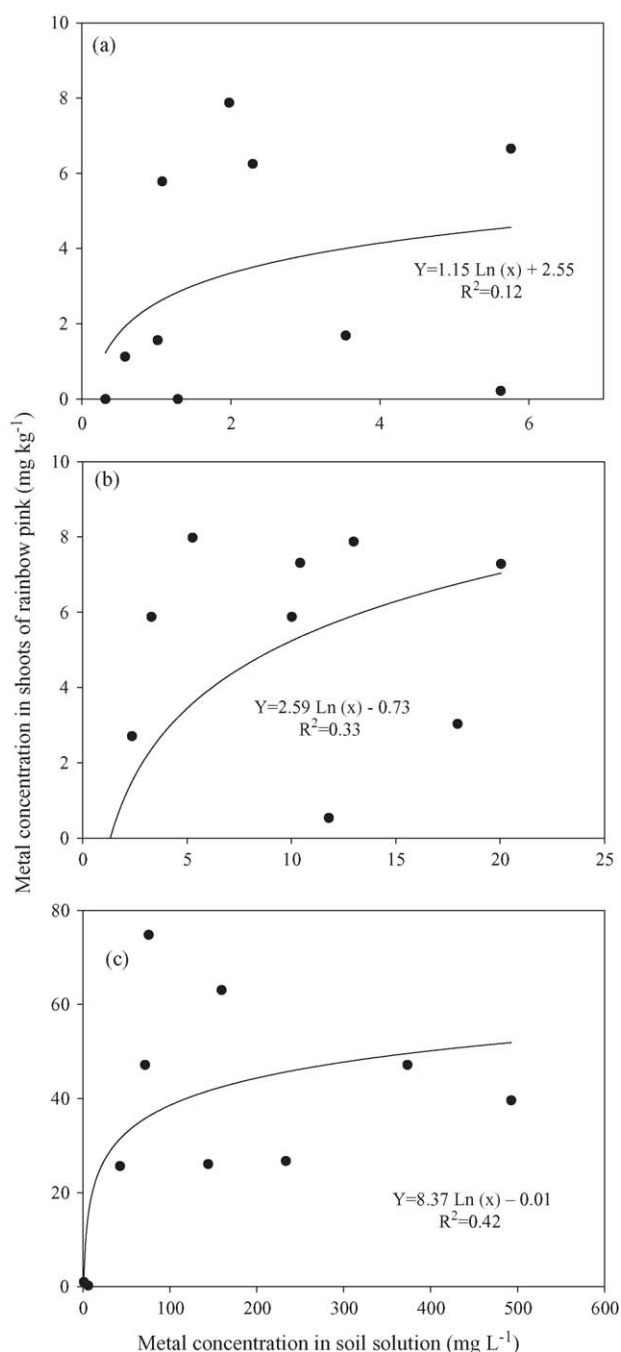


Fig. 5. The relationship between the concentrations of: (a) Cd, (b) Zn, and (c) Pb in the soil solution and in the shoot of rainbow pink in the presence of EDTA.

5. Conclusion

Experimental result of the phytoextraction capacity study showed that rainbow pink has the potential to accumulate high concentrations of metals from metal-contaminated soils after growing for 50 days. In single or combined metal-contaminated soils used in interaction study, the application of 5 mmol EDTA kg⁻¹ soil significantly increased the Cd, Zn, and Pb concentrations in the soil solution ($p < 0.05$). The application rate of 2 mmol EDTA kg⁻¹ soil for silty clay soil utilized in this study was too low to increase the concentrations of metals in

soil solutions. However, EDTA should be used as a reference compound and is not recommended to be in situ used in the contaminated site because it will be resistant to biodegradation.

Without applying EDTA, Pb had an EH effect on the uptake of Cd in rainbow pink; the EH effect was not affected by the application of 2 mmol EDTA kg⁻¹ soil. Cadmium had an IH effect on the uptake of Zn in rainbow pink in control without applying EDTA, but had NE after applying 2 or 5 mmol EDTA kg⁻¹ soil. Because of the greater formation constant of EDTA–Pb complex compared to Cd–EDTA and Zn–EDTA and the sufficient amounts of added EDTA, the existence of Cd or Zn at the same time inhibited the uptake of Pb in rainbow pink after applying 5 mmol EDTA kg⁻¹ soil. The experimental results of this study revealed that some interactions existed in the combined metal-contaminated soils and that the interactions of these metals affected the result of phytoremediation.

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