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Research Article

Phytoextraction of Cu, Zn, and Pb Enhanced by Chelators with Vetiver (*Vetiveria zizanioides*): Hydroponic and Pot Experiments

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Phytoextraction is a green remediation technology for clean-up contaminated soils. The effect of chelator application including EDTA, EDDS, and citric acid on phytoextraction of Cu, Zn, and Pb into high biomass vetiver (*Vetiveria zizaniodides*) was investigated in the hydroponic experiment and the pot experiment. In the hydroponic test, EDTA induced the most significant toxic symptom on vetiver compared to EDDS and citric acid. Obvious biofilm was attached in the rhizosphere of vetiver with the citric acid addition due to its serving as growth substrate while EDTA posed microbial toxicity to present clear solution. Sequential extraction results demonstrated that EDTA was better than EDDS and citric acid to change the adsorbed metal to loosely bound fraction which is more mobile and bioavailable. In the pot experiment, the critical finding was that vetiver has been demonstrated as a hyperaccumulator for treatment of EDDS with Cu; EDDS, citric acid, and EDTA with Zn; EDTA with Pb. EDDS and EDTA possess the viable phytoextraction ability and can be employed as an remediation alternative, though the groundwater leaching needs to be taken into serious consideration.

1. Introduction

Heavy metals including Cu, Zn, and Pb from anthropogenic industrial operation are major pollution sources to cause soil contamination. Conventional physical-chemical technologies employed for heavy metals remediation including dig-and-dump, soil washing/flushing, and vitrification, are usually quite costly, energy consuming, and harmful to soil properties. In Taiwan, the most commonly used soil remediation for agricultural farmland was composed of vertical/horizontal soil mixing and redistribution for heavy metals (Cu, Zn, Ni, Pb, and Cr), while soil acid washing for health concerned heavy metals (Cd and Hg). Lots of debates concerned the effectiveness and soil property deterioration impact of these remediation technologies [1]. Phytoextraction, a green remediation alternative removing metals by the use of plants, has drawn great attention and has offered a promising technology for heavy metal removal from soil [2, 3]. Phytoextraction can employ hyperaccumulators or plants with high biomass assisted by chelators. The success of phytoextraction employing hyperaccumulators is simply based on the ability to uptake and to retain metals within aerial parts. Hyperaccumulator species are plant aerial tissues that can contain >1000 mg/kg Cu, or Pb, and >10000 mg/kg Zn when grown in metal-rich soils. Hyperaccumulation species generally are slow growing and have small biomass [4].

Vetiver is known for its effectiveness in soil erosion control due to its unique morphological and physiological characteristics. Vetiver is also a high biomass plant with remarkable photosynthetic efficiency that renders it tolerant against various harsh environmental conditions. Vetiver with deep roots and higher water use can effectively stabilize soluble metals in soils [5]. These properties enable vetiver to be an ideal candidate for phytoextraction.

The use of chelators for enhancing phytoextraction of metals has been the recent attention [6]. EDTA (ethylene diamine tetraacetic acid) has been employed for soil remediation due to its strong complexes-forming ability. EDTA is poorly biodegraded in the soils though its effectiveness at complexing metals. Excess amounts of EDTA may leach to groundwater and cause subsurface water contamination.

The alternative employed is a biodegradable chelator such as EDDS (ethylene diamine disuccinate), which is an EDTA isomer. EDDS is a naturally occurring substance in soil, which is easily decomposed into less detrimental byproducts. Another biodegradable, citric acid, has also been employed due to its complexation ability and its enhancement of metal mobility in soil has been reported.

A previous study was conducted to investigate EDTA, EDDS, and citric acid on phytoextraction of Cu by *Brassica rapa*. EDTA and EDDS demonstrated comparable metal enhancement while citric acid effect was not significant, which was attributed to fast microbial degradation of citrate and metal-citrate complex. Results of that study also indicated that biodegradable EDDS was effective for prevention of metal leaching during chelator-induced phytoextraction [10].

To our best literature review of recent studies (Table 1), our research is unprecedented to investigate three chelators and three metals in hydroponic and pot experiments to compare their phytoextration efficiency in vetiver. The objectives of this study were aimed to investigate the phytoextration efficiency including root uptake and aerial transportation assisted by chelator EDDS, citric acid, and EDTA in vetiver.

2. Materials and Methods

2.1. Hydroponic Experiment. Vetiver was collected from the University of Kaohsiung campus wetlands (22°73′N, 120°28′E) precultured for 5 days and carefully washed with distilled water. These plants were transferred in 2 L experimental tanks filled with 1.5 L of Hoagland's solution. Nutrient solution pH was adjusted to 6.0 with 0.1 M NaOH or 0.1 M HCl. Three individual plants were placed in each tank. Compressed air was gently applied to provide vetiver with adequate oxygen. Analytical grade of chemical was used to prepare stock solution. Three heavy metals, Cu, Zn, and Pb (prepared as $Pb(NO_3)_2$, $CuSO_4$, and $ZnCl_2$), were prepared as 5 mg/L while three chelators EDDS, citric acid, and EDTA were with the concentration 5 mM. The initial average height and weight of vetiver were 30 cm and 18 g, respectively. The experiment was then conducted in a green house with the temperature, humidity, and light intensity controlled at 33°C, 60%, 1500 LUX, respectively. The growth responses of vetiver and metal removal efficiency were recorded. The plants were collected for metal uptake evaluation after 7 days.

2.2. Pot Cultural Experiment. Sediment samples were collected from local farmland. The properties of soil and the background metal concentrations are shown in Table 2. Collected soil was artificially spiked with $CuSO_4$, $ZnCl_2$, and $Pb(NO_3)_2$, mixed well, and air-dried for 5 days to mimic the local contaminated levels which were 1000, 8000, and 8000 mg/kg, for Cu, Zn, and Pb, respectively. These pollution levels were equivalent to 2.5, 4, and 4 times of Taiwan soil pollution control standard for Cu, Zn, and Pb, respectively. Three vetivers were transferred to each pot, filled with 1.5 kg and 14 cm of air-dried soil in a pot with the depth of 17 cm and the diameters of 18 cm for the following treatments. Three chelators including EDTA, EDDS, and citric acid with the same concentration 5 mmol/kg were applied to each pot of soil at once initially. The distribution and chemical fraction of heavy metals retained in soil were examined to investigate the bioavailability of adsorbed metals. The fractionation of heavy metals was investigated by a sequential extraction technique. Soil samples were placed in a plastic bottle then shaked for proper mixing. Soil samples were then subjected to a five-step serial extraction procedure. The procedure of sequential chemical extraction used in this study includes a series of reagents which are represented as exchangeable (1 M KNO_3), inorganically bound (0.5 M KF), organically bound (0.1 M $Na_4P_2O_7$), Fe and Mn-oxide bound (0.3 M $Na_3C_6H_5O_7$, 1 M $NaHCO_3$, and 0.5 g $Na_2S_2O_4$), and sulfide (6 M HNO_3) forms, respectively [11].

2.3. Plant Tissue and Soil Analysis. Vetiver was harvested, carefully washed, and air-dried for both hydroponic and pot tests. Plant samples were dried at 103°C in an oven until completely dried. Dried plant samples were divided into root, stem, and leave for metal accumulation assessment. These pretreated plants were digested in a solution containing 11 : 1 HNO_3 : HCl solution via a microwave digestion apparatus (Mars 230/60, CEM Corporation) and diluted to 100 mL with deionized water. In the hydroponic test, water samples of 1 mL were taken from the experimental tank and were diluted to 50 mL for heavy metal analysis. In the pot test, 0.2 g of dried soil adding *aqua regia* rending for microwave digestion and 2.5 g of dried for sequential extraction experiments. Metals analyses were conducted via an atomic absorption spectrophotometry (AAS, Perkin Elmer).

2.4. Data and Statistical Analysis. Data were evaluated relative to the control to understand their statistical variation. Metal concentration of plants was recorded as mg of metal per kilogram of dry biomass. The bioaccumulation coefficient (BCF; $C_{\text{roots}}/C_{\text{soil or water}}$) was described as the heavy metal concentration in plant divided by the heavy metal concentration in the solution or soil for hydroponic and pot experiments, respectively. To quantify the translocation of heavy metals from roots to aerial parts, it the translocation factor (TF) was used. TF ($C_{\text{shoots}}/C_{\text{roots}}$) was depicted as the ratio of concentration of metal in shoot to its concentration in root. It was calculated by dividing the metal concentration in shoot by the metal concentration in root. With reference to remediation factor in Sun et al.'s study [9], phytoremediation factor (PEF) was defined as $M_{\text{shoots}}C_{\text{shoots}}/M_{\text{soil}}C_{\text{soil or water}}$ in this study to consider both background metal levels and vetiver uptake where C and M represent concentration and mass, respectively. PEF was calculated for both hydroponic and pot experiment results to illustrate the phytoextration efficiency. Schematic diagram of the pot experiment and BCF, TF, and PEF are shown in Figure 1. A triplicate of water and soil samples from each treatment were recorded and used for statistical analyses. Statistical significance was assessed using mean comparison test. Differences between treatment concentration means of parameters were determined by

Table 1: Plant uptake and transportation in recent study.

Reference	Plant species	Plant uptake concentration (mg/kg)	TF	BCF	Chelator concentration
Doumett et al. [7]	*Paulownia tomentosa*	Root, shoot: Cu: 570, 46 Zn: 750, 149 Pb: 750, 149	Cu: 0.08 Zn: 0.2 Pb: 0.1	Cu: 0.27 Zn: 0.16 Pb: 0.06	EDTA 5 mmol/kg
Epelde et al. [8]	*Cynara cardunculus*	Root, shoot: Pb: EDDS (4165, 310) EDTA (6695, 1332)	EDDS: 0.02 EDTA: 0.20	EDDS: 0.83 EDTA: 1.34	EDDS 10 mmol/kg EDTA 10 mmol/kg
Sun et al. [9]	*Sedum alfredii*	Root, stem, leaf, shoot: Cu: CA (32, 10, 11, 11) EDTA (25, 12, 12, 12) Pb: CA (39, 18, 18, 18) EDTA (68, 39, 43, 40) Zn: CA (680, 2000, 1950, 1930) EDTA (380, 2030, 2000, 2030)	Cu: CA (0.03) EDTA (0.57) Zn: CA (2.88) EDTA (5.34) Pb: CA (0.45) EDTA (0.61)	Cu: CA (0.03) EDTA (0.57) Zn: CA (12.6) EDTA (10.8) Pb: CA (0.29) EDTA (0.7)	Citric acid 5 mmol/kg EDTA 5 mmol/kg
Li et al. (2009)	*Vetiveria zizanioides*	Root, shoot: Zn: 150, 82	Zn: 0.55	Zn: 0.85	EDTA 0.8 mmol/kg
This study	*Vetiveria zizanioides*	Root, stem, leaf: Cu: EDDS (1818, 1459, 361) CA (926, 56, 15) EDTA (2080, 954, 86) Zn: EDDS (16388, 12412, 12036) CA (14444, 12420, 10821) EDTA (12899, 9891, 12552) Pb: EDDS (4343, 280, 197) CA (4914, 388, 103) EDTA (4632, 1878, 340)	Cu: EDDS (0.51) CA (0.04) EDTA (0.25) Zn: EDDS (0.7) CA (0.82) EDTA (0.86) Pb: EDDS (0.06) CA (0.05) EDTA (0.24)	Cu: EDDS (1.97) CA (0.88) EDTA (2.22) Zn: EDDS (1.95) CA (1.67) EDTA (1.5) Pb: EDDS (0.63) CA (0.67) EDTA (0.58)	EDDS 5 mmol/kg Citric acid 5 mmol/kg EDTA 5 mmol/kg

Table 2: Soil properties and background metal concentrations.

	Soil value
pH (H_2O)	6.58 ± 0.44
Organic matter (%)	3.43 ± 0.13
Clay (%) <2 μm	12.10
Silt (%) 2–50 μm	82.20
Sand (%) 50–2000 μm	5.70
Background Cu concentration (mg/kg)	23.26 ± 4.72
Background Zn concentration (mg/kg)	121.55 ± 6.34
Background Pb concentration (mg/kg)	76.55 ± 12.68

Student's t test. A level of $P < 0.05$ considered statistically significant was used in all comparisons. Means are reported ± standard deviation. All statistical analyses were performed with Microsoft Office EXCEL 2003.

3. Results and Discussion

3.1. The Growth and Toxicity Symptom of Vetiver in Hydroponic Experiments. The increasing height of Cu+EDDS, Cu+EDTA, Zn+EDDS, and Zn+citric acid were 19, 16, 20, and 18 cm, respectively, while Zn+EDTA and Pb+EDTA only increased 5 cm after 7 days, indicating that EDTA posed adverse effect with the least height increase. Pb+citric acid presented the most prominent growth with the increased height of 30 cm. The great propagation might be due to citric acid serving as a growth nutrient.

Observation of root of citric acid and EDTA are shown in Figure 2. The figure showed obvious biofilm attached in the rhizoshpere of vetiver with the citric acid addition while EDTA posed microbial toxicity to present clear solution relative to a citric acid treatment. Tandy et al.'s previous study presented similar creamy white substances in root zone of sunflower with EDDS treatment. Their results also demonstrated that 500 μM EDDS did not reduce shoot or root biomass compared to the control. EDDD alleviated the observed symptoms of the shoot and root toxicity when added to the Cu, Zn, or Pb solution [12].

EDTA visibly affected plant growth within 1-2 d, and the leaves appeared damaged and started to wilt, with leaf abscission several days later. EDTA exhibited chlorosis, necrosis, and wilt indicating phytotoxicity. No significant toxic symptom was detected in the experiment with EDDS and citric addition. The results depicted that morphological symptoms including yellowing and chlorosis of leaves and root shedding due to metal toxicity were particularly evident

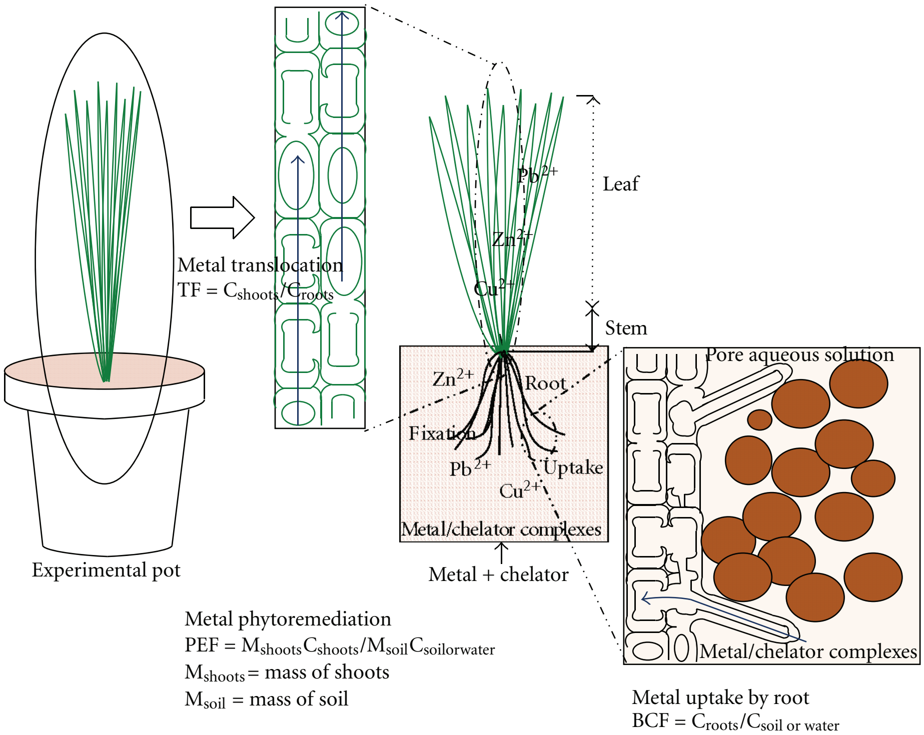

Figure 1: Schematic diagram of the pot experiment.

(a) (b)

Figure 2: Comparison of the root of vetiver in hydroponic experiments, (a) Pb+Citric acid, (b) Pb+EDTA.

in vetiver exposed to EDTA. The finding was consistent with the research conducted on the hydroponic tests on the Siam weed [13]. Our results of hydroponic tests demonstrated that vetiver growth with addition of biodegradable chelators, namely EDDS and citric acid, has better results than the EDTA.

3.2. Hydroponic Heavy Metal Removal Performance. Heavy metal removal by hydroponic experiments in different treatment conditions is shown in Table 3. After 7 days of investigation, citric acid had the highest metal removal with respect to the control, while EDDS and EDTA had less metal removal, in particular for Cu and Zn. The adverse effects have been observed with the addition of chelators compared to control. For Cu, control, EDDS, citric acid, and EDTA had the removal rate of 36.7, 9.1, 33.2, and 4.1%, respectively. For Zn, control, EDDS, citric acid, and EDTA had the removal rate of 15.3, 6.9, 14.3, and 3.3%, respectively. For Pb, control, EDDS, citric acid, and EDTA had the removal rate of 72.9, 57.1, 70.9, and 6.9%, respectively. The removal of metals was in the decrease sequence Pb > Cu > Zn. For those data did not show in Table 3 was caused by the treatment was ceased due the level off of solution concentration. Discrepancy of data might be explained by the metal adherence to the experimental tank.

3.3. The Impact of Chelator on the Uptake and Translocation of Metals in Hydroponic Experiments. Metal accumulation

Table 3: Heavy metal decreasing concentration and the removal efficiency in hydroponic experiments. unit: mg/L.

Treatment condition	Operation time (day) 0	1	2	3	4	5	6	7	Removal efficiency (%)
Cu	4.58	2.47	2.97	2.58	2.60	1.85	2.38	2.90	36.70
Cu+EDDS	5.63	5.12	5.40	—	—	—	—	—	9.10
Cu+CA	5.58	2.05	4.07	4.07	4.48	3.60	4.03	3.73	33.2
Cu+EDTA	5.32	2.95	5.10	—	—	—	—	—	4.10
Zn	4.52	4.12	4.43	3.83	—	—	—	—	15.3
Zn+EDDS	5.10	5.28	5.6	4.75	—	—	—	—	6.90
Zn+CA	5.25	4.85	5.5	4.5	—	—	—	—	14.3
Zn+EDTA	4.53	4.37	—	4.38	—	—	—	—	3.30
Pb	5.65	2.89	2.59	2.56	1.73	1.59	1.91	1.53	72.9
Pb+EDDS	5.69	5.15	5.39	5.05	3.14	4.8	4.51	2.44	57.1
Pb+CA	5.42	3.83	3.66	2.79	3.27	3.29	3.04	1.58	70.9
Pb+EDTA	5.51	4.83	5.30	4.59	5.56	6.28	6.23	5.13	6.90

concentrations in different parts of vetiver are shown in Table 4. For Cu, the accumulation of heavy metal in whole plants of vetiver increased in the sequence citric acid > control > EDDS > EDTA. For Zn, total accumulation concentrations were in the increase order of citric acid > control > EDTA > EDDS. For Pb, metal accumulated in the whole vetiver increased in the sequence control > EDDS > citric acid > EDTA. Regardless of the different treatments, the root parts were the plant most accumulation areas while concentrations in other parts were in the decrease sequence root > stem > leave.

A previous study was conducted using EDDS for the essential Cu and Zn and nonessential Pb uptake by sunflower in a hydroponic experiment. EDDS alleviated the phytotoxicity when added to the Cu and Zn solution due to the reduction in the free metal concentration forming EDDS metal complexes in solution [12]. Jean et al.'s study reported that the translocation from root to stem was associated with metal chelation enhancing its transport to stem by reducing the affinity for the binding site in the cell walls. Metal complex was not retained by ion exchange as compared to free metal ions [14].

The mechanism of metal complex transportation enables the understanding of uptake effectiveness. The passive apoplastic transportation has been proposed as a possible mechanism [15]. The addition of chelators has generally prevented metal precipitation and formed metal complex compound. Apoplast is the free diffusional space outside of the plasma membrane that has high content of carboxylic groups, can act as effective cation exchanger. The negatively charged chelator complexes prevented binding to the cell walls of the roots and allowed complexes to enter into the cells. Metal chelator complexes were subsequently translocated to the aerial part of plant through passive apoplastic pathway.

3.4. Hydroponic Bioconcentration Factor (BCF), Translocation Factor (TF), and Phytoremediation Factor (PEF). Metal uptake results calculated by BCF, TF, and PEF parameters are shown in Figure 3. BCF indicates root metal concentration divided by the solution concentration. The value was used to explain the uptake of vetiver in roots. For Cu, EDDS, citric acid, EDTA were 0.3, 0.8, and 0.2 times decreasing compared to the control ($P = 2.1 \times 10^{-5}$, 0.0057, and 9.3×10^{-6}), respectively. Citric acid presented statistically significant results in root accumulation compared to the other two chelators (P = EDDS: 0.002; EDTA: 0.009). Cu accumulation levels in root were in the decreasing sequence citric acid > EDDS > EDTA. For Zn, EDDS, citric acid, and EDTA were 0.6-, 0.9-, and 0.7-folds (P = 0.056, 0.46, and 0.068) decreased relative to the control BCF value, respectively. Three chelators compared with each other did not present a significant variation ($P > 0.05$). For Pb, EDDS, citric acid, and EDTA were 1, 1.1, and 0.3 times (P = 0.99, 0.58, and 2.9×10^{-5}) compared to the control BCF value, respectively. Citric acid did not show statistical difference compared to EDDS ($P = 0.66$). A previous hydroponic study had demonstrated that the accumulation pattern within plant *Polygonum thunbergii* increased in the sequence of Cd < Pb < Zn < Cu, and bioaccumulation coefficients increased in the order of Cd (2.0) < Pb (3.2) < Zn (13.1) < Cu (17.2) [16]. The discrepancies of BCF from our results might be due to variation in heavy metal concentration and plant species. BCF results showed overall negative effect in addition of EDTA in our study.

TF has been defined as the concentration of aerial parts divided by the concentration of the root. The ratio can be used to evaluate the translocation effects with vetiver. TF for each treatment was less than 1 indicating that the remaining metals were primarily located in the roots. For Cu, EDDS, citric acid, and EDTA were 3.3-, 1.2-, and 7.7-fold (P = 0.009, 0.48, and 0.003) increased relative to the control, respectively. EDTA presented statistically significant (P = 0.018 and 0.0029) translocation ability compared to EDDS and citric acid, respectively. For Zn, EDDS, citric acid, and EDTA were 1.0-, 1.2-, and 1.1-folds compared to the control TF value, respectively. Three chelators did not

Table 4: Heavy metal accumulation and translocation in vetiver in hydroponic experiments. unit: mg/kg.

Treatment condition	Metal accumulation			Whole plant accumulation
	Root	Shoot		
		Stem	Leaf	
Cu	258.16 ± 4.98	21.6 ± 3.58	10.41 ± 2.28	290.17
		(15.96)*		
Cu+EDDS	91.72 ± 6.82	20.46 ± 3.54	16.15 ± 5.77	128.33
		(18.31)		
Cu+CA	274.35 ± 38.52	20.95 ± 0.88	12.79 ± 2.77	308.09
		(16.88)		
Cu+EDTA	46.5 ± 4.76	20.44 ± 3.58	21.24 ± 2.3	88.18
		(20.90)		
Zn	124.2 ± 7.53	67.15 ± 4.12	37.63 ± 4.20	228.98
		(52.30)		
Zn+EDDS	89.08 ± 18.27	49.79 ± 20.10	40.53 ± 7.60	179.40
		(45.20)		
Zn+CA	128.5 ± 15.81	91.18 ± 11.82	44.5 ± 11.84	264.18
		(67.80)		
Zn+EDTA	82.1 ± 18.64	67.37 ± 18.82	43.85 ± 14.27	193.32
		(55.60)		
Pb	276.98 ± 21.16	93.3 ± 7.78	8.47 ± 3.11	378.75
		(50.32)		
Pb+EDDS	279.51 ± 68.34	61.51 ± 29.38	ND	341.02
		(61.51)		
Pb+CA	291.23 ± 78.1	18.16 ± 7.54	14.09 ± 1.64	323.48
		(16.13)		
Pb+EDTA	75.58 ± 4.55	37.94 ± 5.78	30.82 ± 5.77	144.34
		(34.38)		

* () means the metal concentrations in shoot.

present significant variation ($P > 0.05$). For Pb, EDDS, citric acid, and EDTA were 1.2-, 0.3-, and 2.6-fold compared to the control, respectively. EDTA showed the significant difference when it compared to other two chelators (P = EDDS: 0.008; EDTA: 0.0008). Based on these TF analyses, EDTA demonstrated better translocation for Cu and Pb compared to the control.

PEF indicates the concentration in the aerial parts divided by the concentration in solution and both concentrations times the vetiver mass and water weight, respectively. For Cu, PEF value for control, EDDS, citric acid, and EDTA was 3.64, 3.25, 3.02, and 3.94, respectively. PEF did not present significant variations ($P > 0.05$) among three chelators when they were compared with each other with t-test. For Zn, PEF for control, EDDS, citric acid, and EDTA was 11.62, 8.86, 12.93, and 12.27, respectively while for Pb for control, EDDS, citric acid, and EDTA it was 8.90, 10.73, 3.01, and 6.24, respectively.

Total accumulation concentrations indicated that vetiver had the better Pb uptake when it was compared to Zn and Cu without chelator addition. EDTA did not enhance the metal concentration significantly when it was compared with the other two chelators, possibly due to the toxic effect. Hydroponic experiments had some discrepancy results and might not be directly extrapolated to real field conditions. Nevertheless, the hydroponic tests still provide valid approaches for the screening of the feasible chelators for metal uptake enhancement. This hydroponic test was used to observe changes in rhizosphere of plants that affected the efficiency of phytoextraction. The root rhizosphere of citric acid treatment had shown the growth of biofilm possibly due to its serving as a growth substrate for microorganism. Hydroponic results will be further tested for metal phytoextraction in the following pot tests because hydroponic screening has inherent limitations to reflect real-world conditions.

3.5. Sequential Extraction Results of Soil Used for Pot Tests. The sequential extraction results of Cu, Zn, and Pb using three chelators are shown in Figure 4. Initial total metal concentrations of soil were extracted by *aqua regia* aided with microwave heating. Total concentrations of Cu, Zn, and Pb were approximately 1000, 8000, and 8000 mg/kg, respectively which is higher than that most recent phytoextraction studies. Heavy metals associated with different fractions have varied soil mobility and plant uptake efficiency. The latter three fractions, namely organic, Fe- and Mn-hydroxide,

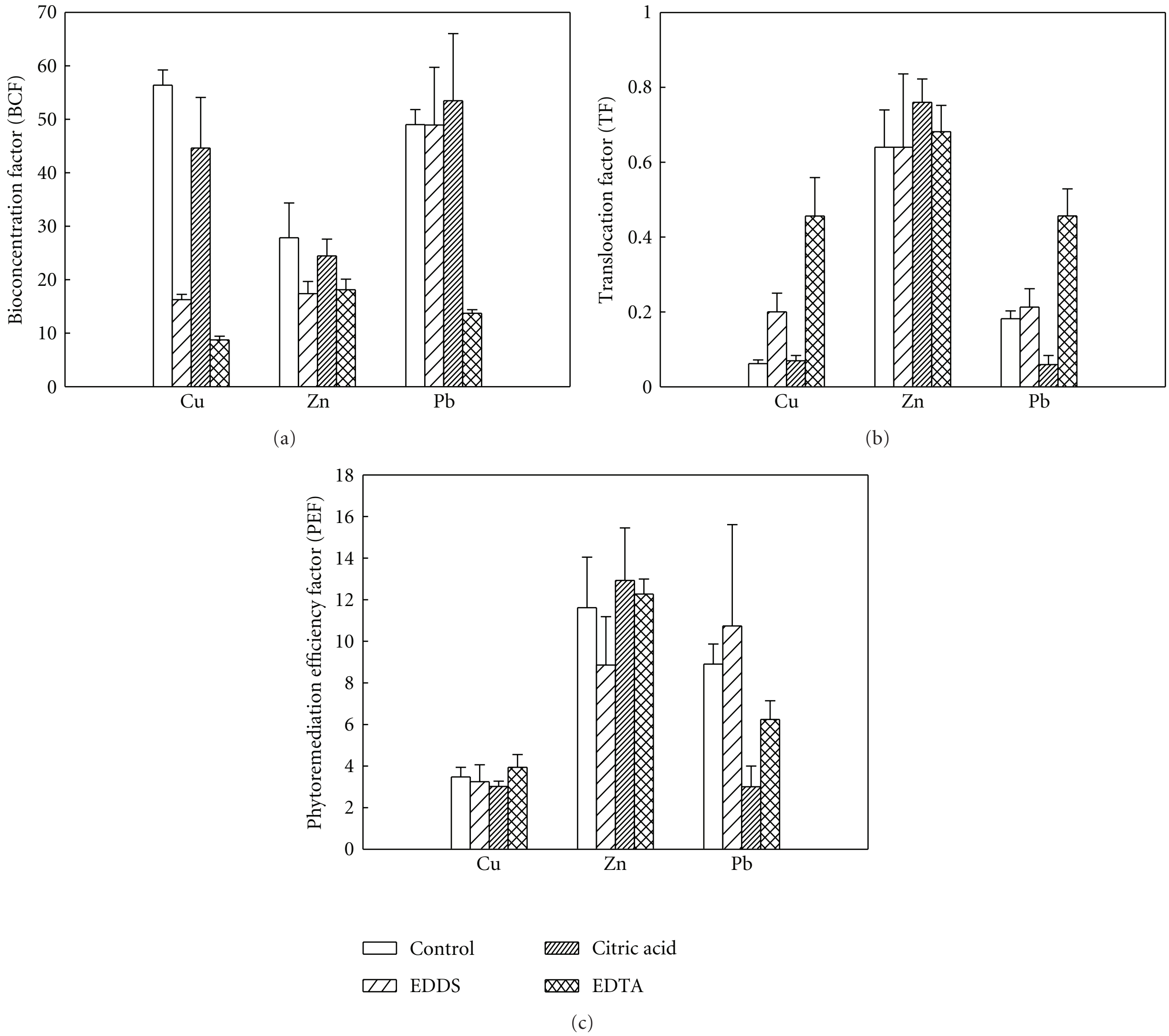

Figure 3: Comparison of (a) BCF, (b) TF, and (c) PEF of different treatment conditions in hydroponic experiments.

and sulfide are more inert, permanently bound to soils, and less bioavailable while the first two fractions including exchangeable and inorganic have been defined as loosely bound.

For Cu, the first two fractions of control, Cu+EDDS, Cu+citric acid, and Cu+EDTA were 1.3, 40.6, 11.1, and 49.7% of total extracted metals, respectively. The mobility effects of three chelators to Cu were in the decreasing order EDTA > EDDS > citric acid. EDTA increased the amounts of loosely bound Cu relative to control that indicated soil Cu mobility was enhanced. For Zn, the first two fractions of control, Zn+EDDS, Zn+citric acid, and Zn+EDTA were 56.5, 57.4, 63.4, and 65.6% of total extracted metals, respectively. The percentages of the first two fractions of control and three chelators were showing no significant difference. The loosely bound percentages for Zn+EDDS, Zn+citric acid, and Zn+EDTA only presented 1.0, 1.2, and 1.3 times relative to the control. Three chelators did not demonstrate significant enhancement for Zn mobility. The reason might be that Zn is commonly treated as a loosely adsorbed metal which led to less firm attachment in soil matrix. For Pb, first two fractions of control, Pb+EDDS, Pb+citric acid, and Pb+EDTA were 5.1, 10.5, 6.8, and 24.8% of total extracted metals, respectively. The first two fraction percentages for Pb+EDDS, Pb+citric acid, and Pb+EDTA presented 1.8-, 1.2-, and 4.6-fold compared to the control. EDDS, citric acid, and EDDA were significantly different with the P value 0.001, 0.024, 1.1×10^{-5}, respectively, with respect to the control. The mobility effects of three chelators to Pb are in the increase order of EDTA > EDDS > citric acid, which is similar to that of Cu.

A previous study investigated the use of EDTA for Pb phytoextraction and found that EDTA was highly effective in solubilizing Pb from soil. From their sequential extraction results, Pb distribution fraction in soil after chelator addition followed the sequence exchangeable > organic matter > iron oxides. 5 mmol/kg of EDTA increased the loosely bound Pb [17]. Another study demonstrated that the mobility and

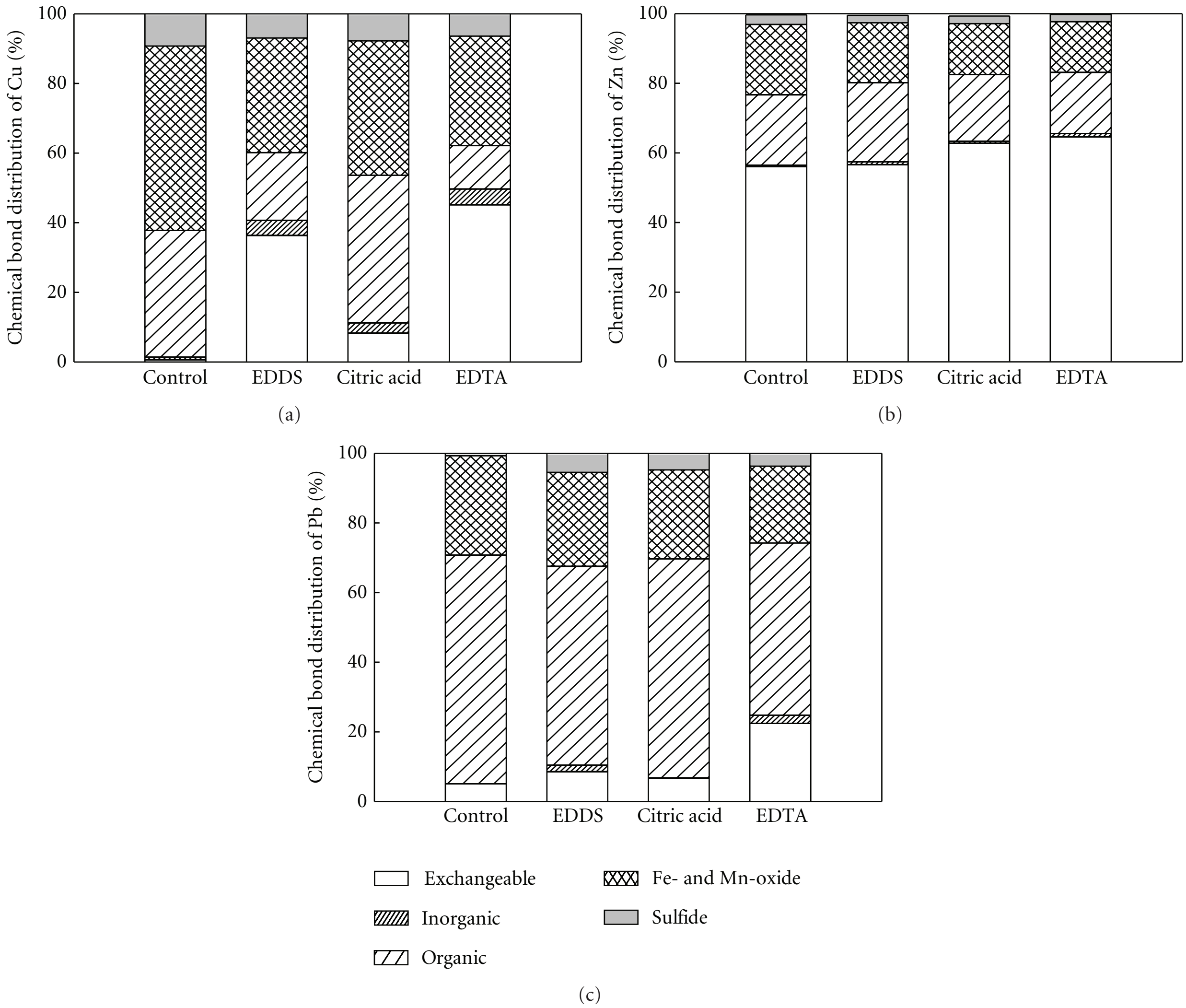

Figure 4: Chemical bond distribution results of (a) Cu, (b) Zn, and (c) Pb of soil in sequential extraction experiment.

bioavailability of heavy metals in soils were correlated with soil texture, surface area, cation exchange capacity, and soil pH [18].

In this study, EDTA and EDDS have promoted the change of the strongly adsorbed fractions to loosely retained fractions for Cu and Pb relative to control. Metal mobility was generally correlated to the stability constants of chelators and metals. Chelators have been commonly employed for ex situ soil washing and in situ soil flushing to decontaminate metal-polluted sites. The effectiveness is determined by the ability to form metal complexes that are mobile and bioavailable.

3.6. The Growth and Toxicity Symptoms of Vetiver in Pot Experiments. The increased heights of vetiver for three chelators of pot experiment are shown in Table 5. The growth of vetiver was observed for 24 days to study the toxic effect of chelators. The initial average length and weight of vetiver were approximately 30 cm and 18 g, respectively. For Cu, the increased height of vetiver for control, EDDS, citric acid, and EDTA was 14.4, 1.2, 9.0, and 0.7 cm, respectively. For Zn, the increased height of vetiver for control, EDDS, citric acid, and EDTA was 0.3, 0.2, 0.5, and 0.2 cm, respectively. For Pb, the increased height of vetiver for control, EDDS, citric acid, and EDTA was 1.3, 2.0, 2.3, and 0.8 cm, respectively. The results of Zn did not present significant growth in chelator amended soils.

Table 5: Increased height of vetiver due to different chelator induced effect in pot experiments.

Treatment condition	Plant growth length (cm)		
	Cu	Zn	Pb
Control	14.4 ± 18.8	0.3 ± 0.3	1.3 ± 0.8
EDDS	1.2 ± 2.0	0.2 ± 0.8	2.3 ± 0.9
CA	9.0 ± 8.5	0.5 ± 0.0	2.0 ± 0.2
EDTA	0.7 ± 0.6	0.2 ± 0.8	0.8 ± 0.8

Cu+EDDS and Cu+EDTA both presented yellowing and chlorosis of leaves at the 12th day. The control and citric acid presented less toxic symptom. For Zn, the control, Zn+EDDS, Zn+citric acid, and Zn+EDTA all showed the yellowing at the 8th day of treatment. All Zn-treated vetiver presented serious chlorosis and wilt symptom at the 14th day. For Pb, all treatments presented yellowing at the 10th day and the toxic effect was in the order of EDTA > citric acid > EDDS. According to the aforementioned results, the toxic effect of EDTA was more significant than that of the other two chelators.

3.7. The Impact of Chelator on the Uptake and Translocation of Metals in Pot Test. The results of the uptake and translocation of metals are shown in Table 6 For Cu, total metal accumulation concentrations of EDDS, citric acid, and EDTA were 14-, 4-, and 12-fold (P = 0.002, 0.02, and 3.5×10^{-6}) increased compared to the control, respectively. The translocation to aerial parts was significant for EDDS, citric acid, and EDTA showing in shoot Cu concentrations raised 151-, 6- and 84-fold (P = 0.004, 4.76×10^{-5}, and 0.002) compared to control, respectively. The results demonstrated that EDDS and EDTA statistically significantly increased total metal concentration and metal in the aerial parts of vetiver. In particular, the shoot concentration of Cu+EDDS was 936 ± 274 mg/kg, which was around the hyperaccumulator level (1000 mg/kg). For Zn, the whole plant accumulation concentrations of EDDS, citric acid, and EDTA were 1.2-, 1.1-, and 1.1-fold compared to control, respectively. The statistical analysis compared with the control did not present significant difference (P = 0.52, 0.88, and 0.77) for the three chelators. However, the Zn concentration in all the aerial parts all achieved hyperaccumulator levels for the three chelator treatment (10000 mg/kg). For Pb, the whole plant accumulation concentrations of EDDS, citric acid, and EDTA were 1.1-, 1.3-, and 1.6-fold (P = 0.55, 0.128, and 0.045) increased relative to control plants, respectively. EDTA presented significant difference ($P < 0.05$) with respect to the control. The other two chelators did not show clear uptake enhancement. EDTA also improved Pb uptake in aerial parts to reach the hyperaccumulator levels (1000 mg/kg). The prominent uptake of Pb by EDTA can be explained by the stability constant $\log K_s = 17.88$ with Pb while the constants for biodegradable chelators EDDS and citric acid were $\log K_s = 18.4$ and $\log K_s = 6.5$, respectively. The critical results of our current research were the achievement of vetiver as a hyperaccumulator.

Another similar research showed prominent metal uptake and translocation of Pb with EDTA. They explained this by its effect on enhancing the solubility of Pb and absorption of the Pb-EDTA complex by the plant *Brassica napus* [19]. In Lin et al's study, EDTA was applied to soil by using sunflower. Pb concentration in the shoot of plants was found directly proportional to the amount of EDTA added to soil. The soil concentration of soluble Pb was correlated with the Pb concentration in plants grown on the soil [20]. Another investigation also demonstrated that EDTA bound Pb was less toxic to free Pb ions and might induce less stress on plants. Pb complexes with phytochelatins were the possible Pb tolerance mechanisms. The results showed that vetiver accumulated by 19800 and 3350 mg/kg in root and shot tissues, respectively [21]. A discrepancy study demonstrated that EDDS caused 2.54-, 2.74-, and 4.3-fold increase in Cd, Zn, and Pb shoot metal concentration, respectively, as compared to control plants. Their study also reported that EDTA induced 1.77-, 1.11-, and 1.87-fold increase in Cd, Zn, and Pb shoot metal concentration, respectively as compared to control plants. Their results demonstrated that EDDS was more effective than EDTA in stimulating the translocation of metals from roots to shoots [22]. Research has reported that the treatment with 5 mmole/kg EDDS in soil resulted in accumulation of 157, 129, and 122 mg/kg of Cu, Zn, and Pb in whole plant, respectively. The concentration in *Brassica carinata* shoots was 2- to 4-fold increased compared to control. Comparing to NTA, the results showed that EDDS in soil degraded rapidly, reducing the risks associated with the leaching of metals to the groundwater [23]. Other researches studied the EDDS enhancement of phytoextration of Cu, Zn, and Pb by maize. The results indicated that soil treated with EDDS significantly increased the concentration of metal in maize shoots (increments of 66%, 169%, and 23% for Cu, Zn, and Pb) with respect to the control [24]. Wang et al. [25] suggested that for phytoremediation of high Pb soil, EDDS would be better at concentration of 5 mmole in a single dosage. Citric acid showed less obvious effect, which might be related to its easy biodegradation in the soil in their study. Rescarach demonstrated that the accumulation of metals in the plant fractions was in the descending sequence Cr > Zn > Cu > Pb. The presence of either compost or *B. licheniformis* BLMB1 strain enhanced metal by accumulation *B. napus*, Cr in particular, in the experimental conditions used (Brunetti, et al, 2011).

Our results for EDTA addition revealed the concentration of Cu, Zn, and Pb of 521, 11233, and 1125 mg/kg in shoot, respectively. The discrepancy compared to other studies might be due to the variation of plant species, initial total metal concentration, and metal bound fraction in soil. In particular, the metal concentration in soil was higher than that of most of the research reported in our study.

3.8. BCF, TF, and PEF Factors in Pot Cultural Experiments. BCF, TF, and PEF in pot experiments of different treatment conditions are depicted in Figure 5. BCF values in the pot experiment can be referred to for evaluatation of vetiver accumulation and adsorption at its root rhizosphere. For Cu, the values of EDDS, citric acid, and EDTA were 1.97, 0.88, and 2.22 equivalent to 9, 4, 10 times of control, respectively. Based on t-test analysis, the variation between control and three chelators presented significant difference (P = 0.00084, 0.022, and 8×10^{-7}). The three chelators all showed the significant enhancement of root Cu uptake. For Zn, the BCF values of control, EDDS, citric acid, EDTA were 2.24, 1.95, 1.67, and 1.50, respectively. Three chelators did not present statistical difference compared to control (P = 0.48, 0.22, and 0.09). For Pb, the BCF values of control, EDDS, citric acid, and EDTA were 0.51, 0.63, 0.67, and 0.58, respectively. Similar to Cu results, Pb with three chelators treatment also did not present statistical difference compared with the

Table 6: Heavy metal accumulation and translocation in vetiver with different treatment conditions in pot experiments unit: mg/kg.

Treatment condition	Metal accumulation			Whole plant accumulation
	Underground	Shoot		
	Root	Stem	Leaf	
Cu	248.70 ± 28.94	6.54 ± 1.88	5.92 ± 1.67	261.16
		(6.21 ± 1.48)*		
Cu+EDDS	1817.60 ± 310.28	1458.58 ± 665.55	360.66 ± 196.08	3636.84
		(936.72 ± 274.41)		
Cu+CA	925.63 ± 330.19	56.33 ± 3.03	14.79 ± 3.10	996.75
		(35.64 ± 2.28)		
Cu+EDTA	2080.00 ± 57.51	953.79 ± 219.53	86.32 ± 5.50	3120.11
		(521.42 ± 108.73)		
Zn	17448.49 ± 4354.89	7700.61 ± 2630.69	8205.86 ± 2470.96	33354.96
		(7954.86 ± 2504.60)		
Zn+EDDS	16387.96 ± 2935.19	12411.55 ± 2111.88	12036.03 ± 3082.94	40835.54
		(12227.15 ± 2568.39)		
Zn+CA	14444.23 ± 3299.45	12420.07 ± 3147.30	10820.70 ± 1055.39	37685.00
		(11609.24 ± 1282.80)		
Zn+EDTA	12898.57 ± 932.03	9891.43 ± 2739.03	12552.38 ± 4360.14	35342.38
		(11233.93 ± 3480.02)		
Pb	4069.25 ± 680.80	67.82 ± 15.70	69.46 ± 19.23	4206.53
		(68.53 ± 9.52)		
Pb+EDDS	4342.94 ± 1038.66	279.67 ± 108.61	197.28 ± 91.01	4819.89
		(238.15 ± 87.84)		
Pb + CA	4913.77 ± 709.89	388.10 ± 179.03	103.03 ± 40.80	5404.90
		(249.04 ± 110.47)		
Pb+EDTA	4632.17 ± 512.71	1877.61 ± 287.53	339.26 ± 172.00	6849.04
		(1125.97 ± 195.30)		

* () means the metal concentrations in shoot.

control ($P = 0.296$, 0.1, and 0.29). The three tested chelators only have significant effect on Cu. The variation might be due to the metal complex property with chelators and total metal concentration in soil.

TF ratio can be used to evaluate the translocation effects in vetiver. High TF can be explained as prominent transfer from root to aerial parts of plant. For Cu, TF of the control, EDDS, citric acid, and EDTA were 0.03, 0.51, 0.04, and 0.2. EDDS, citric acid, and EDTA treatments were equivalent to 17-, 1.3-, 8-fold ($P = 0.0003$, 0.18, and 0.0022) TF increase relative to the control treatment, respectively. For Zn, TF values of the control, EDDS, citric acid, and EDTA were 0.64, 0.74, 0.82, and 0.86, which indicated the TF of EDDS, citric acid, and EDTS was equivalent to 1.2, 1.3, and 1.3 times of control ($P = 0.027$, 0.034, and 0.05), respectively. EDDS, citric acid, and EDTA all revealed statistical difference relative to control ($P < 0.05$). For Pb, the TF values of the control, EDDS, citric acid, and EDTA were 0.02, 0.06, 0.05, and 0.24. These TF values of EDDS, citric acid, and EDTA were equivalent to 3-, 2.5-, 12-fold ($P = 0.08$, 0.1, and 5×10^{-5}) increase compared to the control, respectively. Only EDTA revealed statistical difference when compared with the control. PEF was calculated by the concentrations and weights of soil and shoot. The P values of EDDS, citric acid, and EDTA compared to the control were Cu: 0.004, 4×10^{-5}, and 0.001, Zn: 0.17, 0.19, and 0.39, and Pb: 0.025, 0.04, and 0.0007, respectively.

In our study, the critical finding is that vetiver has been demonstrated as a hyperaccumulator for treatment of EDDS with Cu; EDDS, citric acid, EDTA with Zn; and EDTA with Pb. The other important message is using PEF value to predict the required duration for soil remediation. The remediation time required for phytoextraction reference to PEF can be predicted by the following formula: phytoextraction time (yr) = (metal concentration (mg/kg) in soil needed to decrease × soil mass (kg))/(metal concentration in plant shoot (mg/kg) × plant shoot biomass × the frequency of harvested (number of harvest/yr)). This information is paramountly crucial for a project engineer to design an in situ phytoremediation. In this study, EDTA has been showed

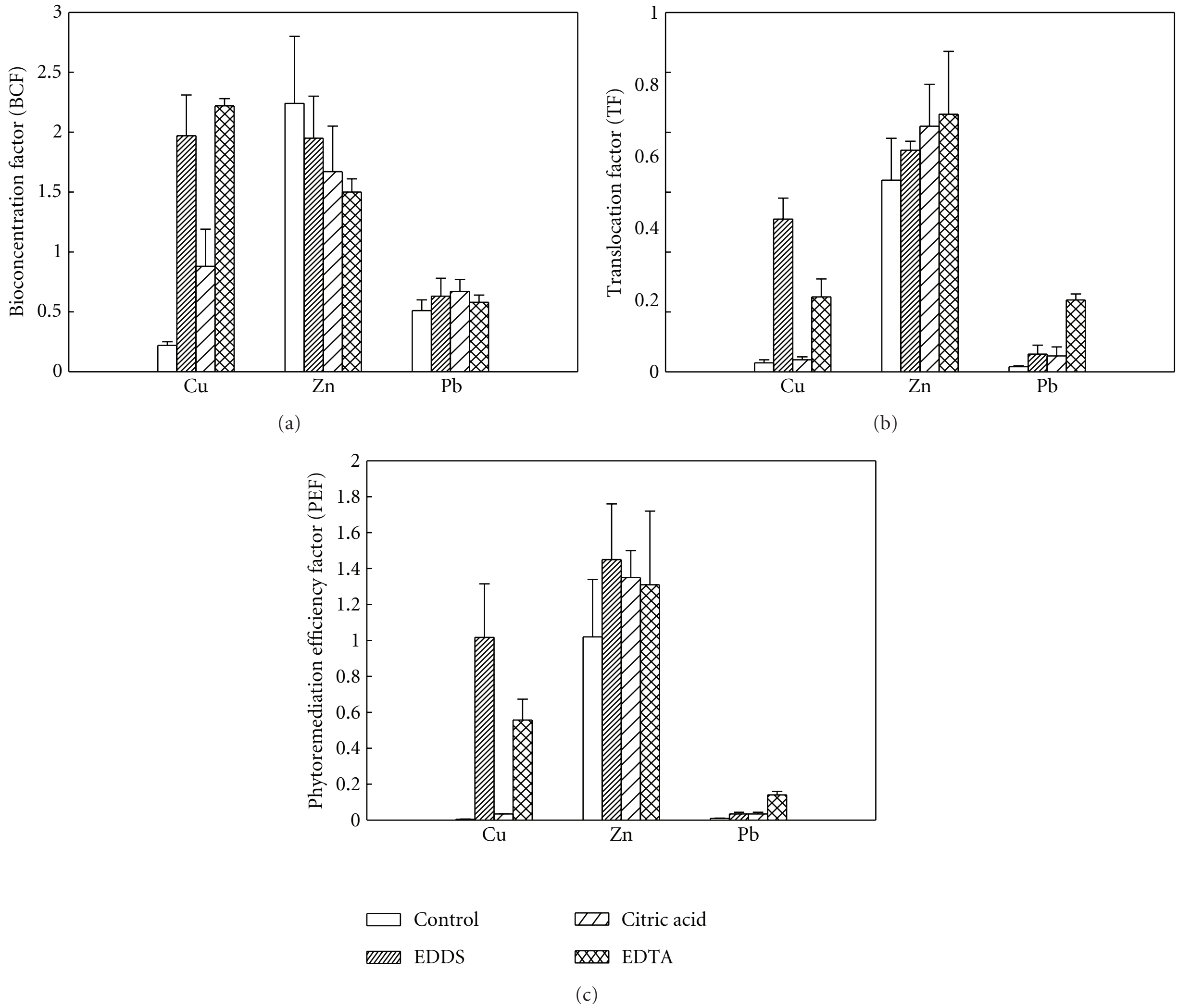

Figure 5: Comparison of (a) BCF, (b) TF, and (c) PEF with different chelator treatments in pot experiments.

to be an effective chelator though its toxic effect and possible leaching to subsurface to induce groundwater contamination. EDDS has a comparable effect with that of EDTA, but it might be more pricy than EDTA. The alternative might chose biodegradable EDDS if groundwater leaching was a major concern. Future study should be focused on the synergistic effect of muti-metal contamination which is more realistic for the real word application.

4. Conclusion

Vetiver was demonstrated as a valid candidate for chelator = assisted phytoextraction in our study. In the hydroponic experiment, root zone observation showed obvious biofilm attached in the rhizoshpere of vetiver with the citric acid addition due to its serving as growth substrate while EDTA posed microbial toxicity to present clear solution. Hydroponic tests can be employed to screen the toxicity of chelator and metal uptake rate. In the pot experiment, the unprecedented finding of this study is that vetiver has achieved the hyperaccumulator in the condition of EDDS addition for Cu, all three chelators for Zn, and EDTA for Pb. Vetiver possesses the great biomass propagation, profuse root which can penetrate to the deeper contaminated soil. Chelator-assisted phytoextraction with vetiver can be a green alternation to conventional costly and not environmental friendly physical-chemical technologies.

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