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Phytoassessment of Vetiver grass enhanced with EDTA soil amendment grown in single and mixed heavy metal–contaminated soil

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Abstract Over the years, ethylene-diamine-tetra-acetate (EDTA) has been widely used for many purposes. However, there are inadequate phytoassessment studies conducted using EDTA in Vetiver grass. Hence, this study evaluates the phytoassessment (growth performance, accumulation trends, and proficiency of metal uptake) of Vetiver grass, *Vetiveria zizanioides* (Linn.) Nash in both single and mixed heavy metal (Cd, Pb, Cu, and Zn)—disodium EDTA-enhanced contaminated soil. The plant growth, metal accumulation, and overall efficiency of metal uptake by different plant parts (lower root, upper root, lower tiller, and upper tiller) were thoroughly examined. The relative growth performance, metal tolerance, and phytoassessment of heavy metal in roots and tillers of Vetiver grass were examined. Metals in plants were measured using the flame atomic absorption spectrometry (F-AAS) after acid digestion. The root-tiller (R/T) ratio, biological concentration factor (BCF), biological accumulation coefficient (BAC), tolerance index (TI), translocation factor (TF), and metal uptake efficacy were used to estimate the potential of metal accumulation and translocation in Vetiver grass. All accumulation of heavy metals were significantly higher ($p < 0.05$) in both lower and upper roots and tillers of Vetiver grass for Cd + Pb + Cu + Zn + EDTA treatments as compared with the control. The single Zn + EDTA treatment accumulated the highest overall total amount of Zn (8068 ± 407 mg/kg) while the highest accumulation for Cu (1977 ± 293 mg/kg) and Pb (1096 ± 75 mg/kg) were recorded in the mixed Cd + Pb + Cu + Zn + EDTA treatment, respectively. Generally, the overall heavy metal accumulation trends of Vetiver grass were in the order of Zn >>> Cu > Pb >> Cd for all treatments. Furthermore, both upper roots and tillers of Vetiver grass recorded high tendency of accumulation for appreciably greater amounts of all heavy metals, regardless of single and/or mixed metal treatments. Thus, Vetiver grass can be recommended as a potential phytoextractor for all types of heavy metals, whereby its tillers will act as the sink for heavy metal accumulation in the presence of EDTA for all treatments.

Keywords Vetiver grass · Lower root · Upper root · Lower tiller · Mixed heavy metal · Enhanced accumulation · Contaminated soil

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Introduction

Heavy metals occur naturally as elemental components in the Earth's crust (Demirbas 2008; Chopra et al. 2009). Some heavy metal such as copper (Cu), cobalt (Co), iron (Fe), manganese (Mn), nickel (Ni), and zinc (Zn) are essentially required by all living organisms in trace amount for biological metabolism and growth. In contrast, many other heavy metals such as cadmium (Cd), mercury (Hg), lead (Pb), and tin (St) have no essential biological function and can be freely bioaccumulated through the food chain (Prasad and Strzałka 1999; Kabata-Pendias 2010).

For years, heavy metal soil contamination has been a global environmental issue as human activities have continuously released these pollutants into the surroundings via agrochemical leaching, disposal of toxic wastes and effluents, and the atmospheric deposition from industrial activities (Bradl 2005; Meuser 2010; Hasanuzzaman and Fujita 2013). Long-term exposure via direct respiration (inhalation), drinking water, and/or ingestion of food contaminated with heavy metals may be adversely harmful to both environmental health (living ecosystem) and human well-being when the tolerance levels are exceeded (Järup 2003; Duruibe et al. 2007).

Various types of soil remediation including physical (dig-and-dump, thermal desorption, fracturing, and soil washing), chemical (solidification-stabilization, reduction-oxidation, etc.), and biological (biosorption, bioleaching, and biofiltration) techniques for heavy metal removal reported over the past decades (Mulligan et al. 2001; van Deuren et al. 2002; Sherameti and Varma 2010; Anjum et al. 2012). Nonetheless, most of these remediation technologies are considerably complicated and cost ineffective and are technically difficult to conduct. As a result, phytoremediation has turned out to be the most viable strategy using plants to clean up heavy metals in contaminated soil. Garbisu and Alkorta (2001), McIntyre (2003), and Ali et al. (2013) suggested that the application of phytoremediation would be esthetically non-destructive to the surrounding, environmentally pleasing, and often required minimum cost for operation and maintenance.

Among numerous types of plants tested for phytoremediation, Vetiver grass, *Vetiveria zizanioides* (Linn.) Nash proven to be an effective species with quick growth, deep fibrous root system, and high adaptability and tolerance to many extreme environmental stresses including the elevated high concentration levels of heavy metals (Truong et al. 2008; Danh et al. 2009; Truong and Danh 2015; Ng et al. 2017, 2018). To further enhance the accumulation of heavy metals in plants, assorted enrichment materials for instance, disodium ethylene-diamine-tetra-acetate (EDTA) were expansively used as an effective low-cost metal chelating agent for phytoremediation purposes (Han et al. 2004; Luo et al. 2005; Hovsepyan and Greipsson 2005; Seth et al. 2011; Ng et al. 2016). Due to its high efficiency to solubilize metals and metalloids in the soils, EDTA is extensively used to facilitate heavy metal phytoremediation (Shahid et al. 2014; Suthar et al. 2014; Luo et al. 2016a; Jiang et al. 2019). Nevertheless, recent studies (Han et al. 2004; Sinegani et al. 2015; Özkan et al. 2016; Vargas et al. 2016; Chen et al. 2018; Luo et al. 2018) indicated that synthetically designed chemical chelators including EDTA able to enhance metal accumulation and translocation in different plant parts for single heavy metal soil contamination.

However, at present, there is still a lack of information on EDTA-enhanced phytoremediation of mixed heavy metal–polluted soils with Vetiver grass. Most of the past studies emphasized solely on the application of EDTA to improve the heavy metal accumulation without considering the influences of single and mixed contaminations of heavy metal uptake by different plant parts (lower root, upper root, lower tiller, and upper tiller) in Vetiver grass (Andra et al. 2011; Luo et al. 2016a; Anning and Akoto 2018; Wasino et al. 2019). To address these uncertainties, this study was conducted to analyze the growth performance, accumulation trend, and capability of metal uptake from both single and mixed Cd-, Pb-, Cu-, and Zn-contaminated soils enhanced with EDTA using Vetiver grass.

Materials and methods

Site location and experimental setup

The greenhouse pot experiments were carried out at the Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur. Vetiver grass, *Vetiveria zizanioides* (Linn.) Nash was selected for this experiment and placed under nine different types of single and mixed heavy metal enhanced treatments (Table 1). All treatments were conducted with triplicates ($n = 3$) under the completely randomized design (CRD).

Soil management and sampling preparation

Top soil (0–20 cm) was collected from the field site (3° 7′ N latitude; 101° 39′ E longitude) situated within the University of Malaya, Kuala Lumpur, for planting purposes. The collected soil underwent preliminary physico-chemical soil characterization (Table 2). Soil was air-dried for a week followed with passing through < 4 mm sieve to eliminate large non-soil components and gravels. The dull reddish brown soil composed of 84.6% sand, 10.5% silt, and 4.9% clay.

Vetiver grass seedlings were purchased from Humibox Malaysia whereby fresh plantlets with a uniform height (20–25 cm) were selected for this study. Each Vetiver grass was carefully grown in a plastic pot (0.18 m diameter × 0.16 m depth) filled with 2 kg of soil for all treatments. All treatments were watered uniformly by using a 50 mL glass beaker of tap water once a day. Plant growth performances such as tiller number, height, and percentage plant survivor rate were continuously recorded all over the entire 60 days of experiment.

The artificially spiked single and mixed heavy metal soils were adjusted using cadmium nitrate tetrahydrate ($Cd(NO_3)_2 \cdot 4H_2O$), copper(II) sulfate ($CuSO_4$), lead(II) nitrate ($Pb(NO_3)_2$), and zinc sulfate heptahydrate ($ZnSO_4 \cdot 7H_2O$) salt compounds as well as the disodium ethylene-diamine-tetra-acetate, $C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$ (EDTA). The concentration of single and mixed heavy metal soils were determined based on the maximum allowable naturally occurring levels set by the Canadian Council of Ministers of Environment (CCME 1999), Department of Environment, Malaysia (DOE 2009), and European Union (Lado et al. 2008) soil contamination guidelines.

Table 1 Greenhouse design with treatment variables

Treatment	Spiked heavy metal (mg/kg) and EDTA (mmol/kg)
Control	No heavy metal and EDTA added
EDTA	10 EDTA
Cd + EDTA	20 Cd + 10 EDTA
Pb + EDTA	200 Pb + 10 EDTA
Cu + EDTA	100 Cu + 10 EDTA
Zn + EDTA	200 Zn + 10 EDTA
Cd + Pb + EDTA	20 Cd + 200 Pb + 10 EDTA
Cu + Zn + EDTA	100 Cu + 200 Zn + 10 EDTA
Cd + Pb + Cu + Zn + EDTA	20 Cd + 200 Pb + 100 Cu + 200 Zn + 10 EDTA

Table 2 Physico-chemical properties of selected soils

Parameter (unit)		Mean
Soil texture		
Sand (%)		84.58
	Very coarse sand (%)	9.16
	Coarse sand (%)	31.02
	Medium coarse sand (%)	42.21
	Fine sand (%)	15.54
	Very fine sand (%)	3.07
Silt (%)		10.48
Clay (%)		4.94
Temperature (°C)		30.3 ± 4.5
pH		5.28 ± 1.73
Color (Munsell color charts)	Dull reddish brown	2.5YR 5/4
Water content (%)		5.72 ± 1.03
Field capacity (%)		40.93 ± 2.45
Saturation level (%)	Dry	13.97
Bulk density (g/cm^3)		1.62 ± 0.78
Porosity (%)		38.87 ± 4.39
Metal contents (mg/kg)		
	Cd	1.15 ± 0.59
	Pb	32.55 ± 8.01
	Cu	11.94 ± 4.32
	Zn	60.22 ± 18.73

Mean ± standard deviation

In terms of soil amendment, although the possible outcomes for heavy metal phytoaccumulation may increase with EDTA, a standard composition of 10 mmol EDTA/kg was selected in this study based on the research findings obtained in Grčman et al. (2001) and Ng et al. (2016). The artificially spiked soil was then repeatedly stirred and incubated for a fortnight to achieve the homogeneity of the desired single and mixed heavy metal soils are obtained.

Sample and chemical analyses

At the end of 60-day experimental period, all Vetiver treatments were harvested and pre-washed in running tap and filter water, followed by deionized water to eliminate all forms of soil adhering material before separating the Vetiver grass into four different parts (lower and upper sections of roots and tillers) (Fig. 1). All plant samples were oven-dried for 72 h until a constant dry weight were obtained in order to determine the dry matter content (g/m^2) of the plant samples before it was homogenized using mortar and pestle.

Approximately, 0.5 g of the homogenized dried plant and soil samples underwent acid digestion with hydrogen peroxide (H_2O_2), nitric acid (HNO_3), and hydrochloric acid (HCl) as accordance to Method 3050B (US EPA 1996) and subsequently with Method 7000B (US EPA 2007) using a Perkin-Elmer AAnalyst 400 flame atomic absorption spectrometer (F-AAS) for the total recoverable elemental analysis. All chemicals used were of analytical reagent standard or of the best grade available. The German Federal Institute for Materials Research and Testing (BRM#12-mixed sandy soil) certified reference material was utilized to control the highly precision techniques of chemical analysis with an average metal recovery rate for Cd (96.1%), Pb (106.9%), Cu (102.9%), and Zn (96.8%), respectively.

Data calculation and statistical analyses

The growth performance of Vetiver grass was assessed using the tolerance index (TI) and root-tiller (R/T) quotient while the ability for metal translocation and accumulation were evaluated by the biological concentration factor (BCF), biological accumulation coefficient (BAC), translocation factor (TF), and percentage of metal uptake efficacy (Kabata-Pendias 2010; Alloway 2013; Ali et al. 2013; Ng et al. 2016) as follows:

R/T quotient = dry matter content in roots/dry matter content in tillers
TI = total dry matter content in heavy metal treatments/total dry matter content in control
TF = heavy metals concentration in tillers/heavy metals concentration in roots
BCF = heavy metals concentration in roots/heavy metals concentration in soil
BAC = heavy metals concentration in tillers/heavy metals concentration in soil
Metal uptake efficacy (%) = (heavy metals concentration in tillers/total heavy metals concentration accumulated in Vetiver grass) × 100

All recorded data were analyzed by using the one-way analysis of variance (ANOVA) and Fisher's least significant difference (LSD) tests for significant differences among treatment means at the 95% level of confidence with by employing the Microsoft Excel Office 365 versions 2016 software.

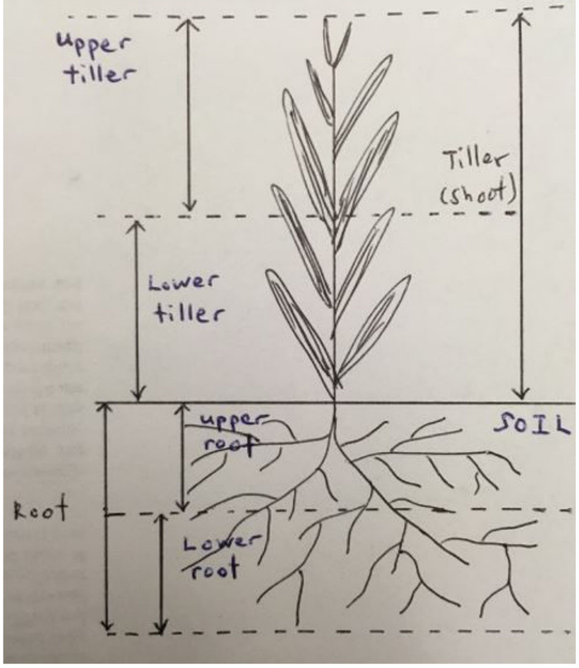

Fig. 1 Plant cross-section between the roots and shoots (tillers) of Vetiver grass

Results

Plant growth performance

The initial soil pH varied from 4.19 to 6.17 where the Cd + Pb + Cu + Zn + EDTA treatment recorded the lowest pH of 4.19 while the highest pH of 6.17 was observed in the control (Fig. 2). Upon harvesting, Cd + EDTA, Pb + EDTA, Cu + EDTA, Zn + EDTA, and Cd + Pb + Cu + Zn + EDTA treatments showed an increased in pH ranging from 4.70 to 5.54, where the highest pH increment (+ 0.98 pH units) was observed in the Cd + Pb + Cu + Zn + EDTA treatment. The soil pH levels in all single and mixed heavy metal treatments were significantly ($p < 0.05$) affected compared to the control. The application of both single and mixed heavy metals substantially influenced the overall change in soil pH in all treatments.

Table 3 shows significant differences ($p < 0.05$) in tiller number, plant height, and percentage of survival of Vetiver grass among all single and mixed heavy metal treatments. All treatments with the exception of EDTA (27.0) and Pb + EDTA (27.7) treatments exhibited significantly lower ($p < 0.05$) tiller number compared with the control. Both of the mixed Cu + Zn + EDTA (12.8) and Cd + Pb + Cu + Zn + EDTA (13.5) treatments recorded the lowest tiller number among all the treatments, respectively. Similarly, all treatments with the exception of Pb + EDTA (56.02 cm) displayed significantly lower ($p < 0.05$) plant height as compared with the control. Control plant height (69.74 cm) was 52.3% higher than the Cd + Pb + Cu + Zn + EDTA treatment which recorded the lowest plant height of 33.25 cm. On the other hand, EDTA (96.7%) and Pb + EDTA (77.3%) treatments showed no significant difference ($p > 0.05$) of percentage survivor rate with the control. Conversely, the percentage of survival among all the other single and mixed heavy metal treatments (69.3–74.7%) were significantly affected ($p < 0.05$) compared to the control, with Cu + Zn + EDTA treatment recording the lowest (67.3%) percentage survivor rate.

The dry matter contents of tiller and total Vetiver grass in all treatments were significantly lower ($p < 0.05$) compared to the control (Table 4). The Cu + EDTA treatment displayed the lowest total dry matter content (9.67 ± 0.11 g/m^2) with an average of 41.2% reduction compared to the control. The single metal treatments recorded comparatively higher dry matter contents than the mixed heavy metal treatments. In contrast, no significant difference ($p > 0.05$) was found in the root-tiller (R/T) quotient, tolerance index (TI), and dry matter content in the roots of Vetiver grass in all the treatments.

Accumulation of heavy metals

The concentration of Cd, Pb, Cu, and Zn accumulations in the roots, tillers, and overall plant of Vetiver grass for all single and mixed heavy metal treatments are shown in Tables 5, 6, 7, and 8. The accumulation of all four heavy metals in the lower and upper parts of both roots and tillers for all treatments was comparatively variable.

With regard to Cd accumulation, all the Cd + EDTA, Cd + Pb + EDTA, and Cd + Pb + Cu + Zn + EDTA treatments recorded significantly higher ($p < 0.05$) Cd in both lower and upper roots and tillers of Vetiver grass compared to the control (Table 5). Similarly, the total roots, total tillers, and overall total accumulation for Cd + EDTA, Cd + Pb + EDTA, and Cd + Pb + Cu + Zn + EDTA treatments exhibited significantly greater ($p < 0.05$) Cd among all other treatments. The highest accumulation of Cd was found in the upper tillers of Cd + Pb + Cu + Zn + EDTA (128.03 ± 17.95 mg/kg) followed by the lower roots of Cd + EDTA (119.60 ± 20.43 mg/kg) treatments. Between roots and tillers, unlike Pb, Cu, and Zn, the accumulation of Cd was noticeably higher in the tillers than in the roots with the exception of the Cd + EDTA treatment and the control. A relatively higher Cd accumulation was demonstrated in the upper roots and upper tillers of the Cd + Pb + EDTA treatment compared with its lower plant parts, respectively. In contrast, the accumulation of Cd was appreciably higher in the lower roots and lower tillers in the Cd + EDTA treatment compared to its upper plant parts. Nonetheless, the order of Cd accumulation among single and mixed Cd treatments was in the order of Cd + Pb + EDTA > Cd + Pb + Cu + Zn + EDTA > Cd + EDTA >> other treatments.

Similarly, with regard to Pb accumulation, the Pb + EDTA, Cd + Pb + EDTA, and Cd + Pb + Cu + Zn + EDTA treatments recorded significantly higher ($p < 0.05$) Pb in both lower and upper roots and tillers of Vetiver grass compared to the control (Table 6). A significantly higher ($p < 0.05$) amounts of Pb accumulation was observed in the total roots, total tillers, and overall total accumulation for Pb + EDTA, Cd + Pb + EDTA, and Cd + Pb + Cu + Zn + EDTA treatments among all other treatments. The upper tillers for Cd + Pb + Cu + Zn + EDTA (531.67 ± 36.19 mg/kg) and Cd + Pb + EDTA (368.80 ± 15.09 mg/kg) treatments recorded the highest accumulation of Pb among all treatments. Between roots and tillers, the accumulation of Pb was remarkably higher in the tillers than in the roots among all treatments. The upper roots and upper tillers for Cd + Pb + EDTA treatment as well as the upper tillers for Pb + EDTA and Cd + Pb + Cu + Zn + EDTA treatments accumulated considerably higher Pb compared with different plant parts. The accumulation trend for Pb among the different treatments was in the following order: Cd + Pb + Cu + Zn + EDTA > Cd + Pb + EDTA > Pb + EDTA >> other treatments.

A significantly higher ($p < 0.05$) Cu accumulation was found in both lower and upper roots and tillers of Vetiver grass for Cu + EDTA, Cu + Zn + EDTA, and Cd + Pb + Cu + Zn + EDTA treatments compared to the control (Table 7). Similarly, the total roots, total tillers, and overall total Cu accumulation for Cu + EDTA, Cu + Zn + EDTA, and Cd + Pb + Cu + Zn + EDTA treatments demonstrated significantly higher ($p < 0.05$) Cu among all the treatments. The upper tillers for Cd + Pb + Cu +

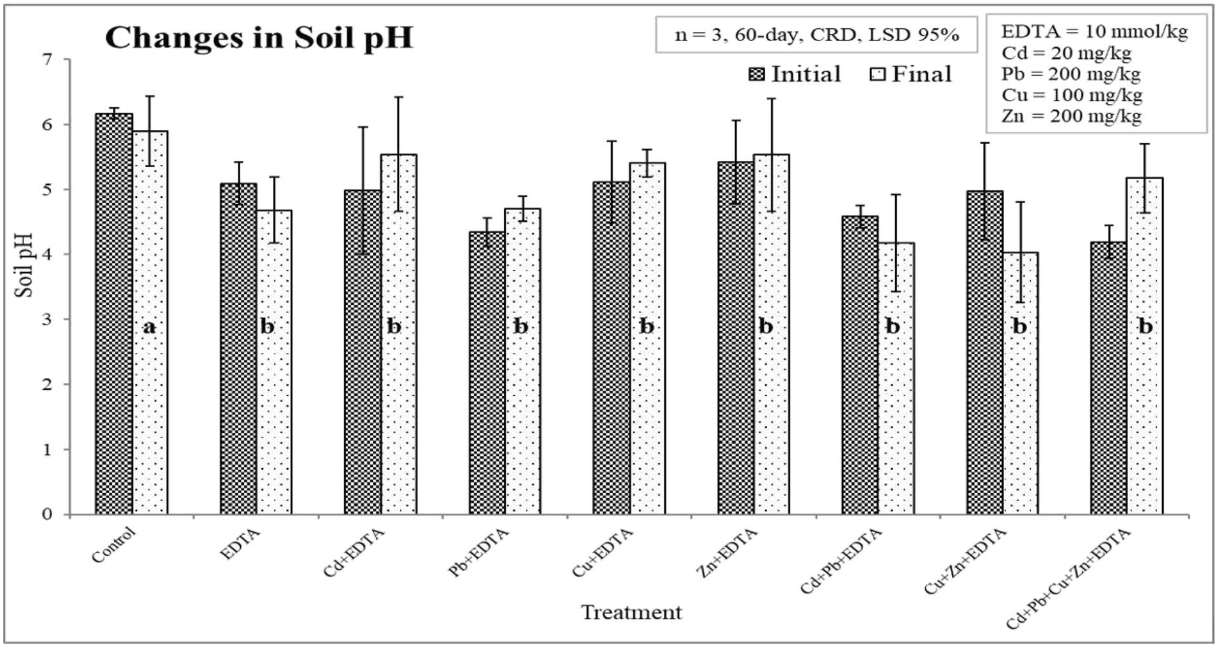

Fig. 2 Changes in soil pH of Vetiver grass in single and mixed heavy metal treatments. Vertical bars represented standard deviation while the same letters indicated no significant difference among all treatments at 0.05 probability level

Zn + EDTA (862.40 ± 231.34 mg/kg) and Cu + Zn + EDTA (538.97 ± 41.88 mg/kg) recorded the highest accumulation of Cu. Between roots and tillers, the accumulation of Cu was substantially higher in the tillers than in the roots among all treatments. The accumulation of Cu in the lower roots and upper tillers for Cd + Pb + Cu + Zn + EDTA and Cu + Zn + EDTA treatments were reasonably greater than other plant parts, respectively. Among the different Cu treatments, the accumulation of Cu was in the order of Cd + Pb + Cu + Zn + EDTA > Cu + Zn + EDTA > Cu + EDTA >> other treatments.

The accumulation of Zn was significantly higher ($p < 0.05$) in the Zn + EDTA, Cu + Zn + EDTA, and Cd + Pb + Cu + Zn + EDTA treatments in both lower and upper roots and tillers of Vetiver grass than the control (Table 8). The Zn + EDTA, Cu + Zn + EDTA, and Cd + Pb + Cu + Zn + EDTA treatments exhibited a significantly higher ($p < 0.05$) Zn in the total roots, total tillers, and overall total Zn accumulation among all other treatments. The upper tillers of Cd + Pb + Cu + Zn + EDTA (3504.80 ± 353.40 mg/kg) and Zn + EDTA (3399.87 ± 485.06 mg/kg) recorded the highest accumulation of Zn

Table 3 Tiller number, plant height (cm), and plant survivor rate (%) of Vetiver grass in single and mixed heavy metal treatments

Treatment	Tiller number	Plant height (cm)	Plant survivor (%)
Control	27.5 ± 1.3 a	69.74 ± 6.45 a	100.00 ± 0.00 a
EDTA	27.0 ± 0.8 a	50.24 ± 3.77 b	96.67 ± 2.27 ab
Cd + EDTA	16.8 ± 0.5 bc	41.13 ± 1.83 b	72.67 ± 4.78 bc
Pb + EDTA	27.7 ± 7.8 a	56.02 ± 13.21 ab	77.33 ± 11.36 abc
Cu + EDTA	14.5 ± 1.5 c	41.71 ± 2.95 b	69.33 ± 9.69 c
Zn + EDTA	16.7 ± 8.3 c	41.86 ± 7.75 b	70.67 ± 2.94 c
Cd + Pb + EDTA	22.5 ± 2.3 ab	49.73 ± 4.46 b	74.67 ± 1.58 bc
Cu + Zn + EDTA	12.8 ± 0.9 c	40.56 ± 2.74 b	67.33 ± 3.74 c
Cd + Pb + Cu + Zn + EDTA	13.5 ± 0.2 c	33.25 ± 6.03 b	71.34 ± 4.60 c

Mean ± standard deviation followed by the same letters were not significantly different at 0.05 probability level

Table 4 Dry matter content (g/m^2), root-tiller quotient (R/T), and tolerance index (TI) of Vetiver grass in single and mixed heavy metal treatments

Treatment	Dry matter content (g/m^2)				
	Vetiver			R/T	TI
	Root	Tiller	Total		
Control	6.75 ± 1.13 a	9.68 ± 1.37 a	16.44 ± 0.35 a	0.718 a	
EDTA	6.06 ± 0.61 a	5.81 ± 0.38 b	11.87 ± 0.27 bc	1.049 a	0.718 a
Cd + EDTA	5.24 ± 0.65 a	4.49 ± 0.92 b	9.72 ± 1.55 c	1.183 a	0.589 a
Pb + EDTA	6.25 ± 0.95 a	5.01 ± 1.06 b	11.26 ± 2.00 b	1.260 a	0.682 a
Cu + EDTA	5.36 ± 1.06 a	4.31 ± 1.11 b	9.67 ± 0.11 c	1.341 a	0.585 a
Zn + EDTA	5.44 ± 0.30 a	4.37 ± 0.47 b	9.82 ± 0.27 c	1.258 a	0.594 a
Cd + Pb + EDTA	5.90 ± 0.42 a	4.52 ± 1.30 b	10.42 ± 1.34 bc	1.380 a	0.631 a
Cu + Zn + EDTA	5.37 ± 0.93 a	4.57 ± 0.87 b	9.94 ± 1.31 bc	1.202 a	0.602 a
Cd + Pb + Cu + Zn + EDTA	5.50 ± 1.08 a	4.45 ± 1.32 b	9.95 ± 0.55 bc	1.351 a	0.602 a

Mean ± standard deviation followed by the same letters were not significantly different at 0.05 probability level

among all the treatments. Between roots and tillers, the accumulation of Zn was markedly greater in the tillers than the roots for all treatments. The lower roots and upper tillers for Zn + EDTA, Cu + Zn + EDTA, and Cd + Pb + Cu + Zn + EDTA treatments accumulated substantially higher amounts of Zn compared to the other plant parts. The accumulation trend for Zn was in the following order: Zn + EDTA > Cd + Pb + Cu + Zn + EDTA > Cu + Zn + EDTA >> other treatments.

Between single and mixed metal treatments, mixed Cd + Pb + Cu + Zn + EDTA treatment exhibited appreciably higher accumulation of Cu and Pb while mixed Cd + Pb + EDTA treatment was higher in Cd accumulation than the single metal treatments. On the other hand, Zn + EDTA was the only single metal treatment that showed higher accumulation of Zn than the other mixed metal treatments. The findings obtained indicated that the single Zn + EDTA treatment accumulated the highest overall Zn (8068.13 ± 407.35 mg/kg) while the highest accumulation of Cu (1977.47 ± 293.68 mg/kg) and Pb (1096.57 ± 75.60 mg/kg) was recorded in the mixed Cd + Pb + Cu + Zn + EDTA treatment, respectively. The mixed Cd + Pb + EDTA treatment demonstrated the highest overall total amount for Cd (302.97 ± 29.44 mg/kg). Generally, the trend of heavy metal accumulation for all treatments were in the order of Zn >>> Cu > Pb >> Cd regardless of the total amount of heavy metal put into the soil.

Heavy metal uptake and translocation

Tables 9, 10, 11, 12, and 13 show the association of soil-plant accumulation for all single and mixed metal treatments in terms of translocation factors (TF), biological concentration factors (BCF), biological accumulation coefficients (BAC), and percentages of metal uptake efficacy.

The ability for translocation of heavy metal from soil to root in plant is assessed using the BCF coefficient. Both lower (7.116–12.008) and upper (4.733–6.529) roots for single and mixed Zn treatments showed significantly higher ($p < 0.05$) BCF values than the other treatments. Despite the tolerably higher accumulation of heavy metals in tillers than roots, both lower and upper roots for the single and mixed Cd (1.837–5.980), Cu (1.376–3.931), and Zn (4.733–12.008) treatments recorded relatively high BCF values > 1, suggesting that uptake of Cd, Cu, and Zn from soil to roots was substantially greater and the roots acted as the sink for accumulation of these heavy metals.

Nevertheless, the BAC, TF, and percentages of metal efficacy were employed to evaluate the capabilities and competency of heavy metal uptake and transport from roots to tillers. Similarly, the lower and upper tillers in all the specified single and mixed heavy metal treatments showed appreciably BAC values > 1 compared with the other treatments. The BAC values > 1 in lower and upper tillers for

Table 5 Concentration of Cd (mg/kg) in both lower and upper roots and tillers of Vetiver grass in single and mixed heavy metal treatments

Treatment	Concentration of Cd (mg/kg)						
	Root			Tiller			Overall **total**
	Lower	Upper	Total	Lower	Upper	Total	
Control	1.83 ± 0.31 c	1.67 ± 0.74 d	3.50 ± 0.90 d	0.75 ± 0.42 c	0.44 ± 0.21 d	1.19 ± 0.22 d	4.69 ± 1.12 c
EDTA	0.05 ± 0.02 c	0.55 ± 0.18 d	0.61 ± 0.20 d	2.30 ± 1.11 c	2.50 ± 1.11 d	4.80 ± 2.23 d	5.40 ± 2.41 c
Cd + EDTA	119.60 ± 20.43 a	60.10 ± 11.26 b	179.70 ± 31.15 a	51.13 ± 12.77 ab	30.07 ± 6.95 c	81.20 ± 6.67 c	260.90 ± 37.34 b
Pb + EDTA	0.06 ± 0.03 c	1.43 ± 0.50 d	1.50 ± 0.49 d	2.12 ± 0.59 c	2.87 ± 1.53 d	4.99 ± 1.15 d	6.49 ± 0.76 c
Cu + EDTA	0.47 ± 0.14 c	1.19 ± 0.40 d	1.66 ± 0.51 d	1.40 ± 0.98 c	2.10 ± 1.41 d	3.50 ± 0.62 d	5.16 ± 0.56 c
Zn + EDTA	0.58 ± 0.18 c	1.41 ± 0.19 d	1.98 ± 0.16 d	1.04 ± 0.49 c	1.55 ± 1.00 d	2.60 ± 1.48 d	4.58 ± 1.43 c
Cd + Pb + EDTA	49.43 ± 8.96 b	97.57 ± 5.45 a	147.00 ± 14.08 b	49.63 ± 16.70 ab	106.33 ± 21.37 b	155.97 ± 38.05 b	302.97 ± 29.44 a
Cu + Zn + EDTA	0.08 ± 0.05 c	1.14 ± 0.37 d	1.22 ± 0.32 d	0.49 ± 0.26 c	5.77 ± 0.91 d	6.26 ± 0.68 d	7.48 ± 0.38 c
Cd + Pb + Cu + Zn + EDTA	44.93 ± 8.73 b	36.73 ± 3.43 c	81.67 ± 10.86 c	52.10 ± 14.73 a	128.03 ± 17.95 a	180.13 ± 4.99 a	261.80 ± 7.28 b

Mean ± standard deviation followed by the same letters were not significantly different at 0.05 probability level

Table 6 Concentration of Pb (mg/kg) in both lower and upper roots and tillers of Vetiver grass in single and mixed heavy metal treatments

Treatment	Concentration of Pb (mg/kg)						
	Root			Tiller			Overall total
	Lower	Upper	Total	Lower	Upper	Total	
Control	17.70 ± 3.60 d	14.40 ± 2..46 d	32.10 ± 5.99 d	1.93 ± 0.38 d	1.90 ± 0.54 d	3.83 ± 0.17 d	35.93 ± 6.10 c
EDTA	1.27 ± 0.37 d	2.35 ± 0.52 e	3.63 ± 0.86 d	15.47 ± 2.54 d	5.65 ± 0.67 d	21.12 ± 3.21 d	24.74 ± 2.46 c
Cd + EDTA	12.80 ± 1.67 d	10.26 ± 2.56 de	23.06 ± 1.46 d	13.83 ± 2.51 d	22.20 ± 2.82 d	36.03 ± 5.33 d	59.09 ± 4.65 c
Pb + EDTA	78.33 ± 8.99 c	69.97 ± 12.44 c	148.30 ± 21.42 c	134.23 ± 7.75 c	237.70 ± 19.22 c	371.93 ± 12.56 c	520.23 ± 9.86 b
Cu + EDTA	9.24 ± 3.77 d	3.10 ± 0.89 de	12.34 ± 4.62 d	28.70 ± 8.61 d	25.37 ± 6.74 d	54.07 ± 5.06 d	66.40 ± 5.08 c
Zn + EDTA	15.50 ± 4.78 d	5.25 ± 0.42 de	20.75 ± 4.40 d	17.70 ± 3.18 d	15.53 ± 0.86 d	33.23 ± 4.04 d	53.98 ± 4.75 c
Cd + Pb + EDTA	184.30 ± 25.88 a	199.20 ± 10.51 a	383.50 ± 36.24 a	300.17 ± 19.75 b	368.80 ± 15.09 b	668.97 ± 32.88 b	1052.47 ± 6.47 a
Cu + Zn + EDTA	10.97 ± 1.99 d	2.41 ± 0.30 e	13.37 ± 2.27 d	12.20 ± 2.27 d	14.00 ± 4.10 d	26.20 ± 2.44 d	39.57 ± 4.62 c
Cd + Pb + Cu + Zn + EDTA	120.60 ± 19.26 b	105.97 ± 7.61 b	226.57 ± 26.87 b	338.33 ± 12.62 a	531.67 ± 36.19 a	870.00 ± 48.79 a	1096.57 ± 75.60 a

Mean ± standard deviation followed by the same letters were not significantly different at 0.05 probability level

Table 7 Concentration of Cu (mg/kg) in both lower and upper roots and tillers of Vetiver grass in single and mixed heavy metal treatments

Treatment	Concentration of Cu (mg/kg)						
	Root			Tiller			Overall total
	Lower	Upper	Total	Lower	Upper	Total	
Control	15.73 ± 5.41 d	12.67 ± 1.72 c	28.40 ± 7.12 c	4.53 ± 0.80 d	2.90 ± 1.40 d	7.43 ± 0.68 c	35.83 ± 7.66 d
EDTA	2.21 ± 0.34 d	3.89 ± 1.64 c	6.10 ± 1.59 c	7.55 ± 0.78 d	12.07 ± 3.05 d	19.61 ± 3.81 c	25.72 ± 5.34 d
Cd + EDTA	3.69 ± 0.64 d	6.13 ± 1.65 c	9.82 ± 1.98 c	18.53 ± 0.68 d	23.77 ± 3.99 d	42.30 ± 3.32 c	52.12 ± 5.28 d
Pb + EDTA	1.34 ± 0.54 d	11.13 ± 1.81 c	12.47 ± 2.35 c	24.70 ± 3.70 d	30.32 ± 6.38 d	55.02 ± 9.80 c	67.49 ± 12.00 d
Cu + EDTA	253.00 ± 24.78 c	137.60 ± 28.05 b	390.60 ± 52.31 b	429.20 ± 33.51 b	381.03 ± 22.66 c	810.23 ± 55.73 b	1200.83 ± 108.04 c
Zn + EDTA	3.41 ± 0.83 d	8.64 ± 0.57 c	12.06 ± 1.32 c	15.87 ± 1.56 d	36.63 ± 6.19 d	52.50 ± 7.75 c	64.56 ± 8.57 d
Cd + Pb + EDTA	5.42 ± 0.53 d	5.73 ± 2.45 c	11.15 ± 1.96 c	12.73 ± 4.45 d	40.63 ± 4.12 d	53.37 ± 8.53 c	64.52 ± 6.79 d
Cu + Zn + EDTA	393.12 ± 9.65 a	157.03 ± 32.90 b	550.15 ± 28.73 a	351.53 ± 32.06 c	538.97 ± 41.88 b	890.50 ± 14.21 b	1440.65 ± 39.57 b
Cd + Pb + Cu + Zn + EDTA	365.33 ± 18.68 b	214.40 ± 18.86 a	579.73 ± 1.24 a	535.33 ± 62.21 a	862.40 ± 231.34 a	1397.73 ± 293.47 a	1977.47 ± 293.68 a

Mean ± standard deviation followed by the same letters were not significantly different at 0.05 probability level

Table 8 Concentration of Zn (mg/kg) in both lower and upper roots and tillers of Vetiver grass in single and mixed heavy metal treatments

Treatment	Concentration of Zn (mg/kg)						
	Root			Tiller			Overall total
	Lower	Upper	Total	Lower	Upper	Total	
Control	215.23 ± 35.35 c	135.30 ± 21.33 c	350.53 ± 50.59 c	49.97 ± 5.07 c	55.50 ± 15.30 c	105.47 ± 19.40 c	456.00 ± 42.90 c
EDTA	62.70 ± 13.90 c	98.85 ± 23.79 c	161.55 ± 37.45 c	102.60 ± 8.09 c	71.03 ± 15.04 c	173.63 ± 13.69 c	335.19 ± 49.02 c
Cd + EDTA	55.83 ± 14.00 c	34.07 ± 6.94 c	89.90 ± 7.64 c	222.23 ± 33.67 c	94.30 ± 4.12 c	316.53 ± 35.02 c	406.43 ± 40.89 c
Pb + EDTA	82.97 ± 10.46 c	96.83 ± 9.23 c	179.80 ± 15.53 c	244.87 ± 42.48 c	107.30 ± 17.66 c	352.17 ± 25.02 c	531.97 ± 12.62 c
Cu + EDTA	51.37 ± 17.50 c	39.13 ± 4.40 c	90.50 ± 19.58 c	256.33 ± 38.50 c	81.67 ± 5.68 c	338.00 ± 43.56 c	428.50 ± 60.19 c
Zn + EDTA	2401.57 ± 484.77 a	1305.80 ± 131.61 a	3707.37 ± 367.76 a	1115.43 ± 168.62 b	3399.87 ± 485.06 a	4515.30 ± 649.08 b	8222.67 ± 431.78 a
Cd + Pb + EDTA	47.47 ± 16.05 c	51.80 ± 10.18 c	99.27 ± 26.12 c	294.33 ± 23.79 c	151.53 ± 25.58 c	445.87 ± 3.86 c	545.13 ± 24.47 c
Cu + Zn + EDTA	1520.97 ± 71.04 b	998.00 ± 176.43 b	2518.97 ± 116.88 b	2575.33 ± 478.90 a	1552.70 ± 202.46 b	4128.03 ± 277.23 b	6647.00 ± 203.87 b
Cd + Pb + Cu + Zn + EDTA	1423.17 ± 268.01 b	946.50 ± 52.05 b	2369.67 ± 319.66 b	2193.67 ± 422.23 a	3504.80 ± 353.40 a	5698.47 ± 116.93 a	8068.13 ± 407.35 a

Mean ± standard deviation followed by the same letters were not significantly different at 0.05 probability level

Table 9 Biological concentration factor (BCF) of Cd, Pb, Cu, and Zn accumulations in the lower and upper root of Vetiver grass in single and mixed heavy metal treatments

Treatment	BCF (Root)							
	Cd accumulation		Pb accumulation		Cu accumulation		Zn accumulation	
	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper
Control	1.594 c	1.449 cd	0.544 bc	0.442 b	1.318 c	1.061 cd	3.574 c	2.247 c
EDTA	0.046 d	0.481 e	0.039 f	0.072 e	0.185 d	0.326 f	1.041 d	1.642 cd
Cd + EDTA	5.980 a	3.005 b	0.393 cde	0.315 c	0.309 d	0.514 ef	0.927 d	0.566 e
Pb + EDTA	0.055 d	1.246 cd	0.392 cde	0.350 c	0.112 d	0.932 d	1.378 d	1.608 cd
Cu + EDTA	0.406 d	1.035 de	0.284 e	0.095 d	2.530 b	1.376 bc	0.853 d	0.650 e
Zn + EDTA	0.501 d	1.223 cd	0.476 bcd	0.161 d	0.286 d	0.724 de	12.008 a	6.529 a
Cd + Pb + EDTA	2.472 b	4.878 a	0.922 a	0.996 a	0.454 d	0.480 ef	0.788 d	0.860 de
Cu + Zn + EDTA	0.072 d	0.988 de	0.337 de	0.074 de	3.931 a	1.570 b	7.605 b	4.990 b
Cd + Pb + Cu + Zn + EDTA	2.247 bc	1.837 c	0.603 b	0.530 b	3.653 a	2.144 a	7.116 b	4.733 b

Mean ± standard deviation followed by the same letters were not significantly different at 0.05 probability level

single and mixed Cd (1.503–6.402), Pb (0.671–2.658), Zn (7.764–16.999), and Cu (3.515–8.624) treatments indicated that the tillers acted as the sink for their accumulation due to the fairly effective translocation of these heavy metals from roots to tillers.

On the other hand, despite the relatively higher accumulation of heavy metal in tillers than roots, TF values < 1 were recorded in the lower and upper tillers for all the single and mixed heavy metal treatments. However, the mixed Cd + Pb + Cu + Zn + EDTA treatment exhibited TF values > 1 in both lower (1.503) and upper (2.356) tillers for Pb accumulation compared to the other treatments.

In terms of percentages of metal efficacy, the upper tillers for mixed Cd + Pb + Cu + Zn + EDTA treatment exhibited the highest metal efficacy for Cd (49.1%) and Pb (48.5%) among all treatments. In contrast, the lower tillers of mixed Cu + Zn + EDTA (38.6%) followed by the single Cu + EDTA (35.8%) treatment recorded the highest metal efficacy for Zn and Cu, respectively. Between single and mixed

Table 10 Biological accumulation coefficient (BAC), translocation factor (TF), and metal uptake efficacy (%) of Cd accumulation in the lower and upper tiller of Vetiver grass in single and mixed heavy metal treatments

Treatment	Cd accumulation					
	BAC (tiller)		TF (tiller)		Efficacy (tiller)	
	Lower	Upper	Lower	Upper	Lower	Upper
Control	0.652 de	0.383 d	0.203 d	0.142 d	15.151 cd	10.448 d
EDTA	2.000 abc	2.171 c	3.700 a	4.057 ab	42.099 a	46.284 b
Cd + EDTA	2.557 a	1.503 cd	0.282 cd	0.175 d	19.402 cd	11.919 cd
Pb + EDTA	1.846 abcd	2.493 c	1.460 b	2.357 bc	33.132 ab	43.214 b
Cu + EDTA	1.217 bcde	1.826 c	0.779 c	1.507 cd	26.903 bc	40.933 b
Zn + EDTA	0.907 cde	1.351 cd	0.530 cd	0.797 d	22.087 bc	31.616 bc
Cd + Pb + EDTA	2.482 a	5.317 ab	0.345 cd	0.734 d	16.156 cd	34.904 bc
Cu + Zn + EDTA	0.426 e	5.014 ab	0.381 cd	5.095 a	6.651 d	76.873 a
Cd + Pb + Cu + Zn + EDTA	2.605 a	6.402 a	0.630 cd	1.605 cd	19.806 bcd	49.055 b

Mean ± standard deviation followed by the same letters were not significantly different at 0.05 probability level

Table 11 Biological accumulation coefficient (BAC), translocation factor (TF), and metal uptake efficacy (%) of Pb accumulation in the lower and upper tiller of Vetiver grass in single and mixed heavy metal treatments

Treatment	Pb accumulation					
	BAC (tiller)		TF (tiller)		Efficacy (tiller)	
	Lower	Upper	Lower	Upper	Lower	Upper
Control	0.059 e	0.058 g	0.063 c	0.059 e	5.581 d	5.247 e
EDTA	0.475 cd	0.174 g	4.527 a	1.643 bc	62.257 a	22.807 d
Cd + EDTA	0.425 d	0.682 de	0.604 c	0.968 d	23.289 c	37.480 b
Pb + EDTA	0.671 c	1.189 c	0.913 c	1.637 bc	25.790 c	45.746 a
Cu + EDTA	0.882 b	0.779 d	2.794 b	2.151 ab	43.543 b	37.955 b
Zn + EDTA	0.544 cd	0.477 ef	0.891 c	0.777 d	32.797 bc	28.873 cd
Cd + Pb + EDTA	1.501 a	1.844 b	0.790 c	0.970 d	28.522 c	35.047 bc
Cu + Zn + EDTA	0.375 d	0.430 f	0.945 c	1.033 d	31.378 c	34.952 bc
Cd + Pb + Cu + Zn + EDTA	1.692 a	2.658 a	1.503 bc	2.356 a	30.898 c	48.487 a

Mean ± standard deviation followed by the same letters were not significantly different at 0.05 probability level

treatments, the mixed Cd + Pb + EDTA, Cu + Zn + EDTA, and Cd + Pb + Cu + Zn + EDTA treatments recorded considerably higher percentages of metal efficacy for Cd, Pb, and Zn than the single metal treatments. However, the lower tillers of single Cu + EDTA (35.769%) treatment demonstrated reasonably higher percentage of Cu efficacy than the mixed metal treatments. Generally, the percentages of metal efficacy were remarkably higher in the upper tillers than the lower tillers for accumulation of all heavy metals.

Discussion

The accumulations of all heavy metals were found in both lower and upper parts of roots and tillers in the single and mixed heavy metal treatments. Besides, similar effects of EDTA application were reported in Chen et al. (2004c), Zhao et al. (2011), and Ali and Chaudhury (2016) who observed the accumulation trends of heavy metals in both roots and tillers of the plants.

EDTA was generally used as a common chelating agent for phytoremediation to enhance the bioavailability

Table 12 Biological accumulation coefficient (BAC), translocation factor (TF), and metal uptake efficacy (%) of Cu accumulation in the lower and upper tiller of Vetiver grass in single and mixed heavy metal treatments

Treatment	Cu accumulation					
	BAC (tiller)		TF (tiller)		Efficacy (tiller)	
	Lower	Upper	Lower	Upper	Lower	Upper
Control	0.380 g	0.243 f	0.171 d	0.099 f	13.356 e	7.818 f
EDTA	0.632 fg	1.011 ef	1.278 b	1.991 cd	29.801 bc	46.652 c
Cd + EDTA	1.552 de	1.991 de	1.955 a	2.433 bc	35.901 ab	45.385 c
Pb + EDTA	2.069 d	2.539 cde	1.997 a	2.426 bc	36.744 a	44.767 c
Cu + EDTA	4.292 b	3.810 c	1.104 bc	0.982 e	35.769 ab	31.789 e
Zn + EDTA	1.329 e	3.068 cd	1.322 b	3.043 ab	24.653 cd	56.560 b
Cd + Pb + EDTA	1.066 ef	3.403 cd	1.207 b	3.752 a	19.414 de	62.995 a
Cu + Zn + EDTA	3.515 c	5.390 b	0.642 cd	0.979 e	24.451 cd	37.378 d
Cd + Pb + Cu + Zn + EDTA	5.353 a	8.624 a	0.923 bc	1.487 de	27.156 c	43.101 c

Mean ± standard deviation followed by the same letters were not significantly different at 0.05 probability level

Table 13 Biological accumulation coefficient (BAC), translocation factor (TF), and metal uptake efficacy (%) of Zn accumulation in the lower and upper tiller of Vetiver grass in single and mixed heavy metal treatments

Treatment	Zn accumulation					
	BAC (tiller)		TF (tiller)		Efficacy (tiller)	
	Lower	Upper	Lower	Upper	Lower	Upper
Control	0.830 d	0.922 c	0.146 e	0.162 e	11.064 f	12.241 d
EDTA	1.704 cd	1.180 c	0.660 cde	0.441 de	31.115 de	21.060 bc
Cd + EDTA	3.690 bc	1.566 c	2.471 b	1.051 bc	54.495 ab	23.332 bc
Pb + EDTA	4.066 b	1.782 c	1.382 c	0.595 cde	45.933 c	20.228 c
Cu + EDTA	4.257 b	1.356 c	2.873 ab	0.923 cd	59.793 a	19.202 cd
Zn + EDTA	5.577 b	16.999 a	0.305 e	0.930 cd	13.524 f	41.262 a
Cd + Pb + EDTA	4.888 b	2.516 c	3.070 a	1.653 a	53.935 ab	27.973 b
Cu + Zn + EDTA	12.877 a	7.764 b	1.028 cd	0.615 cde	38.637 cd	23.427 bc
Cd + Pb + Cu + Zn + EDTA	10.968 a	17.524 a	0.921 cd	1.509 ab	27.066 e	43.641 a

Mean ± standard deviation followed by the same letters were not significantly different at 0.05 probability level

of heavy metals for uptake by plants in the soil (Meers et al. 2009; Shahid et al. 2014; Bloem et al. 2017). As a result, the accumulation trends responded differently when EDTA was applied in all the single and mixed heavy metal treatments. The presence of EDTA molecules enhance the extraction of metals from exchangeable sites and subsequently formed soluble metal-EDTA complexes (Hadi et al. 2010; Leleyter et al. 2012; Jean-Soro et al. 2012; Dipu et al. 2012). This indicated that the application of EDTA as soil amendment managed to enhance overall accumulation of Cd, Pb, Cu, and Zn by 1.21- to 2.79-fold from both single and mixed heavy metal contaminated soil. In contrast, Lai and Chen (2004) found no significant influence with the application of EDTA at 5 and 10 mmol/kg soil for both Zn and Pb accumulations in Vetiver grass. However, the application of 5 and 25 mmol/kg of EDTA by Chen et al. (2004c) and Ng et al. (2016) recorded reasonably higher concentrations of Pb and Cd in Vetiver grass, respectively.

Moreover, the findings of this study also showed that soil pH became more acidic in all the single and mixed heavy metal treatments when EDTA was added as compared to the control. This condition could affect the bioavailability of metals as a change in soil pH could conceivably affect the capability of EDTA to form complexes (Peng et al. 2009; Bennedsen et al. 2012). This was suggested by Sommers and Lindsay (1979) and Shahid et al. (2014) that metal-EDTA complexes were predominantly formed between pH 5.2 and 7.7 in most soil conditions due to soil acidification.

In addition to the single and mixed heavy metal treatments, this study included and tested separately the response of sole EDTA treatment by comparing with the control. However, the results showed no major significant findings in terms metal accumulation with the sole EDTA treatment compared to the control. Nevertheless, the single Zn + EDTA treatment accumulated highest Zn compared to the other mixed metal treatments. Furthermore, the single Zn + EDTA as well as mixed Cu + Zn + EDTA and Cd + Pb + Cu + Zn + EDTA treatments recorded > 1000 mg/kg of Zn accumulation in almost all of the lower and upper parts of both roots and tillers.

Recent studies by Antiochia et al. (2007), Danh et al. (2009), and Aksorn and Chitsomboon (2013) reported that Vetiver grass was both Pb and Zn hyperaccumulator plants. However, despite the high accumulation of Zn in both roots and tillers, the results of this study suggested that Vetiver grass may be a Cd hyperaccumulator plant due to its high phytoaccumulation ability in the upper tillers for both mixed Cd + Pb + EDTA and Cd + Pb + Cu + Zn + EDTA treatments. The fundamental characteristics of hyperaccumulator plants (Baker and Brooks 1989; Van der Ent et al. 2013) were that plant species are capable of growing and bioaccumulate under extremely high concentrations of heavy metals greater than 100 mg/kg of Cd; or 1000 mg/kg of Pb and Cu; or 10,000 mg/kg of Zn in its plant tissues.

Similarly, Vetiver grass may be regarded as both competent phytostabilizers and phytoextractors due to its BCF and BAC values being > 1, as well as the high accumulation in the lower and upper parts of roots and tillers for all types of heavy metals. This study demonstrated that the roots and tillers acted as the sink for the accumulation of all heavy metals in the presence of EDTA as a chelator agent to enhance the phytoremediation process in Vetiver grass irrespective of single and/or mixed metal treatments.

Previous studies by Lai and Chen (2005), Wuana et al. (2016), and Luo et al. (2016a, b) used different types of plant species such as rainbow pink (*Dianthus chinensis*), castor (*Ricinus communis*), and chickpea (*Cicer arietinum*) demonstrated similar findings on the enhancement of EDTA. Correspondingly, this study further expanded to cover separate parts of the lower and upper roots and tillers of Vetiver grass in order to provide an extensive phytoevaluation of the translocation of heavy metals upwards from the lower roots through the top of the plant's tillers.

Furthermore, the direct use of Malaysian garden soil spiked with metal salts in pot trial experiments instead of in situ site experiments for this study would inevitably incur unfavorable effects such as additional increase of heavy metal accumulation due to various biotic and abiotic conditions that may influence the overall results of phytoremediation. Consequently, it cannot be ruled out that the experimental design employed with the application of spiked treatments using pot assays may elevate the phytoaccumulation of heavy metals in both the soil-to-roots and roots-to-tillers of Vetiver grass.

Despite its strong phytoaccumulation ability to enhance metal contaminants in plants, both EDTA and metal-EDTA complexes have its drawbacks as they are poorly biodegradable with high toxicity and are extremely persistent in the soils (Oviedo and Rodríguez 2003; European Chemicals Bureau 2004; Goel and Gautam 2010; Zhao et al. 2010; Mühlbachová 2011; Bloem et al. 2017). Additionally, this study also demonstrated that there was a major significant reduction in terms of tiller number, plant height, plant survivor rate, and dry matter content of Vetiver grass when EDTA was applied, irrespective of both single and mixed metal treatments. Thus, it is crucial to note that the application of EDTA could inhibit plant growth performance, as reported by Chen and Cutright (2001) and Chen et al. (2004a, b). As a result, the appropriate management of the use of EDTA concentrations was ultimately vital to optimize metal phytoaccumulation in Vetiver grass as well as to reduce its toxicity, metal leaching, and other potential risks to the environment.

Conclusions

This study revealed that mixed Cd + Pb + EDTA, Cu + Zn + EDTA, and Cd + Pb + Cu + Zn + EDTA treatments were adequately effective to accumulate higher concentration for Cd, Pb, and Cu than the single metal treatments. In contrast, single Zn + EDTA treatment demonstrated the opposite trend, whereby a higher accumulation for Zn was observed among mixed heavy metal treatments. Predominantly, the inclination of heavy metal accumulation in Vetiver grass for all treatments were in the following order of Zn >>> Cu > Pb >> Cd. In terms of different plant parts, both upper roots and tillers of Vetiver grass showed high tendency for the uptake of substantially larger amounts of all heavy metals, regardless of single and/or mixed metal treatments. As a result of the comparably higher concentration in tillers than roots and with BAC values > 1, Vetiver grass may be recommended as a potential phytoextractor for all heavy metals, whereby its tillers acted as the sink for heavy metal accumulation in the presence of EDTA in all treatments.

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References

Aksorn, E., & Chitsomboon, B. (2013). Bioaccumulation of heavy metal uptake by two different vetiver grass (Vetiveria zizanioides and Vetiveria nemoralis) species. *African Journal of Agricultural Research, 8*, 3166–3171.

Ali, S. Y., & Chaudhury, S. (2016). EDTA-enhanced phytoextraction by Tagetes sp. and effect on bioconcentration and translocation of heavy metals. *Environ Process, 3*, 735–746.

Ali, H., Khan, E., & Sajad, M. A. (2013). Phytoremediation of heavy metals - concepts and applications. *Chemosphere, 91*, 869–881.

Alloway, B. J. (2013). Sources of heavy metals and metalloids in soils. In *Heavy metals in soils* (pp. 11–50). Netherlands: Springer.

Andra, S. S., Datta, R., Reddy, R., Saminathan, S. K., & Sarkar, D. (2011). Antioxidant enzymes response in vetiver grass: a greenhouse study for chelant-assisted phytoremediation of lead-contaminated residential soils. *CLEAN–Soil Air Water, 39*, 428–436.

Anjum, N. A., Pereira, M. E., Ahmad, I., Duarte, A. C., Umar, S., & Khan, N. A. (Eds.). (2012). *Phytotechnologies: remediation of environmental contaminants. CRC Press*. United States: Florida.

Anning, A. K., & Akoto, R. (2018). Assisted phytoremediation of heavy metal contaminated soil from a mined site with Typha latifolia and Chrysopogon zizanioides. *Ecotoxicology and Environmental Safety, 148*, 97–104.

Antiochia, R., Campanella, L., Ghezzi, P., & Movassaghi, K. (2007). The use of vetiver for remediation of heavy metal soil contamination. *Analytical and Bioanalytical Chemistry, 388*, 947–956.

Baker, A. J. M., & Brooks, R. (1989). Terrestrial higher plants which hyperaccumulate metallic elements: a review of their distribution, ecology and phytochemistry. *Biorecovery, 1*, 81–126.

Bennedsen, L. R., Krischker, A., Jørgensen, T. H., & Søgaard, E. G. (2012). Mobilization of metals during treatment of contaminated soils by modified Fenton's reagent using different chelating agents. *Journal of Hazardous Materials, 199*, 128–134.

Bloem, E., Haneklaus, S., Haensch, R., & Schnug, E. (2017). EDTA application on agricultural soils affects microelement uptake of plants. *Science of the Total Environment, 577*, 166–173.

Bradl, H. (Ed.). (2005). *Heavy metals in the environment: origin, interaction and remediation* (Vol. 6). Netherlands: Academic Press.

CCME, Canadian Council of Ministers of the Environment. (1999). *Canadian soil quality guidelines for the protection of environmental and human health*. In: Canadian environmental quality guidelines, Canada.

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

Chen, B., Shen, H., Li, X., Feng, G., & Christie, P. (2004a). Effects of EDTA application and arbuscular mycorrhizal colonization on growth and zinc uptake by maize (Zea mays L.) in soil experimentally contaminated with zinc. *Plant and Soil, 261*(1-2), 219–229.

Chen, Y., Li, X., & Shen, Z. (2004b). Leaching and uptake of heavy metals by ten different species of plants during an EDTA-assisted phytoextraction process. *Chemosphere, 57*, 187–196.

Chen, Y., Shen, Z., & Li, X. (2004c). The use of vetiver grass (Vetiveria zizanioides) in the phytoremediation of soils contaminated with heavy metals. *Applied Geochemistry, 19*, 1553–1565.

Chen, Y., Li, X., & Shen, Z. (2018). *Chelant-enhanced phytoextraction of heavy metal-contaminated soils and its environmental risk assessment. Twenty years of research and development on soil pollution and remediation in China* (pp. 509–533). Singapore: Springer.

Chopra, A. K., Pathak, C., & Parasad, G. (2009). Scenario of heavy metal contamination in agricultural soil and its management. *Journal of Applied Natural Science, 1*, 99–108.

Danh, L. T., Truong, P., Mammucari, R., Tran, T., & Foster, N. (2009). Vetiver grass, Vetiveria zizanioides: a choice plant for phytoremediation of heavy metals and organic wastes. *International Journal of Phytoremediation, 11*, 664–691.

Demirbas, A. (2008). Heavy metal adsorption onto agro-based waste materials: a review. *Journal of Hazardous Materials, 157*, 220–229.

Dipu, S., Kumar, A. A., & Thanga, S. G. (2012). Effect of chelating agents in phytoremediation of heavy metals. *Remediation Journal, 22*, 133–146.

DOE, Malaysian Department of Environment. (2009). *Contaminated land management and control guidelines No. 1: Malaysian recommended site screening levels for contaminated land*. Malaysia: Department of Environment, Ministry of Natural Resources and Environment.

Duruibe, J. O., Ogwuegbu, M. O. C., & Egwurugwu, J. N. (2007). Heavy metal pollution and human biotoxic effects. *International Journal of Physical Sciences, 2*, 112–118.

European Chemicals Bureau. (2004). *European Union risk assessment report: edetic acid (EDTA)*. EUR 21314 EN European Commission, Luxembourg

Garbisu, C., & Alkorta, I. (2001). Phytoextraction: a cost-effective plant-based technology for the removal of metals from the environment. *Bioresource Technology, 77*, 229–236.

Goel, S., & Gautam, A. (2010). Effect of chelating agents on mobilization of metal from waste catalyst. *Hydrometallurgy, 101*, 120–125.

Grčman, H., Velikonja-Bolta, Š., Vodnik, D., Kos, B., & Leštan, D. (2001). EDTA enhanced heavy metal phytoextraction: metal accumulation, leaching and toxicity. *Plant and Soil, 235*, 105–114.

Hadi, F., Bano, A., & Fuller, M. P. (2010). The improved phytoextraction of lead (Pb) and the growth of maize (Zea mays L.): the role of plant growth regulators (GA3 and IAA) and EDTA alone and in combinations. *Chemosphere, 80*, 457–462.

Han, F. X., Su, Y., Monts, D. L., & Sridhar, B. B. M. (2004). Distribution, transformation and bioavailability of trivalent and hexavalent chromium in contaminated soil. *Plant and Soil, 265*, 243–252.

Hasanuzzaman, M., & Fujita, M. (2013). Heavy metals in the environment: current status, toxic effects on plants and phytoremediation. In N. A. Anjum, M. E. Pereira, I. Ahmad, A. C. Duarte, S. Umar, & N. A. Khan (Eds.), *Phytotechnologies: remediation of environmental contaminants CRC Press* (pp. 7–73). United States: Florida.

Hovsepyan, A., & Greipsson, S. (2005). EDTA-enhanced phytoremediation of lead-contaminated soil by corn. *Journal of Plant Nutrition, 28*, 2037–2048.

Järup, L. (2003). Hazards of heavy metal contamination. *British Medical Bulletin, 68*, 167–182.

Jean-Soro, L., Bordas, F., & Bollinger, J. C. (2012). Column leaching of chromium and nickel from a contaminated soil using EDTA and citric acid. *Environmental Pollution, 164*, 175–181.

Jiang, M., Liu, S., Li, Y., Li, X., Luo, Z., Song, H., & Chen, Q. (2019). EDTA-facilitated toxic tolerance, absorption and translocation and phytoremediation of lead by dwarf bamboos. *Ecotoxicology and Environmental Safety, 170*, 502–512.

Kabata-Pendias, A. (2010). *Trace elements in soils and plants*. Florida: CRC United States.

Lado, L. R., Hengl, T., & Reuter, H. I. (2008). Heavy metals in European soils: a geostatistical analysis of the FOREGS geochemical database. *Geoderma, 148*, 189–199.

Lai, H. Y., & Chen, Z. S. (2004). Effects of EDTA on solubility of cadmium, zinc, and lead and their uptake by rainbow pink and vetiver grass. *Chemosphere, 55*, 421–430.

Lai, H. Y., & Chen, Z. S. (2005). The EDTA effect on phytoextraction of single and combined metals-contaminated soils using rainbow pink (Dianthus chinensis). *Chemosphere, 60*, 1062–1071.

Leleyter, L., Rousseau, C., Biree, L., & Baraud, F. (2012). Comparison of EDTA, HCl and sequential extraction procedures, for selected metals (Cu, Mn, Pb, Zn), in soils, riverine and marine sediments. *Journal of Geochemical Exploration, 116*, 51–59.

Luo, C., Shen, Z., & Li, X. (2005). Enhanced phytoextraction of Cu, Pb, Zn and Cd with EDTA and EDDS. *Chemosphere, 59*, 1–11.

Luo, J., Qi, S., Gu, X. S., Wang, J., & Xie, X. (2016a). An evaluation of EDTA additions for improving the phytoremediation efficiency of different plants under various cultivation systems. *Ecotoxicol, 25*, 646–654.

Luo, J., Qi, S., Gu, X. S., Hou, T., & Lin, L. (2016b). Ecological risk assessment of EDTA-assisted phytoremediation of Cd under different cultivation systems. *Bulletin of Environmental Contamination and Toxicology, 96*, 259–264.

Luo, J., Cai, L., Qi, S., Wu, J., & Gu, X. S. (2018). Influence of direct and alternating current electric fields on efficiency promotion and leaching risk alleviation of chelator assisted phytoremediation. *Ecotoxicology and Environmental Safety, 149*, 241–247.

McIntyre, T. (2003). Phytoremediation of heavy metals from soils. In D. T. Tsao (Ed.), *Phytoremediation* (pp. 97–123). Berlin Heidelberg: Springer.

Meers, E., Qadir, M., De Caritat, P., Tack, F. M. G., Du, L. G., & Zia, M. H. (2009). EDTA-assisted Pb phytoextraction. *Chemosphere, 74*, 1279–1291.

Meuser, H. (2010). Causes of soil contamination in the urban environment. In H. Meuser (Ed.), *Contaminated urban soils* (pp. 29–94). Netherlands: Springer.

Mühlbachová, G. (2011). Soil microbial activities and heavy metal mobility in long-term contaminated soils after addition of EDTA and EDDS. *Ecological Engineering, 37*, 1064–1071.

Mulligan, C. N., Yong, R. N., & Gibbs, B. F. (2001). Remediation technologies for metal-contaminated soils and groundwater: an evaluation. *Engineering Geology, 60*, 193–207.

Ng, C. C., Boyce, A. N., Rahman, M. M., & Abas, M. R. (2016). Effects of different soil amendments on mixed heavy metals contamination in Vetiver grass. *Bulletin of Environmental Contamination and Toxicology, 97*, 695–701.

Ng, C. C., Boyce, A. N., Rahman, M., & Abas, R. (2017). Tolerance threshold and phyto-assessment of cadmium and lead in vetiver grass, Vetiveria zizanioides (Linn.) Nash. *Chiang Mai Journal of Science, 44*, 1367–1378.

Ng, C. C., Boyce, A. N., Rahman, M. M., Abas, M. R., & Mahmood, N. Z. (2018). Phyto-evaluation of Cd-Pb using tropical plants in soil-leachate conditions. *Air, Soil and Water Research, 11*, 1–9.

Oviedo, C., & Rodríguez, J. (2003). EDTA: the chelating agent under environmental scrutiny. *Quim Nova, 26*, 901–905.

Özkan, A., Günkaya, Z., & Banar, M. (2016). Pyrolysis of plants after phytoremediation of contaminated soil with lead, cadmium and zinc. *Bulletin of Environmental Contamination and Toxicology, 96*, 415–419.

Peng, J. F., Song, Y. H., Yuan, P., Cui, X. Y., & Qiu, G. L. (2009). The remediation of heavy metals contaminated sediment. *Journal of Hazardous Materials, 161*, 633–640.

Prasad, M. N. V., & Strzałka, K. (1999). Impact of heavy metals on photosynthesis. In M. N. V. Prasad & J. Hagemeyer (Eds.), (pp. 117–138). Heidelberg, Germany: Heavy metal stress in plants, Springer, Berlin.

Seth, C. S., Misra, V., Singh, R. R., & Zolla, L. (2011). EDTA-enhanced lead phytoremediation in sunflower (Helianthus annuus L.) hydroponic culture. *Plant and Soil, 347*, 231–242.

Shahid, M., Austruy, A., Echevarria, G., Arshad, M., Sanaullah, M., Aslam, M., Nadeem, M., Nasim, W., & Dumat, C. (2014). EDTA-enhanced phytoremediation of heavy metals: a review. *Soil and Sediment Contamination, 23*, 389–416.

Sherameti, I., & Varma, A. (2010). Soil biology: soil heavy metals. In *Springer*. Heidelberg, Germany: Berlin.

Sinegani, A. A. S., Tahmasbian, I., & Sinegani, M. S. (2015). Chelating agents and heavy metal phytoextraction. In I. Sherameti & A. Varma (Eds.), *Heavy metal contamination of soils* (pp. 367–393). Switzerland: Springer International Publishing.

Sommers, L. E., & Lindsay, W. L. (1979). Effect of pH and redox on predicted heavy metal-chelate equilibria in soils. *Soil Science Society of America Journal, 43*, 39–47.

Suthar, V., Memon, K. S., & Mahmood-ul-Hassan, M. (2014). EDTA-enhanced phytoremediation of contaminated calcareous soils: heavy metal bioavailability, extractability, and uptake by maize and sesbania. *Environmental Monitoring and Assessment, 186*, 3957–3968.

Truong, P., & Danh, L. T. (2015). *The vetiver system for improving water quality: prevention and treatment of contaminated water and land* (2nd ed.). Australia: The Vetiver Network International.

Truong P, Van TT, Pinners E (2008) Vetiver system applications: a technical reference manual. Australia: The Vetiver Network International

US EPA, United States of America Environmental Protection Agency. (1996). *Method 3050B: acid digestion of sediments, sludges and soils*. United States: Environmental Protection Agency.

US EPA, United States of America Environmental Protection Agency. (2007). *Method 7000B flame atomic absorption spectrophotometry*. United States: Environmental Protection Agency.

Van der Ent, A., Baker, A. J., Reeves, R. D., Pollard, A. J., & Schat, H. (2013). Hyperaccumulators of metal and metalloid trace elements: facts and fiction. *Plant and Soil, 362*, 319–334.

Van Deuren, J., Lloyd, T., Chhetry, S., Liou, R., & Peck, J. (2002). Remediation technologies screening matrix and reference guide. In *United States Federal Remediation Technologies Roundtable*.

Vargas, C., Pérez-Esteban, J., Escolástico, C., Masaguer, A., & Moliner, A. (2016). Phytoremediation of Cu and Zn by vetiver grass in mine soils amended with humic acids. *Environmental Science and Pollution Research, 23*, 13521–13530.

Wasino, R., Likitlersuang, S., & Janjaroen, D. (2019). The performance of vetivers (Chrysopogon zizaniodes and Chrysopogon nemoralis) on heavy metals phytoremediation. *International Journal of Phytoremediation, 21*, 624–633.

Wuana, R. A., Eneji, I. S., & Naku, J. U. (2016). Single and mixed chelants-assisted phytoextraction of heavy metals in municipal waste dump soil by castor. *Advances in Environmental Research, 5*, 19–35.

Zhao, Z., Xi, M., Jiang, G., Liu, X., Bai, Z., & Huang, Y. (2010). Effects of IDSA, EDDS and EDTA on heavy metals accumulation in hydroponically grown maize (Zea mays L.). *Journal of Hazardous Materials, 181*, 455–459.

Zhao, S., Lian, F., & Duo, L. (2011). EDTA-assisted phytoextraction of heavy metals by turfgrass from municipal solid waste compost using permeable barriers and associated potential leaching risk. *Bioresource Technology, 102*, 621–626.