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Effects of Different Soil Amendments on Mixed Heavy Metals Contamination in Vetiver Grass

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Abstract Three different types of low cost soil amendments, namely, EDTA, elemental S and N-fertilizer, were investigated with Vetiver grass, *Vetiveria zizanioides* (Linn.) Nash growing under highly mixed Cd–Pb contamination conditions. A significant increase ($p < 0.05$) in Cd and Pb accumulation were recorded in the shoots of all EDTA and N-fertilizer assisted treatments. The accumulation of Cd in 25 mmol EDTA/kg soil and 300 mmol N/kg soil showed relatively higher translocation factor (1.72 and 2.15) and percentage metal efficacy (63.25 % and 68.22 %), respectively, compared to other treatments. However, it was observed that the increased application of elemental S may inhibit the availability of Pb translocation from soil-to-root and root-to-shoot. The study suggests that viable application of 25 mmol EDTA/kg, 300 mmol N/kg and 20 mmol S/kg soil have the potential to be used for soil amendment with Vetiver grass growing under contaminated mixed Cd–Pb soil conditions.

Keywords Mixed contamination · Soil amendment · EDTA · Elemental S · N-fertilizer · Vetiver grass

Soil contamination has become an increasingly important environmental issue in both developed and developing nations. In most cases, soil contamination has been brought about by anthropogenic factors, with humans being the culprit, continuously contaminating the soil in the past and present via industrial and domestic activities. Of these, heavy metal contamination is one of the major types of inorganic soil contamination in the environment. The major contributing factors to anthropogenic heavy metal contamination in soils and the environment include the improper management of agricultural leaching, metalliferous mining and smelting, disposal of metallurgical and electronic commodities, sewage sludge and other chemical manufacturing waste materials (Bradl 2005; Alloway 2013).

Many remediation technologies, such as landfilling, soil washing, bioleaching and excavation have been attempted to resolve soils with contaminated heavy metals. However, all of these strategies are not cost-effective, extremely complicated and are not economically viable in addition to being intrusive to the environment. As a consequence, phytoremediation has emerged to be the green plant based clean-up solution that is able to remove, metabolize and degrade a wide range of hazardous soil heavy metal contaminants with minimum cost required and are non-destructive to the natural ecosystem (Ali et al. 2013). Numerous plants have being studied over the years, with reports suggesting Vetiver grass, *Vetiveria zizanioides* (Linn.) Nash to be one of the most promising plants, with a fast growth rate, and the ability to adapt to many environmental conditions and stress, in addition to being able to tolerate a wide range of extreme heavy metal contamination in soils (Truong et al. 2008; Truong and Danh 2015; Ng et al. 2016a).

Recent studies by Chen et al. (2012), Prasad et al. (2014) and Singh et al. (2015) have solely focused on the phyto-assessment of a single metal accumulation. However, there

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is a growing concern on mixed (Cd–Pb) metal contamination with Vetiver grass, which remain unstudied and require urgent clarification. Both Pb and Cd metals are extremely toxic even at low concentration levels and humans can be easily exposed to these heavy metals through direct inhalation or ingestion of soil and dust, or consumption of contaminated plants, which can substantially affect human health and well-being (Nagajyoti et al. 2010; Ng et al. 2016b). In order to increase the metal accumulation, low cost soil amendments have been used to enhance the phyto-availability of mixed metal uptakes in Vetiver grass (US EPA 2007a; Karami et al. 2011). This study aims to evaluate the trends and effects of heavy metal accumulation and assess the influence and capability of different types and levels of low cost soil amendments to enhance the accumulation of heavy metals by Vetiver grass grown in mixed Cd–Pb contaminated soil conditions.

Materials and Methods

The experiments were conducted in the plant house located at Rimba Ilmu, Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur using pot assays under natural ambient lighting conditions with the average 12-h photoperiod and room temperature ranging between 25.5 and 33.5°C throughout the day. Top soil (0–20 cm depth) for planting was taken from a field situated at the reading of 3° 7′ N latitude and 101° 39′ E longitude and was air-dried for a week before being thoroughly mixed and sieved through <4 mm mesh to remove all non-soil particles to obtain a homogenous soil sample. The soil samples underwent a preliminary physico-chemical soil assessment (Table 1) prior to the preparation of soils with mixed-contamination of Cd (50 mg/kg) and Pb (100 mg/kg), taking into consideration both national (DOE 2009) and international (CCME 1999a, b) permissible soil heavy metals contamination guidelines. The mixed Cd–Pb contamination was artificially spiked using cadmium nitrate tetrahydrate, $Cd(NO_3)_2 \cdot 4H_2O$ and lead(II) nitrate, $Pb(NO_3)_2$ salt compounds before being filled up with two kilograms of soil in plastic pots with height and diameter measurements of 0.18 × 0.16 m, respectively for all treatments. Fresh and healthy 2-week old Vetiver seedlings were collected and placed under different individual experiments, conducted with various types of soil amendments such as disodium ethylene-diamine-tetra-acetate, $C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$ (EDTA), elemental sulfur, S_8 (S) and ammonium nitrate, NH_4NO_3 (N-fertilizer). Four levels of EDTA (1, 5, 10 and 25 mmol EDTA/kg soil), five levels of elemental S (5, 10, 20, 40 and 80 mmol S/kg soil) and six levels of N-fertilizer (10, 25, 50, 100, 200 and 300 mmol N/

Table 1 Physico-chemical properties of growth media soil

Parameter (Unit)	Result
Soil texture	
Sand (%)	93.12
Very coarse sand (%)	1.54
Coarse sand (%)	45.21
Medium coarse sand (%)	21.87
Fine sand (%)	17.58
Very fine sand (%)	6.92
Silt (%)	4.89
Clay (%)	1.99
Bulk density (g/cm^3)	1.34 ± 0.47
Porosity (%)	49.43 ± 3.45
Colour (Munsell colour charts)	Dull reddish brown 2.5YR 5/4
Water content (%)	5.85 ± 1.09
Field capacity (%)	38.59 ± 8.28
Saturation level (%)	15.16 (Dry)
pH	5.11 ± 0.05
Temperature (°C)	29.27 ± 0.45
Metal contents (mg/kg)	
Cd	1.59 ± 0.15
Pb	52.30 ± 2.77

Mean ± standard deviation

Table 2 Soil amendment with treatment variables

Treatment	Description of Cd and Pb (mg/kg soil), EDTA, elemental S and N (mmol/kg soil)
Control	50 Cd + 100 Pb
1EDTA	50 Cd + 100 Pb + 1 EDTA
5EDTA	50 Cd + 100 Pb + 5 EDTA
10EDTA	50 Cd + 100 Pb + 10 EDTA
25EDTA	50 Cd + 100 Pb + 25 EDTA
5S	50 Cd + 100 Pb + 5 elemental S
10S	50 Cd + 100 Pb + 10 elemental S
20S	50 Cd + 100 Pb + 20 elemental S
40S	50 Cd + 100 Pb + 40 elemental S
80S	50 Cd + 100 Pb + 80 elemental S
10N	50 Cd + 100 Pb + 10 N-fertilizer
25N	50 Cd + 100 Pb + 25 N-fertilizer
50N	50 Cd + 100 Pb + 50 N-fertilizer
100N	50 Cd + 100 Pb + 100 N-fertilizer
200N	50 Cd + 100 Pb + 200 N-fertilizer
300N	50 Cd + 100 Pb + 300 N-fertilizer

Table 3 Operating parameters of flame atomic absorption spectrometer (FAAS) and concentrations of certified reference material (CRM) and rate of metal recovery (%) for Cd and Pb

Characteristic (Unit)	Cd	Pb
Operating parameters		
Wavelength (nm)	228.80	283.31
Slit width (nm)	1.35	1.05
Air flow (L/min)	10.00	10.00
Acetylene flow (L/min)	2.50	2.50
Limit of detection (mg/kg)	0.01	0.10
Precision control		
Initial soil (mg/kg)	1.59 ± 0.15	52.30 ± 2.77
Spiked metal (mg/kg)	52.14 ± 7.56	101.88 ± 13.21
CRM[a] (mg/kg)	4.04 ± 0.22	204.0 ± 6.00
Calculated (mg/kg)	3.64 ± 1.45	217.32 ± 14.32
Metal recovery (%)	90.09	106.53

[a]BAM Germany certified reference material BRM#12-mixed sandy soil; Mean ± standard deviation

kg soil) were tested, respectively (Table 2). All soil amendments and heavy metal salt compounds were prepared using the products purchased from the R&M Chemicals. All of the treatments were watered evenly with 50 mL of tap water once a day and their growth performance was continuously monitored throughout the 60-day period of the experiment. The study was conducted under the completely randomized design (CRD) with three replications.

Freshly harvested Vetiver were brought into the laboratory and washed in running filtered water followed by deionized water to remove any adhering soil particles before separating them into roots and shoots (tillers). The fresh weights of plant samples were determined before the samples were oven-dried for 72 h at 70°C until it achieved a constant weight. Then the dry matter yield of the Vetiver samples was determined before it was homogenized in a mortar and pestle. Approximately 0.5 g of the homogenized dried root and shoot samples underwent acid digestion with hydrochloric acid (HCl), hydrogen peroxide (H_2O_2) and nitric acid (HNO_3) as according to Method 3050B (US EPA 1996) followed by Method 7000B (US EPA 2007b) for the elemental analysis using the Perkin-Elmer AAnalyst 400 flame atomic absorption spectrometer (FAAS). The Bundesanstalt für Materialforschung und –prüfung (BAM): German Federal Institute for Materials Research and Testing, certified reference material (BRM#12-mixed sandy soil)

Table 4 Dry matter yield (g/m^2), root-shoot (R/S) ratio and tolerance index (TI) of Vetiver grass as influenced by different treatments of soil amendments

Treatment	Dry matter yield (g/m^2)			R/S ratio	Tolerance Index (TI)
	Root	Shoot	Total		
EDTA					
Control	3.07 ± 0.16 ab	4.92 ± 0.75 ab	7. 99 ± 0.66 ab	0.624 ab	
1EDTA	3.23 ± 0.36 ab	4.83 ± 0.13 ab	8.07 ± 0.31 ab	0.669 ab	1.010 ab
5EDTA	3.29 ± 0.28 ab	4.19 ± 0.36 ab	7.48 ± 0.30 ab	0.785 a	0.936 ab
10EDTA	3.57 ± 0.21 a	4.75 ± 0.24 ab	8.32 ± 0.16 ab	0.752 ab	1.041 ab
25EDTA	3.45 ± 0.35 ab	5.12 ± 0.52 a	8.57 ± 0.86 a	0.674 ab	1.073 a
Elemental S					
Control	3.07 ± 0.16 a	4.92 ± 0.75 a	7. 99 ± 0.66 a	0.624 a	
5S	3.20 ± 0.36 a	4.35 ± 0.58 a	7.54 ± 0.93 a	0.736 a	0.944 a
10S	3.79 ± 0.63 a	4.66 ± 0.75 a	8.45 ± 1.31 a	0.813 a	1.058 a
20S	3.68 ± 0.80 a	4.77 ± 1.11 a	8.46 ± 1.81 a	0.771 a	1.059 a
40S	4.58 ± 1.37 a	5.53 ± 2.03 a	10.11 ± 3.39 a	0.828 a	1.265 a
80S	4.15 ± 0.69 a	5.34 ± 0.55 a	9.48 ± 1.09 a	0.777 a	1.186 a
N-fertilizer					
Control	3.07 ± 0.16 ab	4.92 ± 0.75 ab	7. 99 ± 0.66 a	0.624 a	
10N	3.73 ± 0.46 ab	4.88 ± 1.40 ab	8.61 ± 0.98 a	0.764 a	1.078 a
25N	2.63 ± 1.16 b	3.75 ± 1.48 b	6.38 ± 2.60 a	0.701 a	0.798 a
50N	3.02 ± 0.12 ab	4.97 ± 2.02 ab	7.99 ± 1.93 a	0.608 a	1.000 a
100N	4.28 ± 0.79 a	5.07 ± 1.06 a	9.35 ± 1.62 a	0.844 a	1.170 a
200N	3.89 ± 0.17 ab	4.45 ± 0.79 ab	8.34 ± 0.91 a	0.874 a	1.044 a
300N	2.86 ± 0.52 b	4.11 ± 1.39 b	6.97 ± 1.82 a	0.696 a	0.872 a

Mean ± standard deviations followed by the same letters are not significantly different for each treatment means at 0.05 levels of probability

Table 5 Metal accumulation of cadmium (Cd) and lead (Pb) in the root and shoot of Vetiver grass as influenced by different treatments of soil amendments

Treatment	Cd (mg/kg)			Pb (mg/kg)		
	Root	Shoot	Total	Root	Shoot	Total
EDTA						
Control	153.6 ± 6.1 ab	73.4 ± 7.6 cd	227.0 ± 1.6 cd	165.8 ± 14.0 a	35.5 ± 0.6 d	201.3 ± 14.6 b
1EDTA	133.3 ± 6.0 bcd	79.2 ± 1.1 cd	212.5 ± 4.9 d	80.4 ± 9.0 bc	36.2 ± 0.9 d	116. 6 ± 10.0 c
5EDTA	175.5 ± 6.3 a	91.2 ± 8.3 c	266.7 ± 14.6 abc	65.8 ± 4.4 c	74.1 ± 2.1 c	139.9 ± 6.5 c
10EDTA	147.4 ± 16.7 abc	156.4 ± 10.6 b	303.8 ± 27.3 a	82.5 ± 2.6 bc	126.7 ± 4.8 b	209.2 ± 7.4 b
25EDTA	111.5 ± 11.8 cd	191.8 ± 1.9 a	303.3 ± 9.9 ab	93.2 ± 7.1 b	211.3 ± 12.0 a	304.5 ± 19.1 a
Elemental S						
Control	153.6 ± 6.1 a	73.4 ± 7.6 c	227.0 ± 1.6 abcd	165.8 ± 14.0 a	35.5 ± 0.6 a	201.3 ± 14.6 a
5S	149.7 ± 8.0 ab	129.2 ± 10.7 abc	278.9 ± 18.7 a	118.6 ± 10.6 bc	24.3 ± 4.2 bc	142.9 ± 14.8 b
10S	109.8 ± 6.0 c	144.8 ± 32.9 ab	254.6 ± 26.9 ab	77.5 ± 3.3 d	16.7 ± 1.3 cd	94.2 ± 2.0 c
20S	93.2 ± 18.9 cd	159.3 ± 16.2 a	252.5 ± 35.1 abc	121.1 ± 10.0 b	25.7 ± 3.5 b	146.8 ± 13.5 b
40S	75.6 ± 1.1 d	97.8 ± 15.0 abc	173.4 ± 16.1 d	62.8 ± 3.0 d	11.1 ± 1.5 de	73.9 ± 1.5 c
80S	82.85 ± 7.0 cd	89.3 ± 8.3 bc	172.2 ± 15.4 d	89.0 ± 6.7 cd	5.2 ± 0.3 e	94.2 ± 6.4 c
N-fertilizer						
Control	153.6 ± 6.1 a	73.4 ± 7.6 d	227.0 ± 1.6 a	165.8 ± 14.0 a	35.5 ± 0.6 e	201.3 ± 14.6 bc
10N	140.3 ± 5.2 ab	79.6 ± 10.0 d	219.9 ± 15.2 a	99.7 ± 5.1 bc	187.3 ± 12.0 a	286.9 ± 17.1 a
25N	129.9 ± 20.8 abc	93.7 ± 7.4 bcd	223.6 ± 28.2 a	114.0 ± 14.4 b	163.8 ± 20.0 ab	277.8 ± 5.6 a
50N	109.4 ± 10.0 bcd	116.9 ± 13.4 abcd	226.3 ± 3.4 a	84.5 ± 7.3 bcd	135.6 ± 7.0 bc	220.1 ± 14.3 b
100N	114.7 ± 6.1 bcd	127.3 ± 16.6 abc	242.0 ± 22.7 a	63.9 ± 3.0 de	102.6 ± 8.1 cd	166.5 ± 11.0 c
200N	96.5 ± 5.4 cde	138.1 ± 25.2 ab	234.6 ± 30.6 a	82.9 ± 8.0 cd	78.4 ± 8.0 d	161.3 ± 16.0 c
300N	68.9 ± 9.1 e	147.9 ± 5.6 a	216.8 ± 3.5 a	38.4 ± 3.7 e	71.1 ± 4.1 d	109.5 ± 0.4 d

Mean followed by the same letters are not significantly different for each treatment means at 0.05 levels of probability

was used to validate the precision of the chemical analysis technique whilst the limits of detection and metal recovery rates are recorded in Table 3. Soil samples were also air-dried for 72 h until it reached a constant weight before it was analysed following similar analytical procedures.

The growth performance of Vetiver grass was measured using tolerance index (TI) and root-shoot (R/S) ratio. The ability for heavy metal accumulation and translocation upwards in Vetiver were evaluated by assessing the biological concentration factor (BCF), biological accumulation coefficient (BAC), translocation factor (TF) and metal uptake efficacy as follow:

TI = Total dry matter yield in heavy metal treatment/total dry matter yield in control;
R/S ratio = Dry matter yield in root/dry matter yield in shoot;
BCF = Heavy metal concentration in root/heavy metal concentration in soil;
BAC = Heavy metal concentration in shoot/heavy metal concentration in soil;
TF = Heavy metal concentration in shoot/heavy metal concentration in root; and
Metal uptake efficacy (%) = (Heavy metal concentration in shoot/total heavy metal concentration removed from the soil) × 100 %

Data was analysed by performing one-way analysis of variance (ANOVA) to evaluate the growth performance and metal accumulation in Vetiver growing under different types and levels of treatments. Further statistical validity test for significant differences among treatment means, was carried out using Fisher's least significant difference (LSD) tests at the 95 % level of confidence whilst linear regression analysis was undertaken to assess the relationships between the different types of soil amendments and the accumulation of heavy metal concentration in Vetiver grass.

Results and Discussion

The dry matter yields were not affected by treatment variables (Table 4) as there were no significant differences ($p > 0.05$) found in all of the three different types and levels of soil amendments. These findings indicate that the

Table 6 Metal accumulation of cadmium (Cd) and lead (Pb) in its biological concentration factor (BCF), biological accumulation coefficient (BAC), translocation factor (TF) and metal uptake efficacy (%) of Vetiver grasses as influenced by different treatments of soil amendments

Treatment	Cd accumulation				Pb accumulation			
	BCF	BAC	TF	Efficacy (%)	BCF	BAC	TF	Efficacy (%)
EDTA								
Control	3.072 ab	1.468 c	0.4778 c	32.33 c	1.658 a	0.355 d	0.214 e	17.64 e
1EDTA	2.665 bc	1.584 c	0.594 c	37.28 c	0.804 b	0.362 d	0.451 d	31.06 d
5EDTA	3.510 a	1.824 c	0.520 c	34.20 c	0.658 b	0.741 c	1.126 c	52.97 c
10EDTA	2.948 abc	3.128 b	1.061 b	51.48 b	0.825 b	1.267 b	1.536 b	60.56 b
25EDTA	2.229 c	3.836 a	1.721 a	63.25 a	0.932 b	2.113 a	2.268 a	69.40 a
Elemental S								
Control	3.072 a	1.468 c	0.4778 c	32.33 c	1.658 a	0.355 a	0.214 a	17.64 a
5S	2.994 a	2.583 abc	0.863 bc	46.32 b	1.186 bc	0.243 bc	0.205 a	17.01 a
10S	2.195 b	2.895 ab	1.319 ab	56.88 ab	0.775 c	0.167 cd	0.215 a	17.68 a
20S	1.864 b	3.186 a	1.709 a	63.09 a	1.211 b	0.257 b	0.212 a	17.51 a
40S	1.511 b	1.956 bc	1.295 ab	56.42 ab	0.628 c	0.111 de	0.177 a	15.03 a
80S	1.657 b	1.786 bc	1.078 bc	51.87 ab	0.890 c	0.051 e	0.058 b	5.46 b
N-fertilizer								
Control	3.072 a	1.468 d	0.478 c	32.33 e	1.658 a	0.355 e	0.214 d	17.64 d
10N	2.806 ab	1.592 d	0.567 c	36.20 e	0.997 bc	1.873 a	1.880 ab	65.27 a
25N	2.598 abc	1.874 bcd	0.721 c	41.91 cde	1.140 b	1.638 ab	1.437 abc	58.96 ab
50N	2.187 bcd	2.338 abcd	1.069 bc	51.67 bcd	0.845 bc	1.356 bc	1.605 ab	61.61 ab
100N	2.294 bcd	2.546 abc	1.109 bc	52.60 bc	0.639 de	1.026 cd	1.607 ab	61.64 ab
200N	1.929 cde	2.762 ab	1.432 b	58.88 ab	0.829 cd	0.784 d	0.946 cd	48.62 c
300N	1.378 e	2.958 a	2.147 a	68.22 a	0.384 e	0.711 e	1.854 a	64.96 ab

Mean ± standard deviations followed by the same letters are not significantly different for each treatment means at 0.05 levels of probability

application of mixed Cd–Pb contamination in soil growth media may not have much effect on the overall (roots, shoots and total) dry matter yield for Vetiver grass regardless of the different types and levels of treatment combination. Subsequently, the root-shoot (R/S) ratio and tolerance index (TI) was employed to assess the capability of the Vetiver grass growing under mixed Cd–Pb contamination conditions. Similarly, no significant differences ($p > 0.05$) was observed in the R/S ratio and tolerance index (TI) among the treatment variables. The Vetiver grass showed high tolerant and good adaptability properties to the contaminated mixed Cd–Pb soil conditions as was previously reported in Chen et al. (2004) and Danh et al. (2009).

Metal accumulation for both Cd and Pb in the roots and shoots of Vetiver grass are shown in Table 5. Each level of EDTA, elemental S and N-fertilizer soil amended treatment recorded a distinctive Cd (172.2–303.3 mg/kg) and Pb (73.9–304.5 mg/kg) concentration pattern in the roots and shoots of Vetiver. The 25 mmol EDTA treatment exhibited the highest accumulation of both Pb (211.3 ± 12.0 mg/kg) and Cd (191.8 ± 1.9 mg/kg) in the shoots. Between roots and shoots, the accumulation of both Cd and Pb were comparatively greater in the shoots than the roots for 10 mmol EDTA and 25 mmol EDTA. A significant increase ($p < 0.05$) in Cd and Pb accumulation in the shoots were obtained in both 10 mmol EDTA and 25 mmol EDTA treatments compared to the control. For all types and levels of soil amendments, Pb accumulation in the roots, together with selected Cd roots treatments (25EDTA, 10S, 20S, 40S, 80S, 50N, 100N, 200N and 300N), a significant reduction ($p < 0.05$) was observed compared to the control. With regard to Pb accumulation, significantly lower ($p < 0.05$) uptake was observed in all levels of elemental S treatment irrespective of roots, shoots and total metal accumulation compared to the control. However, a significantly larger ($p < 0.05$) accumulation of Pb in the shoots was observed in all N-fertilizer treatments compared with the control. On the other hand, no significant difference ($p > 0.05$) was found between the total metal accumulation of Cd in both elemental S and N-fertilizer treatments compared with control.

The comparatively lower BCF values were obtained in all types of soil amended treatments compared to the control, probably due to the effects of lower accumulation of both Cd and Pb metals in the roots than shoots (Table 6). Alternatively, all levels of EDTA and N-fertilizer treatments recorded remarkably higher BAC and TF values than the

Table 7 Regression equation, coefficients of determination (R^2) and F values of different parameters in Vetiver grass

Regression equation	R^2	R	F value
Relationship between level of soil amendments and Cd accumulation			
Y_{Cd} (EDTA) = 239.579 + 3.119X_1	0.579	0.761	2.746
Y_{Cd} (S) = 271.075 − 1.445X_1	0.779	0.882	10.557*
Y_{Cd} (N) = 227.831 − 0.006X_1	0.005	0.068	0.019
Relationship between level of soil amendments and Pb accumulation			
Y_{Pb} (EDTA) = 111.152 + 7.939X_1	0.977	0.988	83.180**
Y_{Pb} (S) = 127.273 − 0.546X_1	0.261	0.511	1.061
Y_{Pb} (N) = 268.567 − 0.569X_1	0.849	0.921	22.405**
Relationship between dry matter yield and Cd accumulation			
Y_{Cd} (EDTA) = −60.363 + 40.926X_2	0.132	0.363	0.303
Y_{Cd} (S) = 646.832 − 47.748X_2	0.915	0.956	32.185**
Y_{Cd} (N) = 181.232 + 5.787X_2	0.446	0.668	3.224
Relationship between dry matter yield and Pb accumulation			
Y_{Pb} (EDTA) = 124.487 + 9.154X_2	0.020	0.140	0.040
Y_{Pb} (S) = 344.732 − 26.610X_2	0.668	0.817	6.038
Y_{Pb} (N) = 252.947 − 6.207X_2	0.009	0.096	0.038

X_1 level of soil amendments, X_2 dry matter yield

*Significant at 0.05 level of probability

**Significant at 0.01 level of probability

control in both Cd and Pb accumulation, suggesting that the pathway for metal translocation from soil into shoots were more favourable. The 25 mmol EDTA treatment demonstrated the highest BAC (3.836) and TF (2.268) for Cd and Pb accumulation, respectively. Chiu et al. (2005) and Chen et al. (2012) also reported similar findings in Vetiver grass showing the accumulation of heavy metals is gradually enhanced with the application EDTA. Furthermore, the higher accumulation of heavy metals in the shoots than the roots also suggested that the shoots of Vetiver grass act as the sink for both Cd and Pb accumulation. Among the different types of soil amendments, N-fertilizer (36.20 %–68.22 %), EDTA (34.20 %–63.25 %) and elemental S (46.32 %–63.09 %) recorded higher accumulation efficacy for Cd, compared with other individual levels of treatment, respectively. On the other hand, 25 mmol EDTA (69.40 %), 10 mmol N (65.27 %) and 300 mmol N (64.96 %) exhibited the greatest accumulation efficacy for Pb with more than a two-fold increase compared to the control. However, the application of elemental S showed no significant difference ($p > 0.05$) in the enhancement of Pb accumulation regardless of the different concentrations used, compared to the control. Moreover, the efficiency of Pb metal translocation from soil-to-root and root-to-shoot decreased with increasing amount of elemental S used. These findings are contrary to that reported by Feng et al. (2009), Motior et al. (2011), Rahman et al. (2011) as well as Dede and Ozdemir (2016) which used other types of plant species. Despite the lower Pb accumulation in the elemental S treatments, appreciably higher BAC, TF and metal efficacy for Cd accumulation than the control were detected.

Generally, the inclination trend observed for Cd accumulation, among the different types of soil amendments were in the order of N-fertilizer (300N) > EDTA (25EDTA) > elemental S (20S) for all the treatments. The findings show that application of higher levels of both N-fertilizer and EDTA are likely to enhance the accumulation of Cd in the shoots of Vetiver grass whereas the opposite was found with elemental S. Notwithstanding, the trend for Pb accumulation was in the following order of EDTA (25EDTA) > N-fertilizer >> elemental S among all treatments. The application of higher levels of EDTA and N-fertilizer could have probably increased Pb accumulation in the shoots of Vetiver grass, as similar trends have been reported previously by Nascimento et al. (2006), Chiu et al. (2006) and Rahman et al. (2013). Although higher Pb accumulation was recorded with the application of N-fertilizer in the shoots, relatively all levels of N-fertilizer treatments displayed approximately similar accumulation of Pb. With higher application levels of elemental S, Pb accumulation are more likely to drop in both the roots and shoots of Vetiver grass.

There were strong and significant positive relationships found between the accumulations of Pb in EDTA ($r = 0.998$) and N-fertilizer ($r = 0.921$) treatments with the levels of soil amendments used when grown under the mixed heavy metal contamination (Table 7). Elemental S treatment ($r = 0.956$) exhibited strong negative correlation with regard to dry matter yield and Cd accumulation due to the appreciably decreased metal uptake in the roots and shoots in selected elemental S treatments. The regression equations revealed a positive association with the application of EDTA showing a comparably higher influence on dry matter yield, as well as Cd and Pb accumulation compared to the other two types of soil amendments. The study demonstrates that 25 mmol EDTA, 300 mmol N-fertilizer and 20 mmol elemental S are the best possible soil amendments with Vetiver grass in the mixed Cd–Pb contaminated soil condition.

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