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Contributed Paper

Tolerance Threshold and Phyto-assessment of Cadmium and Lead in Vetiver Grass, *Vetiveria zizanioides* (Linn.) Nash

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ABSTRACT

Various types of plant species have been extensively used for heavy metals phyto-remediation without taking into consideration its tolerance threshold. In this study, Vetiver grass, *Vetiveria zizanioides* (Linn.) Nash was evaluated under five different sets of contaminated spiked cadmium (5Cd, 10Cd, 50Cd, 100Cd and 150Cd mg/kg) and lead (50Pb, 100Pb, 200Pb, 400Pb and 800Pb mg/kg) concentration levels in soil. The growth performance, metal tolerance and phyto-assessment of Cd and Pb in the roots and tillers were assessed using flame atomic absorption spectrometry (FAAS). Tolerance index (TI), translocation factor (TF), biological transfer factor (BTF), biological accumulation coefficient (BAC) and metal uptake efficacy were used to determine the Cd and Pb translocation capability in Vetiver grass. Significantly higher ($p<0.05$) accumulation of Cd and Pb was recorded in the roots of all spiked treatments. Furthermore, strong and significantly positive correlations were exhibited between the increased levels of spiked heavy metal concentrations with both Cd ($r=0.975$) and Pb ($r=0.952$) accumulations. The results of this study showed Vetiver grass as an effective phyto-stabilizer for both Cd and Pb. Nevertheless, the growth of Vetiver grass was restricted when the tolerance threshold of 100 mg/kg (dry weight basis) Cd was exceeded in the contaminated soil.

Keywords: heavy metal, spiked metal, phyto-stablizer, Vetiver grass, threshold

1. INTRODUCTION

Over the years, soil contamination has attracted much global attention as it instigates considerable risks to human health and the environment. Anthropogenic sources of soil contaminants such as heavy metals released by human activities via industrial and agricultural practices, urban activities and transportation have caused serious threats to the environment [1, 2]. The term heavy metal is widely used to describe a large group of elements in the periodic table with an atomic density greater than 6 g/cm^3 but can also be defined in relation to its natural chemical properties [3]. Among various types of heavy metals, both cadmium (Cd) and lead (Pb) are regarded to be highly toxic pollutants even at low concentration levels. Although naturally occurring, both Cd and Pb are often imperceptible, non-biodegradable and are persistent in soils over a long duration [4, 5]. These metals are hazardous to human health as it is easily bio-accumulated via the food chain including direct inhalation, ingestion of soil and/or consumption of contaminated plants [6, 7]. The exposure of plants in heavy metal contaminated soil could possibly affect the inhibition of plant growth; reduced metabolism and lower biomass due to the toxicity effects of both Cd and Pb [8, 9]. As a result, the United States Priority Pollutant List [10] has recognised both Cd and Pb to be among the 126 Toxic and Priority Pollutants due to its lethal characteristics in nature.

There are numerous remediation technologies including both physical (excavation, containment and fracturing) and chemical (soil washing, solidification-stabilization and chemical redox) assisted methods that have been tested to clean up contaminated heavy metals in soils [11, 12]. Nonetheless, phyto-remediation has evolved to be an alternative biological assisted method that is cost-effective, non-destructive and environmentally friendly solution for heavy metals soil contamination compared to other techniques [13-15]. Among various types of plants, Vetiver grass, *Vetiveria zizanioides* (Linn.) Nash has been reported to be one of the most promising species with great potential for heavy metals phyto-remediation due to its fast growth, extensive deep root system and high tolerance to a wide range of adverse soil conditions [16-19]. However, little information is available concerning the effect of different levels of spiked heavy metals in Vetiver grass. Recent studies [20-22] have solely reported on the phyto-extraction and phyto-stabilization effects of heavy metals uptake in Vetiver grass. Nevertheless, there are currently no robust studies that have been tested to evaluate the comparative and empirical phyto-assessment using Vetiver grass growing under different levels of spiked Cd and Pb conditions. Hence, the purpose of this study was to (i) evaluate the growth performance; (ii) assess the metal uptake ability and accumulation trends; and (iii) examine the phyto-tolerance and threshold limits of Vetiver grass growing under different levels of spiked Cd and Pb contaminated soil conditions.

2. MATERIALS AND METHODS

2.1 Samples Preparation and Experimental Design

The study was conducted using pot experiments in the planthouse of the Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur with the average room temperature ranging between 27°C and 36°C throughout the day. Top soil (0-20cm) collected from uncontaminated field located in the University of Malaya, Kuala Lumpur (3° 7′ N latitude and 101° 39′ E longitude) was used as the tested soil in the experiment after undergoing preliminary physico-chemical soil assessment (Table 1). The dull reddish brown soil composed of 90.47% sand, 7.89% silt and 1.64% clay. All collected soil was air-dried for a week followed by < 4mm sieving using test sieve to remove gravels and large non-soil particles. The artificially spiked heavy metal treatments were prepared using cadmium nitrate tetrahydrate [$Cd(NO_3)_2.4H_2O$] and lead (II) nitrate [$Pb(NO_3)_2$] salt compounds that exceed the median permissible in the natural occurring levels of the Malaysian [23], Canadian [24] and European Union [25] soil contamination guidelines. The amended soil was then continuously stirred and incubated for a week to ensure that the homogeneousity of the desired spiked heavy metal concentration is obtained.

The control and five different levels of spiked Cd and Pb treatments (Table 2) were tested using Vetiver grass, *Vetiveria zizanioides* (Linn.) Nash in plastic pots (0.18m diameter × 0.16m depth) filled with 2kg of soil, respectively. Vetiver saplings were obtained from Humibox Malaysia whereby only fresh and healthy plant saplings with an initial average tiller number (10-15), plant height (30-35cm) and basal diameter (2.5-3.5cm) were selected for the experiment. All of the spiked heavy metal treatments were watered evenly with 50mL of tap water once a day and the plant growth parameters such as height, tiller number, basal diameter and percentage plant survivorship were continuously monitored throughout the 60-day period of the experiment. The study was conducted under the completely randomized design (CRD) with three replications.

Table 1. Physico-chemical parameters of growth media soil.

Parameters (Unit)		Mean
pH		6.62 ± 0.09
Soil texture		
Sand (%)		90.47
	Very coarse sand (%)	1.93
	Coarse sand (%)	50.45
	Medium coarse sand (%)	32.19
	Fine sand (%)	10.22
	Very fine sand (%)	5.21
Silt (%)		7.89
Clay (%)		1.64
Metal contents (mg/kg)		
	Cd	1.23 ± 0.46
	Pb	9.25 ± 1.42
Bulk density (g/cm^3)		1.49 ± 0.56
Porosity (%)		43.77 ± 2.14
Field capacity (%)		24.32 ± 2.16
Saturation level (%)	Dry	15.83
Colour (Munsell colour charts)	Dull reddish brown	2.5YR 5/4

Mean ± standard deviation

Table 2. Treatment variables.

Treatment	Detail of spiked heavy metal
Control	No heavy metal added
5Cd	5 mg/kg of Cd
10Cd	10 mg/kg of Cd
50Cd	50 mg/kg of Cd
100Cd	100 mg/kg of Cd
150Cd	150 mg/kg of Cd
50Pb	50 mg/kg of Pb
100Pb	100 mg/kg of Pb
200Pb	200 mg/kg of Pb
400Pb	400 mg/kg of Pb
800Pb	800 mg/kg of Pb

2.2 Samples and Chemical Analyses

At the end of the 60-day of the experimental period, all Vetiver treatments were uprooted and brought into the laboratory for chemical analysis. Freshly harvested Vetiver were washed in running water followed by deionized water to remove any adhering soil particles before it was sectioned into parts of roots and tillers (shoots). All soil and plant samples were oven-dried for 72 hours at 70°C to obtain a constant weight of dry matter content before it was homogenized in a mortar and pestle. Approximately, 0.5g of the homogenized samples underwent acid digestion with hydrochloric acid (HCl), nitric acid (HNO_3) and hydrogen peroxide (H_2O_2) according to Method 3050B [26] followed by Method 7000B [27] for total recoverable elemental analysis of both Cd and Pb using the Perkin-Elmer AAnalyst 400 flame atomic absorption spectrometer (FAAS). The highly precise technique of determining the spiked heavy metal concentrations and chemical analysis was controlled using the BAM Germany (BRM#12-mixed sandy soil) certified reference material with an average rate of metal recovery for Cd (93.46%) and Pb (108.25%), respectively (Table 3).

Table 3. Concentrations of initial and spiked heavy metal (mg/kg), certified reference material (CRM) and metal recovery (%) for Cd and Pb metals.

Treatment	Initial soil (mg/kg)		Spiked metal (mg/kg)		CRM* (mg/kg)	Measured (mg/kg)	Metal recovery (%)
	Cd	Pb	Cd	Pb			
Control	1.23 ± 0.46	9.25 ± 1.42	NA	NA	NA	NA	NA
Spiked Cd					4.04 ± 0.22	3.78 ± 1.42	93.46
5Cd	1.23 ± 0.46	9.25 ± 1.42	5.31 ± 0.94	NA	NA	NA	NA
10Cd	1.23 ± 0.46	9.25 ± 1.42	13.83 ± 2.76	NA	NA	NA	NA
50Cd	1.23 ± 0.46	9.25 ± 1.42	51.77 ± 5.32	NA	NA	NA	NA
100Cd	1.23 ± 0.46	9.25 ± 1.42	107.68 ± 8.90	NA	NA	NA	NA
150Cd	1.23 ± 0.46	9.25 ± 1.42	155.14 ± 4.61	NA	NA	NA	NA
Spiked Pb					204.00 ± 6.00	220.83 ± 34.92	108.25
50Pb	1.23 ± 0.46	9.25 ± 1.42	NA	52.18 ± 4.82	NA	NA	NA
100Pb	1.23 ± 0.46	9.25 ± 1.42	NA	102.44 ± 8.31	NA	NA	NA
200Pb	1.23 ± 0.46	9.25 ± 1.42	NA	207.63 ± 3.19	NA	NA	NA
400Pb	1.23 ± 0.46	9.25 ± 1.42	NA	404.94 ± 8.09	NA	NA	NA
800Pb	1.23 ± 0.46	9.25 ± 1.42	NA	811.76 ± 13.98	NA	NA	NA

*BAM Germany (BRM#12-mixed sandy soil) certified reference material

Mean ± standard deviation; NA = Not applicable

2.3 Statistical Analyses and Data Interpretation

The plant growth performance were evaluated using the root-tiller (R/T) ratio and tolerance index (TI) whilst the ability and proficiency for heavy metal accumulation and translocation upwards (soil-to-root and root-to-tiller) in Vetiver grass were assessed by determining the translocation factor (TF), biological transfer factor (BTF), biological accumulation coefficient (BAC) and percentage of metal uptake efficacy [3, 15, 28] as follows:

R/T ratio = Dry matter content in root/Dry matter content in tiller
TI = Total dry matter content in spiked heavy metal treatment/Total dry matter content in control
TF = Concentration of heavy metal in tiller/Concentration of heavy metal in root
BTF = Concentration of heavy metal in root/Concentration of heavy metal in soil
BAC = Concentration of heavy metal in tiller/Concentration of heavy metal in soil
Metal uptake efficacy (%) = [Concentration of heavy metal in tiller/Total concentration of heavy metal accumulated in Vetiver] × 100

All experimental data were analysed by performing the one-way analysis of variance (ANOVA) and further statistical validity test for significant differences among treatment means was conducted by employing the Fisher's least significant difference (LSD) tests at the 95% level of confidence.

3. RESULTS AND DISCUSSION

3.1 Responses of Soil pH and Plant Growth Performance

The initial soil pH varied from 4.12 to 5.57 where control soil recorded the highest pH of 5.57 while the lowest pH of 4.12 was observed in 800Pb treatment (Figure 1). Upon harvesting, all spiked Cd and Pb treatments, except for 800Pb treatment, showed a decline in pH ranging from 4.20 to 4.62, where the highest pH reduction (-1.03 pH units) was recorded in 50Pb treatment. No significant difference (*F=1.45; d.f.=35; p>0.05*) in soil pH was observed among all Cd spiked treatments. On the other hand; 100Pb, 200Pb, 400Pb and 800Pb treatments significantly (*F=4.05; d.f.=35; p<0.05*) affected the soil pH levels compared to the control. The changes in soil pH observed could be related to the application of high levels of spiked Pb as more positively charge (proton ions) are present in the soils. Similar studies [29, 30] have revealed that the bioavailability of metal uptake in plants is strongly associated with the soil pH conditions.

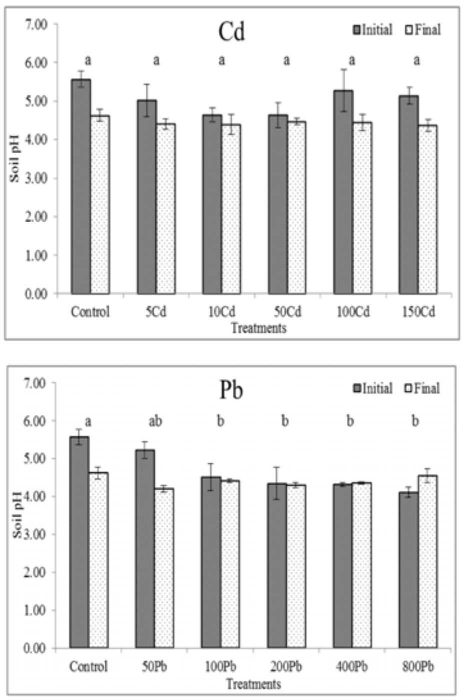

Figure 1. Changes in soil pH in Vetiver as influenced by different levels of spiked Cd and Pb concentrations. Vertical bars represent standard deviation in treatment means and same letters are not significantly different at 0.05 levels of probability.

Table 4 shows that a significantly lower (*F=5.98, 8.42; d.f.=35; p<0.05*) tiller number and plant height were obtained in 150 Cd treatment compared to other spiked Cd treatments and control. Both 100Cd (64.44%) and 150Cd (37.22%) treatments demonstrated significantly lower (*F=13.09; d.f.=35; p<0.05*) percentage of survival compared with the control and other spiked Cd treatments. Among spiked Cd treatments, 100Cd and 150Cd recorded significantly lower growth performance, as the plants started to wither during the second week of the experimental period due to the presence of high concentrations of spiked Cd in the soils. In contrast, there was no significant difference (*F=0.80, 0.35, 0.02, 1.05; d.f.=35; p>0.05*) in terms of tiller number, plant height, basal diameter and percentage survivorship recorded in all spiked Pb treatments compared with the control. The findings demonstrated adverse growth reduction in terms of tiller number (27.6-71.0%), plant height (26.8-77.8%) and plant survivorship (35.6-62.8%) when high amounts of spiked 100Cd and 150Cd treatments were applied to Vetiver grass, respectively. With regard to Pb, similar past studies [17, 31] had reported that Vetiver grass has progressive outgrowth under all different levels of spiked Pb concentrations.

Table 4. The tiller number, plant height, basal diameter and plant survivorship of Vetiver as influenced by different levels of spiked heavy metal concentrations.

Treatment	Number of tillers	Plant height (cm)	Basal diameter (cm)	Plant survivorship (%)
Control	27.9 a	61.49 ab	7.73 ab	100.00 a
5Cd	26.9 a	73.26 a	8.71 ab	98.89 a
10Cd	32.6 a	75.58 a	9.64 a	98.33 a
50Cd	21.2 ab	61.93 ab	7.95 ab	97.78 a
100Cd	20.2 b	45.02 b	6.82 ab	64.44 b
150Cd	8.1 c	13.64 c	3.38 b	37.22 c
F-value	5.984	8.423	1.472	13.087
LSD 95%	12.075	27.486	6.203	25.072
Control	27.9 a	61.49 a	7.73 a	100.00 a
50Pb	21.0 a	74.07 a	8.28 a	99.44 a
100Pb	19.0 a	79.92 a	8.37 a	98.89 a
200Pb	21.4 a	75.89 a	8.37 a	98.89 a
400Pb	21.1 a	75.77 a	8.44 a	98.33 a
800Pb	23.0 a	80.56 a	8.13 a	98.33 a
F-value	0.802	0.351	0.019	1.051
LSD 95%	11.868	40.371	6.557	2.192

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

The roots, tillers and total dry matter contents in all spiked Pb treatments were not significantly affected (*F=0.92, 0.14, 0.32; d.f.=17; p>0.05*) with the application of different levels of Pb concentrations (Table 5). Only the 150Cd treatments recorded a significantly lower (*F=4.66, 3.41; d.f.=35; p<0.05*) tiller number and total dry matter content. The 150Cd (9.87 ± 0.12 g/m^2) treatment displayed the lowest total dry matter content with an average of 31.3% reduction as compared to the control. The application of both root-tiller (R/T) ratios and tolerance index (TI), employed to assess the tolerance ability of Vetiver grass to grow under different levels of spiked heavy metal concentrations, showed that there was no significant difference observed in the R/T ratios (*F=0.58, 1.07; d.f.=17; p>0.05*) among all spiked Cd and Pb treatments. Furthermore, although no significant difference in TI (*F=1.49, 0.23; d.f.=14; p>0.05*) was observed in both spiked Cd and Pb treatments, all spiked Pb treatments recorded remarkably high TI values > 1 (1.021-1.099). The results indicated that the different levels of spiked Pb concentrations had no direct influence on the overall growth performance and dry matter contents in Vetiver grass. As the TI (1.001-1.099) was relatively > 1, Vetiver grass can be regarded to have good adaptability and high tolerance proficiency in Pb concentrated soils. Conversely, it can be concluded that Vetiver grass is not suitable to be used as a phyto-remediator for soils contaminated with Cd exceeding the threshold level of 100 mg/kg (dry weight basis). This is in agreement with the findings of past studies [17, 32-33].

Table 5. Dry matter content, root-tiller ratio and tolerance index of Vetiver as influenced by different levels of spiked heavy metal concentrations.

Treatment	Dry matter content (g/m^2)			R/T ratio	TI
	Root	Shoot	Total		
Control	8.20 ± 0.51 a	6.17 ± 0.93 a	14.37 ± 1.43 a	1.343 a	
5Cd	8.10 ± 1.15 a	6.11 ± 0.74 a	14.21 ± 1.14 a	1.341 a	1.001 a
10Cd	8.04 ± 1.68 a	6.06 ± 0.79 a	14.10 ± 2.32 a	1.322 a	0.994 a
50Cd	7.98 ± 1.56 a	5.83 ± 0.33 a	13.81 ± 1.53 a	1.375 a	0.969 a
100Cd	7.56 ± 1.33 a	5.03 ± 0.87 ab	12.60 ± 2.19 ab	1.502 a	0.890 a
150Cd	5.98 ± 0.40 a	3.89 ± 0.48 b	9.87 ± 0.12 b	1.565 a	0.692 a
F-value	1.467	4.662	3.408	0.578	1.488
LSD 95%	2.411	1.445	3.251	0.458	0.385
Control	8.20 ± 0.51 a	6.17 ± 0.93 a	14.37 ± 1.43 a	1.343 a	
50Pb	9.00 ± 0.81 a	5.66 ± 0.80 a	14.66 ± 1.50 a	1.599 a	1.021 a
100Pb	9.73 ± 1.62 a	5.86 ± 0.41 a	15.60 ± 2.03 a	1.652 a	1.084 a
200Pb	9.78 ± 1.70 a	5.81 ± 0.81 a	15.59 ± 2.47 a	1.680 a	1.087 a
400Pb	9.54 ± 0.50 a	6.01 ± 1.39 a	15.56 ± 1.46 a	1.640 a	1.086 a
800Pb	9.51 ± 0.68 a	6.14 ± 0.87 a	15.65 ± 1.27 a	1.564 a	1.099 a
F-value	0.919	0.141	0.320	1.067	0.229
LSD 95%	2.178	1.829	3.484	0.411	0.236

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

3.2 Distribution of Metals in Plant

The accumulation of both Cd and Pb in the roots and tillers of Vetiver grass was varied (Table 6). The roots (*F=38.38; d.f.=17; p<0.05*) and total accumulation (*F=124.89; d.f.=17; p<0.05*) of Cd in 50Cd, 100Cd and 150Cd treatments were found to be significantly higher than the control, 5Cd and 10Cd treatments. There was no Cd accumulation recorded in the tillers for both control and 5Cd treatments as its detection limits were lower than 0.01 mg/kg (dry weight basis), whilst the tillers of 150Cd treatment (66.85 ± 9.73 mg/kg) showed the highest accumulation Cd. Between roots and tillers, Cd accumulations were reasonably greater in the roots (1.28-112.67 mg/kg) than the tillers (4.16-66.85 mg/kg) in all treatments. The accumulation trend of Cd in Vetiver grass was in the following order: 150Cd > 100Cd > 50Cd > 10Cd > control.

Similarly, with regard to Pb accumulation, the roots (*F=160.28; d.f.=17; p<0.05*) and total accumulation (*F=228.32; d.f.=17; p<0.05*) of Pb in 100Pb, 200Pb, 400Pb and 800Pb treatments were found to be significantly greater than the control and 50Pb treatments. A significantly higher accumulation of Pb was observed in the tillers (*F=42.74; d.f.=17; p<0.05*) of both 400Pb and 800Pb treatments compared to the control and other spiked Pb treatments. Between roots and tillers, Pb accumulated substantially higher in the roots (4.27-396.60 mg/kg) compared to the tillers (1.79-122.94 mg/kg) in all treatments. The trend for Pb accumulation in Vetiver grass was in the following order of 800Pb > 400Pb > 200Pb > 100Pb > 50Pb > control. In both spiked heavy metal treatments, Vetiver grass accumulated the highest total amount of Cd (179.52 ± 16.74 mg/kg) and Pb (519.54 ± 24.93 mg/kg) in the spiked 150Cd and 800Pb treatments, respectively. The results demonstrated that the accumulation of both Cd and Pb in Vetiver grass increases with the application of higher levels of spiked heavy metals concentrations. Both 100Cd and 150Cd treatments recorded fairly higher accumulation of Cd than the other spiked Cd treatments and the control whereby this was reflected in the noticeable reduction in plant growth. As a result, these findings indicated that the declining plant growth trends do not affect the overall bioavailability uptake of spiked heavy metals in Vetiver grass. On the other hand, the findings are supported by the past similar studies [18, 34] which revealed that the phyto-tolerance and growth threshold of Vetiver grass for Cd and Pb accumulations were 20-60 mg/kg Cd and > 1500 mg/kg Pb in soil, respectively. The obtained results are aligned yet it has been further extemporized to show that the Vetiver grass have limited phyto-tolerance and threshold levels when the accumulation of heavy metal is higher than 100 mg/kg of Cd in soil whilst no specific threshold level of Pb is recorded till present. However, the use of direct pot experiments in this study for the spiked heavy metals instead of in-situ (field) experiments may have possibly attributed to the high heavy metals accumulation in both the roots and tillers of Vetiver grass.

3.3 Association of Metals Uptake in Plant

The plant-soil association of both Cd and Pb accumulated from the spiked heavy metal soils into the roots and the tillers of Vetiver grass are shown in terms of translocation factor (TF), biological transfer factor (BTF), biological accumulation coefficient (BAC) and percentage of metal uptake efficacy in Table 6. Considering the tolerably lower accumulation of spiked heavy metals in the tillers than the roots in Vetiver grass, TF and BAC are employed to evaluate the capability of Vetiver to translocate heavy metals from soils and roots into the tillers. As reported in Table 5, all of the recorded TF and BAC values in both Cd and Pb accumulations were < 1. The 100Cd (0.446) and 150Cd (0.615) treatments recorded significantly higher (*F=10.06; d.f.=17; p<0.05*) TF values whilst the 50Pb (0.145), 100Pb (0.104) and 200Pb (0.145) treatments showed a significant decrease (*F=3.82; d.f.=17; p<0.05*) in TF values compared to the control for both Cd and Pb accumulations, respectively. No significant difference (*F=1.56; d.f.=17; p>0.05*) were found in BAC in all Pb spiked treatments. Meanwhile 10Cd, 50Cd, 100Cd and 150Cd treatments exhibited significantly higher (*F=14.47; d.f.=17; p<0.05*) BAC values than the control.

Table 6. Metal accumulation of cadmium (Cd) and lead (Pb) with its translocation factor (TF), biological transfer factor (BTF), biological accumulation coefficient (BAC) and metal uptake efficacy (%) of Vetiver as influenced by different levels of spiked heavy metal concentrations.

Treatment	Metal concentration (mg/kg)			TF	BTF	BAC	Efficacy
	Root	Shoot	Total				(%)
Cd accumulation							
Control	1.28 ± 0.72 c	ND (< 0.01)	1.28 ± 0.72 d	0.010 c	1.041 ab	0.008 c	0.98 c
5Cd	6.71 ± 2.76 c	ND (< 0.01)	6.71 ± 2.76 d	0.002 c	1.341 ab	0.002 c	0.17 c
10Cd	17.10 ± 6.05 c	4.16 ± 0.97 c	21.26 ± 6.10 d	0.262 bc	1.710 a	0.416 ab	20.49 b
50Cd	59.12 ± 10.59 b	13.13 ± 7.79 c	72.24 ± 13.77 c	0.224 bc	1.182 ab	0.263 b	17.65 b
100Cd	88.57 ± 18.89 a	37.32 ± 10.99 b	125.88 ± 15.50 b	0.446 ab	0.886 ab	0.373 ab	29.89 ab
150Cd	112.67 ± 22.34 a	66.85 ± 9.73 a	179.52 ± 16.74 a	0.615 a	0.751 b	0.446 a	37.57 a
F-value	38.383	46.660	124.885	10.064	1.905	14.472	16.165
LSD 95%	25.952	13.579	22.416	0.263	0.863	0.183	12.986
Pb accumulation							
Control	4.27 ± 0.43 e	1.79 ± 1.16 c	6.06 ± 1.13 e	0.426 a	0.462 d	0.193 a	27.97 a
50Pb	36.94 ± 13.44 e	5.08 ± 1.61 c	42.02 ± 14.65 e	0.145 bc	0.739 bc	0.102 a	12.54 ab
100Pb	120.71 ± 16.02 d	12.52 ± 1.43 c	133.23 ± 16.86 d	0.104 c	1.207 a	0.125 a	9.45 b
200Pb	189.58 ± 11.08 c	27.86 ± 9.22 c	217.44 ± 20.20 c	0.145 bc	0.948 ab	0.139 a	12.63 ab
400Pb	235.25 ± 28.49 b	86.57 ± 11.03 b	321.82 ± 37.04 b	0.369 ab	0.588 cd	0.216 a	26.91 a
800Pb	396.60 ± 30.82 a	122.94 ± 29.11 a	519.54 ± 24.93 a	0.314 abc	0.496 cd	0.154 a	23.64 a
F-value	160.283	42.741	228.324	3.826	13.824	1.563	8.710
LSD 95%	39.301	26.554	43.935	0.241	0.270	0.119	11.135

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

ND = Not detected

Nevertheless, in terms of BTF, no significant difference (*F=1.90; d.f.=17; p>0.05*) was observed in all spiked Cd treatments. However, 50Pb (0.739), 100Pb (1.207) and 200Pb (0.948) treatments documented significantly higher (*F=13.82; d.f.=17; p<0.05*) values compared to the control. Metal uptake efficacy (%) was used to assess the total efficiency and potential of Cd and Pb uptake in Vetiver grass from the soil to its tillers. A significantly greater (*F=16.17; d.f.=17; p<0.05*) percentage of Cd efficacy was recorded in the 10Cd, 50Cd, 100Cd and 150Cd treatments, whilst only 100Pb (9.45%) treatment showed a significantly lower (*F=8.71; d.f.=17; p<0.05*) percentage of Pb efficacy compared to the control. The overall findings of TF and BAC < 1, whilst BTF > 1, demonstrate that the translocation of both Cd and Pb were more favourably accumulated in the roots than the tillers in Vetiver grass. Besides, the noticeably higher Pb accumulation in the roots than the tillers of Vetiver grass could have caused the tolerably lower percentage of Pb efficacy among all spiked Pb treatments. Vetiver grass can be regarded as a suitable phyto-stabilizer for both Cd and Pb, owing to the BTF >1 value and its considerable ability to immobilize heavy metals in the soil [14, 35-36].

Vetiver grass showed a strong and significantly positive relationship with the levels of spiked concentrations for both Cd (*r=0.975*) and Pb (*r=0.952*) accumulations (Table 7). The slopes indicated that with each application of 1.0 mg/kg (dry weight basis) level of spiked Cd and Pb concentrations in soil, an approximately 1.181 mg/kg (Cd) and 0.617mg/kg (Pb) will be accumulated in Vetiver grass, respectively. However, significantly negative correlation relationships were exhibited between the dry matter content with Cd accumulations (*r=0.491*) and the levels of spiked Cd concentrations (*r=0.508*) in Vetiver grass. Dry matter content was negatively correlated due to the increased level of spiked Cd concentrations in the soil which affected higher accumulation of Cd in Vetiver grass.

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 spiked concentrations and Cd accumulation			
$Y_{Cd} = 6.059 + 1.181X_1$	0.97507	0.98746	586.795**
Relationship between level of spiked concentrations and Pb accumulation			
$Y_{Pb} = 49.690 + 0.617X_1$	0.95158	0.97549	294.792**
Relationship between dry matter contents and Cd accumulation			
$Y_{Cd} = 14.509 - 0.026X_2$	0.49136	0.70097	14.490*
Relationship between dry matter contents and Pb accumulation			
$Y_{Pb} = 380.678 -10.368X_2$	0.00858	0.09262	0.130
Relationship between level of spiked concentrations and dry matter contents			
$Y_{Cd} = 14.509 - 0.026X_1$	0.50833	0.71297	15.508*
$Y_{Pb}= 15.690 - 0.0002X_1$	0.00113	0.09262	0.130

X_1 = Level of spiked concentrations; X_2 = Dry matter contents

* Significant at 0.01 level of probability ** Significant at < 0.001 level of probability

4. Conclusions

The inclination trend of heavy metals accumulation for both Cd and Pb in Vetiver grass were in the order of 150Cd > 100Cd > 50Cd > 10Cd > control and 800Pb > 400Pb > 200Pb > 100Pb > 50Pb > control, respectively. The accumulation of both Cd and Pb in the roots and tillers of Vetiver grass increased when higher levels of spiked heavy metals concentrations were applied into the soil. Vetiver grass can thus be suggested to be an effective Cd and Pb phyto-stabilizer, owing to the considerably high heavy metals accumulation in its roots. However, it is not suitable for phyto-remediation if the level of contamination exceeded the threshold amount of 100 mg/kg Cd (dry weight basis) as it would inhibit overall plant growth. Nonetheless, in terms of Pb accumulation, Vetiver grass could be used as a suitable phyto-remediator as its phyto-tolerance and threshold is expected to be higher than 800 mg/kg (dry weight basis). These findings can be further extended with the increase application of higher spiked concentrations of Pb as well as covering a wider range of highly hazardous heavy metals such as aluminium (Al), arsenic (As) and mercury (Hg).

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