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ORIGINAL ARTICLE

Evaluation of Vetiver Grass Uptake Efficiency in Single and Mixed Heavy Metal Contaminated Soil

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Abstract

Most phyto-remediation studies have been conducted merely on a single type of contaminant element without consideration of the influence of other co-existent contaminants. In this study, *Vetiveria zizanioides* (Linn.) Nash was evaluated in both single and mixed heavy metal (Cd, Pb, Cu and Zn) spiked 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 investigated in detail. The relative growth performance, metal tolerance and phyto-assessment of heavy metal in roots and tillers of Vetiver grass were assessed. Metals in plants were measured using the flame atomic absorption spectrometry (F-AAS) after acid digestion. The root-tiller (R/T) ratio, tolerance index (TI), translocation factor (TF), biological concentration factor (BCF), biological accumulation coefficient (BAC) and metal uptake efficacy were estimated to examine the ability of metal accumulation and translocation in Vetiver grass. No significant difference ($p > 0.05$) of plant height was observed among all single and mixed heavy metal spiked soils compared with the control. However, significantly higher ($p < 0.05$) heavy metal (Cd, Pb, Cu and Zn) accumulations were found in roots, tillers and overall total accumulation of the individual spiked metal as compared with other treatments. Vetiver grass grown in the mixed Cd + Pb + Cu + Zn spiked soils accumulated the highest Zn (3322 ± 21.6 mg/kg) followed by Cu (430 ± 11.4 mg/kg), Pb (197 ± 13.5 mg/kg) and Cd (100 ± 0.7 mg/kg). Vetiver grass grown in mixed Cd + Pb, Cu + Zn and Cd + Pb + Cu + Zn spiked soils accumulated higher heavy metal concentrations than from the single spiked soil with the following order of metals: Zn > > Cu > Pb > Cd. Moreover, lower roots and lower tillers of Vetiver grass revealed a strong tendency for greater uptake and accumulation of all four heavy metals in both single and/or mixed spiked contaminated soils.

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

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1 Introduction

Soil contamination has received global environmental attention as a result of its adverse effects on both human health and the environment (Doran 2002; Azam 2016; Gómez-Sagasti et al. 2016). Soil often becomes contaminated typically due to the past and present emissions from rapidly expanding industrial activities, agricultural chemical runoff and improper disposal of wastes (Waller 1982; Meuser 2010; Van der Perk 2013). The common sources of soil contaminants may include both organic (halogenated volatiles, non-halogenated volatiles, pesticides, dioxin, furan, poly-chlorinated biphenyl and cyanides) and inorganic (volatile metals, non-volatile metals and radioactive materials) components (Harris et al. 1995; Ali and Khan 2017). Among the various types of soil contamination, heavy metal contaminants have become concerns as heavy metals are freely available in soil materials (environment) and are highly hazardous to human health even in trace amounts (Storelli 2008; Martin and Griswold 2009; Clemens and Ma 2016; Ali et al. 2019). Generally, the term heavy metal is widely accepted to describe a group of naturally occurring metals in the periodic table which have an elemental density > 5 g/cm^3 and atomic number > 20, which are often persistent in environmental bodies over a long duration and are mostly lethal (Gomes 2012; Kabata-Pendias 2010; Ali and Khan 2018a, b).

Heavy metals such as copper (Cu), zinc (Zn), iron (Fe), nickel (Ni) and manganese (Mn) are essential soil micronutrients required by living organisms in trace amounts for biological metabolic processes (Pilbeam and Barker 2007). Nevertheless, non-essential heavy metals like cadmium (Cd), lead (Pb), chromium (Cr), arsenic (As) and mercury (Hg) are predominantly hazardous, and are not needed for the growth of living organisms. Naturally occurring heavy metals are usually untraceable, non-biodegradable and can easily bio-accumulate and affect human health through the food chain (Bradl 2005; Kamal et al. 2016; Ali and Khan 2018a, b). Among all the different types of heavy metals, Cd, Pb, Cu and Zn are the few commonly available metal found in the soil (Brümmer 1986; Wuana and Okieimen 2011; Alloway 2013). Soil contaminated by heavy metals may severely contribute to the inhibition of growth and reduced metabolic activities in plants over time (Antonovics et al. 1971; Nagajyoti et al. 2010).

As a consequence, soil remediation techniques (physical, chemical and biological remediation) for heavy metal contamination have been developed over the years (Garbisu and Alkorta 2003; Hasegawa et al. 2016). Nonetheless, phyto-remediation has successfully developed to be one of the most preferred techniques as a result of its simple, cost-effective and environmentally friendly approach (Ali et al. 2013; Mahar et al. 2016). Correspondingly, Vetiver grass, *Vetiveria zizanioides* (Linn.) Nash has been carefully selected among various types of plants based on the earlier research studies (Chen et al. 2004; Ng et al. 2017, 2018) to be the most favourable species due to its fast growing, deep and extensive root system, high tolerance towards environmental stress, and its ability to withstand extreme concentrations of a wide range of contaminant heavy metals (Danh et al. 2009; Truong and Danh 2015; Gnansounou et al. 2017; Raman and Gnansounou 2018; Darajeh et al. 2019; Ng et al. 2019). However, even though there is a growing interest on phyto-remediation of single and mixed heavy metal contaminated soils with Vetiver grass, it remains poorly studied and requires urgent elucidation. Over the years, little evidence has been made available on studies with mixed heavy metal contamination (Khalil et al. 1996; Peralta-Videa et al. 2002; Stolpe and Müller 2016; Yang et al. 2016; Ghadiri et al. 2018). Previous studies have solely emphasized on the limited types of heavy metals and inadequately explained phyto-assessment in the different plant parts.

Therefore, the objectives of this study were to evaluate the growth performance, accumulation trend and efficiency of metal uptake from single and mixed Cd, Pb, Cu and Zn spiked contaminated soils as well as their bioaccumulation in both the lower and upper roots and tillers of Vetiver grass.

2 Materials and Methods

2.1 Site Descriptions and Experimental Design

A pot experimental study was conducted in the greenhouse situated at the Rimba Ilmu, Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur. Vetiver grass *Vetiveria zizanioides* (Linn.) Nash was selected for this experiment. Treatments included eight different types of single and mixed heavy metal spiked conditions (Table 1). All of the treatments were conducted under a completely randomized design (CRD) with three replications ($n = 3$).

2.2 Soil Sampling and Sample Preparation

Top soil (0–20 cm) was collected from a field located in the University of Malaya, Kuala Lumpur situated at the 3° 7' N latitude and 101° 39′ E longitude. The preliminary physico-chemical soil characterization (Table 2) was conducted before soils were air-dried for a week followed with passing through <4 mm sieve to remove gravel and large non-soil objects. The dull reddish brown soil consists of 89.4% sand, 8.3% silt and 2.3% clay. Vetiver grass saplings were purchased from Humibox Malaysia and each fresh plant sapling with a uniform height (20–25 cm) was selected for this study. Each plant was grown in a plastic pot (0.18 m diameter × 0.16 m depth) filled with two kilograms of soil, for all the treatments. All plants were watered evenly with 50 mL of tap water using a glass beaker once a day and their plant growth performance such as height, tiller number and percentage plant survivorship were continuously observed throughout the entire 60-day of the experiment.

The single and mixed heavy metal spiked treatments were prepared using cadmium nitrate tetrahydrate [$Cd(NO_3)_2.4H_2O$], lead (II) nitrate[$Pb(NO_3)_2$], copper (II) sulfate [$CuSO_4$] and zinc sulfate heptahydrate [$ZnSO_4.7H_2O$] salt compounds. The amended soil was then continuously stirred and incubated for two weeks to ensure the homogeneity of the desired single and mixed heavy metal treatments. The concentrations of both single and mixed heavy metal spiked treatments were determined based on the range of heavy metal concentrations exceeding the median permissible in the natural occurring levels by the Department of Environment,

Table 1 Design of treatment variables

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

Table 2 Soil physico-chemical properties

Parameter (Unit)		Mean
Metal contents (mg/kg)		
	Cd	0.87 ± 0.08
	Pb	26.95 ± 1.24
	Cu	7.48 ± 2.35
	Zn	52.51 ± 11.64
Soil texture		
Sand (%)		89.42
	Very coarse sand (%)	4.56
	Coarse sand (%)	39.15
	Medium coarse sand (%)	30.68
	Fine sand (%)	11.55
	Very fine sand (%)	3.48
Silt (%)		8.27
Clay (%)		2.31
Temperature (°C)		32.6 ± 1.2
pH		5.84 ± 0.92
Colour (Munsell colour charts)	Dull reddish brown	2.5YR 5/4
Water content (%)		6.29 ± 1.28
Field capacity (%)		35.16 ± 4.82
Saturation level (%)	Dry	17.89
Bulk density (g/cm^3)		1.96 ± 0.35
Porosity (%)		26.04 ± 3.14

Mean ± standard deviation

Malaysia (DOE 2009), Canadian Council of Ministers of Environment (CCME 1999) and European Union (Lado et al. 2008) soil contamination guidelines.

2.3 Soil and Plant Sample Analyses

At the end of the 60-day of experiment, all freshly harvested plants were brought into the laboratory and washed in running filter water, followed by deionized water to remove any adhering soil particles before separating the plants into four different parts (lower and upper sections of roots and tillers) (Fig. 1). All plant samples were oven-dried for 72 h until obtaining a constant dry weight. The dry matter content (g/m^2) of the plant samples was determined before homogenizing using a mortar and pestle. Approximately, 0.5 g of the homogenized dried samples underwent acid digestion with hydrochloric acid (HCl), nitric acid (HNO_3) and hydrogen peroxide (H_2O_2) according to Method 3050B (US EPA 1996) followed by Method 7000B (US EPA 2007) for the total recoverable elemental analysis using the Perkin-Elmer AAnalyst 400 flame atomic absorption spectrometer (F-AAS). The instrument's limit of detection was less than 0.01 mg/L for Cd and 0.1 mg/L for Pb, Cu and Zn. All chemicals used were of analytical reagent standard or of the best grade available. Similarly, soil samples were air-dried for 72 h until reaching a constant weight before analysis following the analytical procedures. The highly precise technique of chemical analysis was controlled using the Bundesanstalt für Materialforschung und –prüfung (BAM Germany): German Federal Institute for Materials Research and Testing (BRM#12-mixed sandy soil) certified reference material with an average rate of metal recovery for Cd (102.7%), Pb (98.4%), Cu (93.2%) and Zn (105.9%), respectively.

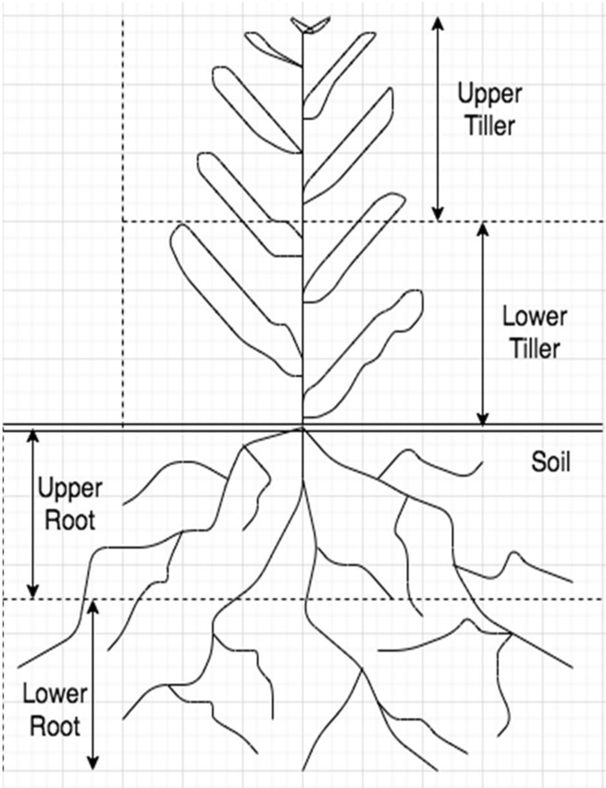

Fig. 1 Plant cross-section between the roots and shoots (tillers) of Vetiver grass

2.4 Statistical Analysis and Data Processing

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

R/T ratio:	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:	Concentration of heavy metals in tillers / Concentration of heavy metals in roots
BCF:	Concentration of heavy metals in roots / Concentration of heavy metals in soil
BAC:	Concentration of heavy metals in tillers / Concentration of heavy metals in soil
Metal uptake efficacy (%):	[Concentration of heavy metals in tillers / Total concentration of heavy metals accumulated in Vetiver grass] × 100

Both commonly used terminologies i.e., accumulation and concentration of heavy metals, are inter-connected and related to each other. The accumulation emphasizes on the outcome of heavy metal accumulated (found) whereby the concentration explains more precisely the amount and quantity of heavy metal (mg/kg) obtained (accumulated) in the soil and/or plant section, respectively.

All experimental data were analysed by performing the one–way analysis of variance (ANOVA) and further statistical validity test for significant differences among treatment was conducted by employing the Fisher's least significant difference (LSD) tests at the 95% level of confidence with the aid of Microsoft Excel Office 365 versions 2016 software.

3 Results

3.1 Responses of Plant Growth

Soil pH was not significantly affected ($p > 0.05$) by the single and mixed spiked heavy metals in all Vetiver treatments (Fig. 2). During the 60-day of the experimental period, all Vetiver treatments recorded fluctuations in the soil pH between initial reading of 4.26–4.95 and final reading of 4.17–5.74. The control treatment recorded the highest pH of 5.74 while the lowest pH of 4.17 was observed in the Cd + Pb treatment. The results obtained for both single and mixed spiked heavy metals did not considerably influence the overall soil pH changes in all treatments.

The relative growth of Vetiver grass in terms of plant height, tiller number and percentage survivorship varied among different types of single and mixed heavy metal spiked treatments (Table 3). There were no significant differences ($p > 0.05$) in the plant height observed among all single and mixed heavy metal spiked treatments compared with the control. Nevertheless, all of the single and mixed heavy metal spiked treatments recorded relatively lower plant height (45.68–68.48 cm) compared to the control (76.88 cm).

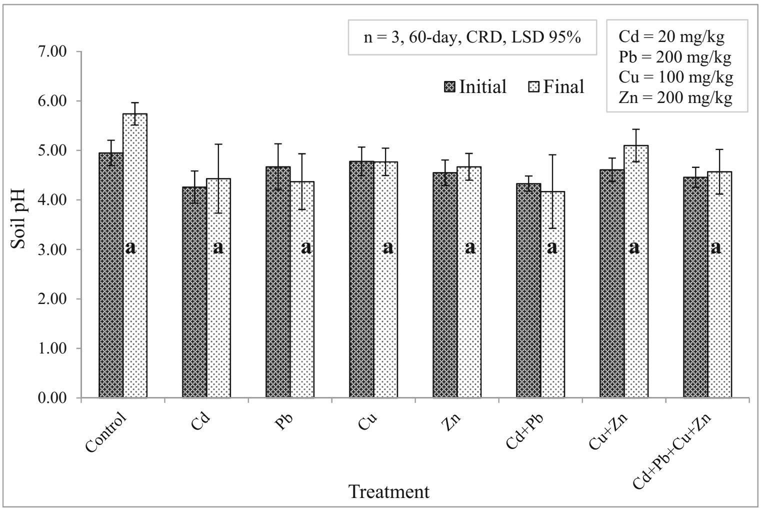

Fig. 2 Changes in soil pH of Vetiver grass in single and mixed heavy metal spiked treatments. Vertical bars represent standard deviation and common letters are not significantly different at the 95% level of confidence by LSD

Table 3 Plant height (cm), tiller number and plant survivorship (%) of Vetiver grass in single and mixed heavy metal spiked treatments

Treatment	Plant height (cm)	Tiller number	Plant survivorship (%)
Control	76.88 ± 12.07 a	26.6 ± 5.5 a	100.00 ± 0.00 a
Cd	60.16 ± 8.40 a	16.8 ± 8.4 b	97.33 ± 14.21 a
Pb	68.48 ± 20.83 a	21.6 ± 11.4 ab	100.00 ± 0.00 a
Cu	52.00 ± 14.95 a	18.0 ± 8.9 b	81.94 ± 5.72 ab
Zn	49.88 ± 11.16 a	17.4 ± 9.3 b	78.67 ± 13.66 b
Cd + Pb	64.48 ± 9.05 a	19.6 ± 7.3 ab	87.33 ± 10.09 ab
Cu + Zn	47.14 ± 22.39 a	17.2 ± 5.4 b	77.34 ± 23.45 b
Cd + Pb + Cu + Zn	45.68 ± 17.73 a	12.2 ± 7.7 b	58.67 ± 19.46 b

Mean ± standard deviation and common letters are not significantly different at the 95% level of confidence using LSD

In contrast, the Cd, Cu, Zn, Zn + Cu and Cd + Pb + Cu + Zn spiked treatments showed significantly lower ($p < 0.05$) tiller number compared to the control. The control recorded the highest tiller number of 26.6 while the lowest tiller number of 12.2 was observed in the Cd + Pb + Cu + Zn treatment. Similarly, with regard to plant survivorship, the Zn, Cu + Zn and Cd + Pb + Cu + Zn spiked treatments demonstrated significantly decreased ($p < 0.05$) percentage of survival compared with the control. Among all spiked treatments, both Cu + Zn (77.34%) and Cd + Pb + Cu + Zn (58.67%) mixed heavy metal treatments recorded the lowest percentage of survivorship.

The single Cu and Zn spiked treatments as well as the mixed Cu + Zn and Cd + Pb + Cu + Zn spiked treatments exhibited significantly lower ($p < 0.05$) dry matter contents in both roots and tillers compared to the control (Table 4). All spiked treatments, with the exception of Pb treatment, showed significantly lower ($p < 0.05$) total dry matter content compared with the control. Between spiked treatments, both single Cd (15.50 ± 1.22 g/m^2) and Pb (17.14 ± 0.69 g/m^2) spiked treatments recorded reasonably higher total dry matter content than the other mixed heavy metal treatments.

Both root-tiller (R/T) ratio and tolerance index (TI) were employed to evaluate the tolerance ability of Vetiver grass growing under various single and mixed heavy metal spiked treatments.

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

Treatment	Dry matter content (g/m^2)				
	Vetiver			R/T ratio	TI
	Root	Tiller	Total		
Control	8.01 ± 1.37 a	11.17 ± 2.87 a	19.18 ± 3.01 a	0.751 a	
Cd	7.00 ± 0.22 abc	8.51 ± 1.21 ab	15.50 ± 1.22 b	0.833 a	0.817 ab
Pb	7.48 ± 0.90 ab	9.66 ± 1.18 a	17.14 ± 0.69 ab	0.790 a	0.914 a
Cu	5.77 ± 0.60 bc	5.95 ± 1.61 bc	11.72 ± 1.56 c	1.034 a	0.625 bcd
Zn	5.27 ± 1.01 c	4.85 ± 1.54 c	10.12 ± 2.35 c	1.136 a	0.546 bcd
Cd + Pb	6.82 ± 0.76 abc	8.36 ± 0.61 ab	15.19 ± 1.30 b	0.815 a	0.803 abc
Cu + Zn	5.26 ± 0.88 c	4.37 ± 1.13 c	9.63 ± 1.68 c	1.241 a	0.520 cd
Cd + Pb + Cu + Zn	5.24 ± 1.65 c	4.34 ± 0.97 c	9.58 ± 0.70 c	1.321 a	0.506 d

Mean ± standard deviation and common letters are not significantly different at the 95% level of confidence by LSD

In terms of R/T ratio, no significant difference ($p > 0.05$) was observed among all treatments. Nonetheless, among all the treatments, the single Pb spiked treatment showed the highest TI value of 0.914 while the lowest TI was recorded in the Cd + Pb + Cu + Zn spiked treatment.

3.2 Heavy Metal Uptake in Plant

Tables 5 to 8 show the concentration of Cd, Pb, Cu and Zn accumulation in roots, tillers and their total for Vetiver grass in all single and mixed heavy metal spiked treatments. The accumulation of all four heavy metals in the lower and upper parts of the roots and tillers was comparatively variable. In terms of Cd (Table 5), all of the Cd, Cd + Pb and Cd + Pb + Cu + Zn spiked treatments showed significantly greater ($p < 0.05$) Cd in both lower and upper roots and tillers of Vetiver grass compared to the control. Similarly, the Cd, Cd + Pb and Cd + Pb + Cu + Zn spiked treatments recorded significantly larger accumulation of Cd ($p < 0.05$) in the total roots, total tillers and overall total among all other treatments. Unlike other types of heavy metals (Pb, Cu and Zn), the highest accumulation of Cd were recorded in the lower tillers for Cd + Pb + Cu + Zn (62.53 ± 5.97 mg/kg) and Cd + Pb (58.33 ± 10.06 mg/kg) spiked treatments. Between roots and tillers, Cd accumulation was considerably greater in roots than in tillers. The accumulation of Cd was relatively higher in the lower roots and lower tillers for Cd, Cd + Pb and Cd + Pb + Cu + Zn spiked treatments compared with the upper plant parts, respectively. Nevertheless, the accumulation of Cd among various single and mixed Cd spiked treatments was in the order of Cd + Pb + Cu + Zn > Cd + Pb > Cd > > other spiked treatments.

With regard to Pb accumulation (Table 6), the Pb, Cd + Pb and Cd + Pb + Cu + Zn spiked treatments exhibited significantly higher ($p < 0.05$) Pb in both the lower and upper roots and tillers of Vetiver grass compared to the control. A significantly greater ($p < 0.05$) Pb accumulation was demonstrated in the total root, total tiller and overall total accumulation for Pb, Cd + Pb and Cd + Pb + Cu + Zn spiked treatments. The lower roots of Cd + Pb + Cu + Zn (177.67 ± 20.01 mg/kg) and Cd + Pb (141.83 ± 9.99 mg/kg) spiked treatments recorded the highest accumulation of Pb among all the treatments.

Between roots and tillers, an appreciably higher accumulation of Pb was found in roots than in the tillers for all treatments. The accumulation of Pb was noticeably greater in the lower roots and lower tillers for both Cd + Pb and Cd + Pb + Cu + Zn spiked treatments compared with the upper plant parts, respectively whilst the vice versa trend was observed for Pb spiked treatment. However, among all single and mixed Pb spiked treatments, the accumulation trend for Pb was in the following order: Cd + Pb + Cu + Zn > Cd + Pb > Pb > > other spiked treatments.

The Cu, Cu + Zn and Cd + Pb + Cu + Zn spiked treatments recorded significantly higher ($p < 0.05$) accumulation of Cu in both the lower and upper roots and tillers of Vetiver grass compared to the control (Table 7). Similarly, the total root, total tiller and overall total accumulation for Cu, Cu + Zn and Cd + Pb + Cu + Zn spiked treatments exhibited significantly greater ($p < 0.05$) Cu than all other treatments. The lower roots (365.64 ± 27.00 mg/kg) and upper roots (308.03 ± 10.74 mg/kg) for Cd + Pb + Cu + Zn spiked treatment recorded the highest accumulation of Cu among all the treatments. Between roots and tillers, the accumulation of Cu was substantially higher in roots than in tillers. The lower roots and lower tillers for Cu, Cu + Zn and Cd + Pb + Cu + Zn spiked treatments accumulated reasonably higher Cu compared with the upper plant parts, respectively. The accumulation trend for Cu among all single and mixed Cu spiked treatments was in the order: Cd + Pb + Cu + Zn > Cu + Zn > Cu > > other spiked treatments.

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

Treatment	Concentration of Cd (mg/kg)						
	Root			Tiller			Overall total
	Lower	Upper	Total	Lower	Upper	Total	
Control	0.52 ± 0.20 c	0.37 ± 0.25 c	0.44 ± 0.23 d	0.49 ± 0.17 c	0.70 ± 0.16 cd	0.60 ± 0.16 c	1.04 ± 0.38 d
Cd	45.87 ± 9.33 b	48.37 ± 7.59 a	47.12 ± 0.88 b	50.27 ± 12.96 b	ND (<0.01)	25.14 ± 6.48 b	72.25 ± 5.62 c
Pb	1.17 ± 0.14 c	0.46 ± 0.13 c	0.82 ± 0.12 d	0.31 ± 0.20 c	0.29 ± 0.09 cd	0.30 ± 0.15 c	1.11 ± 0.27 d
Cu	0.65 ± 0.21 c	ND (<0.01)	0.33 ± 0.11 d	0.40 ± 0.09 c	0.23 ± 0.18 cd	0.32 ± 0.05 c	0.65 ± 0.07 d
Zn	ND (<0.01)	0.50 ± 0.25 c	0.25 ± 0.13 d	0.42 ± 0.15 c	0.55 ± 0.15 cd	0.49 ± 0.15 c	0.74 ± 0.28 d
Cd + Pb	52.17 ± 6.07 ab	37.47 ± 7.91 b	44.82 ± 1.08 c	58.33 ± 10.06 ab	30.43 ± 2.75 a	44.39 ± 4.13 a	89.20 ± 3.64 b
Cu + Zn	0.57 ± 0.27 c	0.95 ± 0.17 c	0.76 ± 0.22 d	1.22 ± 0.27 c	1.17 ± 0.40 c	1.95 ± 0.17 c	3.12 ± 0.14 d
Cd + Pb + Cu + Zn	58.10 ± 1.56 a	55.60 ± 2.12 a	56.85 ± 1.84 a	62.53 ± 5.97 a	23.60 ± 4.06 b	43.07 ± 1.13 a	99.92 ± 0.71 a

Mean ± standard deviation and common letters are not significantly different at the 95% level of confidence by LSD; ND = Not detected

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

Treatment	Concentration of Pb (mg/kg)						
	Root			Tiller			Overall total
	Lower	Upper	Total	Lower	Upper	Total	
Control	8.23 ± 0.85 d	10.53 ± 1.00 d	9.39 ± 0.91 d	0.69 ± 0.20 c	ND (<0.1)	0.35 ± 0.10 c	9.73 ± 1.01 d
Cd	9.67 ± 1.72 d	10.33 ± 1.06 d	10.00 ± 1.22 d	0.63 ± 0.18 c	ND (<0.1)	0.32 ± 0.09 c	10.32 ± 1.27 d
Pb	81.67 ± 7.67 c	83.27 ± 8.94 c	82.47 ± 8.17 c	26.83 ± 2.61 b	29.70 ± 6.32 a	28.27 ± 1.87 b	110.74 ± 9.92 c
Cu	10.89 ± 1.32 d	5.05 ± 0.50 d	7.97 ± 0.81 d	0.50 ± 0.18 c	ND (<0.1)	0.25 ± 0.09 c	8.22 ± 0.73 d
Zn	1.11 ± 0.27 d	9.40 ± 1.10 d	5.26 ± 0.42 d	1.29 ± 0.54 c	ND (<0.1)	0.65 ± 0.27 c	5.90 ± 0.21 d
Cd + Pb	141.83 ± 9.99 b	121.33 ± 18.16 b	131.59 ± 5.16 b	55.67 ± 7.31 a	20.29 ± 2.56 b	37.98 ± 4.65 a	169.56 ± 5.10 b
Cu + Zn	3.79 ± 0.37 d	6.50 ± 0.40 d	5.15 ± 0.06 d	3.14 ± 0.45 c	ND (<0.1)	1.57 ± 0.23 c	6.72 ± 0.25 d
Cd + Pb + Cu + Zn	177.67 ± 20.01 a	138.67 ± 12.53 a	158.17 ± 3.85 a	57.20 ± 13.00 a	20.10 ± 6.58 b	38.65 ± 9.73 a	196.82 ± 13.53 a

Mean ± standard deviation and common letters are not significantly different at the 95% level of confidence by LSD; ND = Not detected

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

Treatment	Concentration of Cu (mg/kg)						
	Root			Tiller			Overall total
	Lower	Upper	Total	Lower	Upper	Total	
Control	11.40 ± 4.69 d	7.56 ± 1.83 d	9.48 ± 1.47 c	2.76 ± 0.85 d	ND (<0.1)	1.38 ± 0.43 d	10.86 ± 1.90 d
Cd	10.97 ± 1.94 d	5.73 ± 2.49 d	8.35 ± 2.07 c	1.92 ± 1.01 d	ND (<0.1)	0.96 ± 0.51 d	9.31 ± 2.54 d
Pb	12.78 ± 4.01 d	8.16 ± 0.77 d	10.47 ± 1.64 c	5.80 ± 1.43 d	3.72 ± 1.43 c	4.76 ± 1.22 d	14.90 ± 2.65 d
Cu	178.77 ± 17.42 c	226.00 ± 18.34 c	202.39 ± 2.99 b	83.07 ± 5.39 c	58.60 ± 14.93 ab	70.84 ± 10.13 c	273.22 ± 10.52 c
Zn	0.80 ± 0.57 d	6.20 ± 0.95 d	3.50 ± 0.76 c	2.95 ± 0.60 d	5.15 ± 0.89 c	4.05 ± 0.18 d	7.55 ± 0.93 d
Cd + Pb	5.03 ± 1.36 d	4.42 ± 1.40 d	4.73 ± 0.10 c	2.34 ± 0.78 d	2.13 ± 1.86 c	2.24 ± 0.64 d	6.96 ± 0.69 d
Cu + Zn	227.67 ± 31.41 b	276.99 ± 13.56 b	252.33 ± 22.31 a	110.80 ± 18.92 b	63.47 ± 11.36 a	87.14 ± 4.36 b	339.47 ± 26.59 b
Cd + Pb + Cu + Zn	365.64 ± 27.00 a	308.03 ± 10.74 a	336.84 ± 9.86 a	136.07 ± 9.06 a	49.27 ± 6.51 b	92.67 ± 1.53 a	429.51 ± 11.39 a

Mean ± standard deviation and common letters are not significantly different at the 95% level of confidence by LSD; ND = Not detected

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

Treatment	Concentration of Zn (mg/kg)						
	Root			Tiller			Overall total
	Lower	Upper	Total	Lower	Upper	Total	
Control	315.67 ± 34.26 c	184.67 ± 8.49 d	250.17 ± 20.80 d	49.23 ± 11.54 d	42.98 ± 2.49 d	46.11 ± 5.21 c	296.27 ± 26.00 c
Cd	148.47 ± 13.83 c	110.47 ± 10.49 de	129.47 ± 1.68 d	56.70 ± 4.09 d	23.36 ± 6.62 d	40.03 ± 5.10 c	169.50 ± 6.78 cd
Pb	245.03 ± 41.41 c	181.77 ± 16.73 d	213.41 ± 20.45 d	46.73 ± 9.96 d	38.60 ± 10.45 d	42.67 ± 9.27 c	256.07 ± 29.64 cd
Cu	209.53 ± 27.79 c	83.90 ± 8.54 e	146.72 ± 18.17 d	117.07 ± 15.66 d	33.87 ± 9.20 d	75.47 ± 11.33 c	222.19 ± 29.07 cd
Zn	1945.53 ± 144.65 b	1173.27 ± 96.01 c	1559.40 ± 115.79 c	1342.17 ± 130.21 c	1449.40 ± 153.58 a	1395.79 ± 140.46 ab	2955.19 ± 51.53 b
Cd + Pb	200.33 ± 20.48 c	38.87 ± 9.25 e	119.60 ± 6.90 d	56.17 ± 5.97 d	18.60 ± 1.47 d	37.39 ± 3.72 c	156.99 ± 8.46 d
Cu + Zn	2188.00 ± 167.78 a	1276.23 ± 66.60 b	1732.12 ± 115.66 b	1921.90 ± 130.97 a	1060.77 ± 73.63 b	1491.34 ± 65.10 a	3223.45 ± 176.54 a
Cd + Pb + Cu + Zn	2191.33 ± 145.06 a	1849.90 ± 77.04 a	2020.62 ± 108.01 a	1703.13 ± 170.40 b	897.27 ± 48.98 c	1300.20 ± 109.45 b	3322.49 ± 21.64 a

Mean ± standard deviation and common letters are not significantly different at the 95% level of confidence by LSD

Similarly, in terms of Zn accumulation (Table 8), a significantly higher ($p < 0.05$) accumulation was found in both the lower and upper roots and tillers of Vetiver grass for Zn, Cu + Zn and Cd + Pb + Cu + Zn spiked treatments compared to the control. A significantly greater ($p < 0.05$) concentration of Zn was observed in the total roots, total tillers and overall total accumulation for Zn, Cu + Zn and Cd + Pb + Cu + Zn spiked treatments than all other treatments. The lower roots of Cd + Pb + Cu + Zn (2191.33 ± 145.06 mg/kg) and Cu + Zn (2188.00 ± 167.78 mg/kg) recorded the highest accumulation of Zn among all the treatments. Between roots and tillers, the Zn accumulation was noticeably greater in roots than in tillers. A considerably higher accumulation of Zn was recorded in the lower roots and lower tillers for Zn, Cu + Zn and Cd + Pb + Cu + Zn spiked treatments compared with their upper plant parts, respectively. Among all single and mixed Zn spiked treatments, the accumulation of Zn was in the order of Cd + Pb + Cu + Zn > Cu + Zn > Zn > > other spiked treatments.

Based on the results obtained, the mixed Cd + Pb + Cu + Zn spiked treatment accumulated the highest overall total amount of Zn (3322.49 ± 21.64 mg/kg) followed by Cu (429.51 ± 11.39 mg/kg), Pb (196.82 ± 13.53 mg/kg) and Cd (99.92 ± 0.71 mg/kg). The general trends of heavy metal accumulation for all treatments were in the order of Zn > > Cu > Pb > Cd regardless of the total amount of spiked heavy metals in the soil. On the other hand, between single and mixed spiked treatments, the accumulation for mixed Cd + Pb, Cu + Zn and Cd + Pb + Cu + Zn spiked treatments recorded remarkably higher accumulation compared to all of the single spiked treatments.

3.3 Heavy Metal Translocation

For all single and mixed spiked heavy metals biological concentration factors (BCF) as well as the biological accumulation coefficients (BAC), the translocation factors (TF) and the metal uptake efficacy (%) are presented in Tables 9, 10, 11, 12 and 13.

Relatively higher BCF values were found in both lower and upper roots of all single and mixed Cd (1.873 – 2.905), Pb (0.408 – 0.888), Cu (1.788 – 3.656) and Zn (5.866 – 10.957) spiked treatments, respectively, compared with other treatments. Among all the treatments, the lower roots for mixed Cd + Pb + Cu + Zn spiked treatment exhibited the highest BCF value. Considering the BCF values >1 for Cd, Cu and Zn accumulation, all single and mixed spiked

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 spiked metal treatments

Treatment	BCF (Root)							
	Cd accumulation		Pb accumulation		Cu accumulation		Zn accumulation	
	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper
Control	0.594 d	0.421 de	0.306 d	0.391 bc	1.524 c	1.011 c	6.012 c	3.517 c
Cd	2.293 b	2.418 a	0.359 cd	0.383 bc	1.467 c	0.766 cd	2.827 e	2.104 d
Pb	1.341 c	0.529 d	0.408 c	0.416 b	1.709 bc	1.091 c	4.666 d	3.462 c
Cu	0.747 d	0.011 e	0.404 cd	0.188 d	1.788 bc	2.260 b	3.990 de	1.598 d
Zn	0.011 e	0.579 d	0.041 f	0.349 bc	0.107 d	0.829 cd	9.728 b	5.866 b
Cd + Pb	2.608 ab	1.873 b	0.709 b	0.607 a	0.673 d	0.591 d	3.815 de	0.740 e
Cu + Zn	0.651 d	1.088 c	0.141 e	0.241 d	2.277 b	2.770 a	10.940 a	6.381 b
Cd + Pb + Cu + Zn	2.905 a	2.780 a	0.888 a	0.693 a	3.656 a	3.080 a	10.957 a	9.250 a

Mean followed by common letters are not significantly different at the 95% level of confidence using LSD

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 spiked treatments

Treatment	Cd accumulation					
	BAC (Tiller)		TF (Tiller)		Efficacy (Tiller)	
	Lower	Upper	Lower	Upper	Lower	Upper
Control	0.567 d	0.808 cd	0.597 ab	0.878 a	23.994 bc	35.014 a
Cd	2.513 ab	0.001 f	0.535 b	0.0001 d	34.464 a	0.007 c
Pb	0.352 d	0.333 ef	0.179 c	0.175 cd	12.880 d	12.892 b
Cu	0.464 d	0.268 ef	0.625 ab	0.437 bc	31.198 ab	18.988 b
Zn	0.479 d	0.636 de	0.848 ab	1.153 a	28.427 ab	38.444 a
Cd + Pb	2.917 ab	1.522 a	0.652 ab	0.339 cd	32.601 a	17.091 b
Cu + Zn	1.406 c	1.341 b	0.885 a	0.809 ab	19.517 cd	18.783 b
Cd + Pb + Cu + Zn	3.127 a	1.180 bc	0.551 ab	0.207 cd	31.306 ab	11.803 bc

Mean followed by common letters are not significantly different at the 95% level of confidence using LSD

treatments accumulated appreciably higher metals in roots than tillers suggesting that the transfer of heavy metals from soils to roots was remarkably greater and roots acted as a sink for heavy metal accumulation.

The BAC, TF and metal efficacy were calculated to evaluate the capability and efficiency of heavy metal translocation from roots to tillers. Despite the relatively lower accumulation of all heavy metals in the tillers than in the roots, the BAC values >1 were recorded in both the lower and upper tillers for single and mixed Cd (1.180 – 3.127) as well as Zn (4.486 – 9.610) spiked treatments. Based on the appreciably high BAC values <1 in both lower and upper tillers for single and mixed Pb (0.101 – 0.286) and Cu (0.493 – 0.831) spiked treatments, it is deduced that the translocation pathway for heavy metal accumulation from roots to tillers may be inhibited.

Similarly, with regard to TF values <1, the tolerably lower accumulation in both lower and upper tillers than in roots for all four different types of heavy metals suggested that the movement of metals from the roots to tillers were hindered. Even

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 spiked treatments

Treatment	Pb accumulation					
	BAC (Tiller)		TF (Tiller)		Efficacy (Tiller)	
	Lower	Upper	Lower	Upper	Lower	Upper
Control	0.026 c	0.004 c	0.035 d	0.005 c	3.499 d	0.518 c
Cd	0.023 c	0.004 c	0.030 d	0.005 c	3.033 d	0.490 c
Pb	0.134 b	0.149 a	0.123 bc	0.133 a	12.252 bc	13.318 a
Cu	0.019 c	0.004 c	0.031 d	0.006 c	3.110 d	0.611 c
Zn	0.048 c	0.004 c	0.110 c	0.008 c	10.968 c	0.848 c
Cd + Pb	0.278 a	0.101 b	0.164 b	0.060 b	16.414 b	5.974 b
Cu + Zn	0.116 b	0.004 c	0.233 a	0.007 c	23.297 a	0.745 c
Cd + Pb + Cu + Zn	0.286 a	0.101 b	0.144 bc	0.050 b	14.430 bc	5.047 b

Mean followed by common letters are not significantly different at the 95% level of confidence using LSD

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 spiked treatments

Treatment	Cu accumulation					
	BAC (Tiller)		TF (Tiller)		Efficacy (Tiller)	
	Lower	Upper	Lower	Upper	Lower	Upper
Control	0.369 d	0.013 c	0.125 bc	0.005 d	12.524 bc	0.469 d
Cd	0.256 d	0.013 c	0.099 c	0.006 d	9.879 c	0.562 d
Pb	0.775 c	0.497 ab	0.190 ab	0.120 bc	18.979 ab	12.008 c
Cu	0.831 c	0.586 a	0.152 abc	0.107 bc	15.194 abc	10.667 c
Zn	0.394 d	0.689 a	0.200 a	0.340 a	20.012 a	33.989 a
Cd + Pb	0.312 d	0.285 b	0.171 ab	0.147 b	17.074 ab	21.515 b
Cu + Zn	1.108 b	0.635 a	0.162 abc	0.095 bc	16.246 abc	9.461 cd
Cd + Pb + Cu + Zn	1.361 a	0.493 ab	0.158 abc	0.057 cd	15.830 abc	5.749 cd

Mean followed by common letters are not significantly different at the 95% level of confidence using LSD

though the TF values were < 1, fairly higher TF values in both lower and upper tillers than the other treatments were found for the accumulation of Pb (0.050 – 0.164) and Zn (0.135 – 0.298).

The percentages of metal efficacy in both lower and upper tillers for Pb (5.047 – 16.414%) and Zn (13.509 – 29.800%) accumulation for single and mixed spiked treatments were relatively higher compared with the other treatments, respectively. Despite the considerably lower accumulation of Cd found in tillers compared to the Cu and Zn, the lower tillers for single (34.464%) and mixed (31.306 – 32.601%) Cd spiked treatments recorded the highest percentages of Cd efficacy. Between single and mixed spiked heavy metal treatments, the single spiked treatments for all four different types of heavy metal recorded a relatively higher percentage of metal efficacy compared to the mixed spiked treatments. Nonetheless, the percentages of metal efficacy were remarkably higher in the lower tiller compared to the upper tiller for all four different types of heavy metals.

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 spiked treatments

Treatment	Zn accumulation					
	BAC (Tiller)		TF (Tiller)		Efficacy (Tiller)	
	Lower	Upper	Lower	Upper	Lower	Upper
Control	0.938 e	0.818 d	0.082 e	0.073 d	8.240 e	7.300 d
Cd	1.080 e	0.445 d	0.167 d	0.068 d	16.714 d	6.849 d
Pb	0.890 e	0.735 d	0.091 e	0.075 d	9.060 e	7.463 d
Cu	2.229 d	0.645 d	0.263 b	0.076 d	26.338 b	7.588 d
Zn	6.711 c	7.247 a	0.227 c	0.245 a	22.701 c	24.506 a
Cd + Pb	1.070 e	0.354 d	0.179 d	0.059 d	17.880 d	5.926 d
Cu + Zn	9.610 a	5.304 b	0.298 a	0.165 b	29.800 a	16.487 b
Cd + Pb + Cu + Zn	8.516 b	4.486 c	0.256 b	0.135 c	25.643 b	13.509 c

Mean followed by common letters are not significantly different at the 95% level of confidence using LSD

4 Discussion

The findings of this study indicated that soil pH, plant heights and R/T ratios of Vetiver grass were not affected with single and mixed spiked heavy metal treatments. Nonetheless, the results obtained for tiller number, percentage survivorship and dry matter content in Vetiver grass sharply declined among single and mixed spiked treatments compared to the control.

In the present study, 54.1% and 41.3% reductions were observed in the mixed Cd + Pb + Cu + Zn spiked treatment compared to the control in terms of both tiller number and percentage survivorship, respectively. The significant decrease in tiller number and percentage survivorship in Vetiver grass could be accounted for as a result of the combination application of mixed heavy metal, as was suggested by Chiu et al. (2006). In addition, studies by An et al. (2004) with cucumber (*Cucumis sativus*) also recorded similar findings of lower dry matter contents. However, Huang et al. (2009) reported the opposite results for paddy rice plant (*Oryza sativa* L.) where single and mixed spiked heavy metals accumulations were applied.

In contrast to Pb, Cu and Zn accumulations, there was no accumulation of Cd found in the upper tiller for Cd spiked treatment. The highest accumulation of Cd was recorded in the lower tiller compared to root parts for all mixed spiked treatments, unlike other heavy metals. This trend was supported by earlier studies undertaken by Aibibu et al. (2010), Zhang et al. (2014), Christofilopoulos et al. (2016) and Phusantisampan et al. (2016) whereby generally, most of Cd were more likely to be accumulated in roots compared to tillers. These findings highlighted the fact that Vetiver grass could be a potential Cd phyto-stabilizer with regard to its high accumulation capability in both roots and tillers with BCF and BAC values >1 for single Cd as well as for mixed Cd + Pb and Cd + Pb + Cu + Zn spiked treatments.

Similarly, the high BCF values of >1 in both lower and upper roots demonstrated positive characteristics of phyto-stabilization for all heavy metal treatments. Generally, there are numerous categories of phyto-remediation technology depending on the different types of plants and levels of clean-up required (Padmavathiamma and Li 2007; Tangahu et al. 2011). Phyto-extraction refers to the bioaccumulation and translocation uptake of metal contaminants in the soil via roots into the above ground components (Nascimento et al. 2006; Sheoran et al. 2016). On the other hand, phyto-stabilization uses the plant to immobilize metal contaminants in soil through bioaccumulation and adsorption by roots within the root zones (Berti and Cunningham 2000; Mahar et al. 2016).

Over the past decades, there have been limited studies with emphasis on the comparison between single and mixed heavy metal accumulation in plants. This study demonstrated complex interactions in single and mixed spiked treatments, affecting the overall heavy metal accumulation trends in Vetiver grass. Similar effects of single and mixed spiked treatments were reported by Peralta-Videa et al. (2002), Zhou et al. (2014), Wuana et al. (2016), He et al. (2016), Yang et al. (2016) and Chirakkara et al. (2016). They contributed to the metal accumulation in alfalfa, castor and paddy rice. Compared to the works of Duo et al. (2010), this study was further expanded to cover separate parts of the lower and upper roots and tillers of Vetiver grass in order to provide a more comprehensive phyto-assessment for translocation of heavy metals from the lower root upwards to the top of the tiller.

Notwithstanding, it is important to note that the mixed heavy metal spiked soils showed more complex interaction than with only a single metal contamination. The presence of more than one heavy metal in the soil would possibly affect the overall phyto-remediation ability in the plants (Chirakkara et al. 2016). Many recent studies by Ramamurthy and Memarian (2014), Hechmi et al. (2014) and Chigbo and Batty (2015) reported that the use of two and more different types of soil contaminants could unexpectedly limit their mobility and bioavailability resulting in reduction of phyto-accumulation efficiency in plants. However, this study demonstrated findings to the contrary, with Vetiver grass showing substantially high phyto-accumulation ability under mixed metal spiked treatments as compared to single heavy metal spiked treatments. This scenario is possible as the fates and translocation of metal contaminants under the mixed heavy metal conditions are complex and unpredictable (Reddy 2011). As a result, this study indicates that the presence and combination of different types of heavy metals due to the physico-chemical interactions among metals in the soil and/or within the plant species, may lead to the higher accumulation in all mixed heavy metal treatments as correspondingly observed by Chirakkara et al. (2016).

5 Conclusions

Vetiver grass grown in mixed Cd + Pb, Cu + Zn and Cd + Pb + Cu + Zn spiked treatments was potentially capable of accumulating higher heavy metals than single spiked treatments, in the order of Zn > > Cu > Pb > Cd. Vetiver grass may be regarded as a promising Cd, Cu and Zn phyto-stabilizer due to its high BCF values of >1 and noticeably higher accumulation in roots compared to tillers. In terms of different plant parts, the lower roots and lower tillers of Vetiver grass exhibited a strong tendency for greater uptake and accumulation of all four heavy metals, irrespective of single and/or mixed spiked treatments.

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