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PHYTO-ASSESSMENT OF SOIL HEAVY METAL ACCUMULATION IN TROPICAL GRASSES

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ABSTRACT

Tropical grasses are fast growing and often used for phytoremediation. Three different types of tropical grasses: Vetiver (*V. zizanoides*), Imperata (*I. cylindrical*) and Pennisetum (*P. purpureum*) tested in different growth media of spiked heavy metal contents under the glasshouse environment of RimbaIlmu for 60-day. The growth performance, metals tolerance and phyto-assessment of cadmium (Cd), lead (Pb), zinc (Zn) and copper (Cu) in shoots and roots were assessed using flame atomic absorption spectrometry (FAAS).Tolerance index (TI), translocation factor (TF), biological accumulation coefficient (BAC), biological concentration factor (BCF), and uptake efficacy was applied to evaluate the metal translocation ability among all three grasses. All three grasses showed significantly higher ($p<0.05$) accumulation of the total heavy metals in the spiked metal treatment compared with other tested treatments. Vetiver accumulated remarkably higher total concentration of Cd (93.08 ± 3.81 mg/kg) and Zn (1284.00 ± 234.83 mg/kg) than both Imperata and Pennisetum. The overall trend of heavy metals accumulation for all three grasses followed the order of Zn >Pb> Cd >Cu. The results of study suggested that both Imperata and Pennisetum are commendable and potential phytoextractors for Zn as well as phytostabilizers for Cd, Pb and Cu, respectively.

Key words: Vetiver; Imperata; Pennisetum; Spiked heavy metal; Heavy metal accumulation.

INTRODUCTION

Soil is commonly regarded as one of the significant natural resources that provide numerous essential elements and interrelating functionswhich include as a store for biodiversity, as a natural habitat for living organisms, food and biomass production as well as a relatively stable reservoir for the whole ecosystem. It is a limited resource that can easily deteriorateby both anthropogenicand natural changes. Soil contamination is the form of which pollutant materials present at concentrations above naturally occurring levels andare likely to cause adirectand/or long term danger to humans and the environment (DOE, 2009). Urban soil contamination has greatly affected many countries, including the United States, Germany, United Kingdom, China and India (Belluck *et al.*, 2006;Meuser, 2010) meanwhile heavy metal soil contamination itself has gained a serious attention at the global perspective.

Heavy metal can be very toxic even in low concentration and are not easily degraded or destroyed. It is generally harmful to humans and other living organisms as heavy metals caneasily bio-accumulateand cause food chain contamination. Nevertheless, heavy metals often exist in small amountsin soils and plants as some of thetrace metalsplay an essential role in promoting biological growth. In general, heavy metal can be categorized into essential and non-essential. Essential heavy metals such as nickel (Ni), iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu) are required by living organisms in trace amounts to support their metabolic functions while non-essential heavy metals such as chromium (Cr), arsenic (As), mercury (Hg), lead (Pb) and cadmium (Cd) are not needed for the growth of living organisms (Kabata-Pendias, 2011; Cuypers *et al.*, 2013). Heavy metals such as arsenic (As), chromium (Cr), mercury (Hg), cadmium (Cd), lead (Pb), zinc (Zn) and copper (Cu) are hazardous and the metal toxicity can be severely hazardous if the concentration of heavy metal exceeds its threshold level (DOE, 2009; Ng *et al.*, 2016). And among all heavy metals; cadmium (Cd), lead (Pb), zinc (Zn) and copper (Cu) are the most commonly foundmetals in contaminated sites (Wang *et al.*, 2009).

Many soil remediation technologies have been used over the last few decades,andphytoremediation has emerged to be one of the most cost effective and eco-friendly solution for soil metal contamination (Glass, 2000; Purakayastha and Chhonkar, 2010). In phytoremediation, plants are utilized to remove various hazardous substances present in the environment including organic compounds, inorganic ions, heavy metals and radioactive materials. As a consequence, the phytoremediation approach has gained much attention and numerous plants species have been tested for phytoremediation properties, including vegetable crops, ornamental flowers, trees, weeds and grasses.

Tropical grasses arefast growing plantswith good tolerance for growth under a wide range of soil, rainfall and temperature conditions. Due to its good adaptation to environmental stress, high biomass production and fast growth rate; grasses areoften used to be the preferable choice for phytoremediation compared to shrubs and trees (Ali *et al.*, 2013; Sinha *et al.*, 2013). Hence, three tropical grasses, Vetiver (*V. zizanoides*), Imperata (*I. cylindrical*) and Pennisetum (*P. purpureum*) are carefully selected in this study.

All of these three grasses shared many similar plant physiological and behavioral characteristics in nature (Langeland *et al.*, 2008; MacDonald *et al.*, 2008; Moore *et al.*, 2006).Recent studies of Paz-Alberto *et al.* (2007), Liu *et al.* (2009) and Abdel-Salam (2012) have barely discovered the preliminary phytoextraction ability of a specific type of metal contamination among these grasses in the soils. To augment these findings, this study was specifically designed to (i) determine the ability and tolerance level; (ii) evaluate the accumulation trend of cadmium (Cd), lead (Pb), zinc (Zn) and copper (Cu) heavy metals; and (iii) assess the feasibility and potential of phyto-assessment among all three tropical grasses of Vetiver (*V. zizanoides*), Imperata (*I. cylindrical*) and Pennisetum(*P. purpureum*) growing under contaminated heavy metals soil.

MATERIALS AND METHODS

Site description and experimental design: The study was conducted at the planthouse located in RimbaIlmu, Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur with the average temperature ranging between 23.5°C and 34.5°C and the relative humidity of around 76.0%,asrecorded by a RR Group Data Logger. Top soil (0-20cm) for planting, was taken from the field situated at 3° 7' N latitude and 101° 39' E longitude. The saplings ofall three tropical grasses, (*V. zizanoides*), Imperata (*I. cylindrical*) and Pennisetum **(***P. purpureum*) were placedunder different treatments of spiked heavy metals: Control, Cd (15 mgCd/kg soil), Pb (140 mgPb/kg soil), Zn (250 mgZn/kg soil) and Cu (20 mgCu/kg soil). All of the treatments were conducted under the completely randomized design (CRD) with four replications.

Soil pretreatment and plant preparation: Preliminary soil assessment (physical, biological and chemical) was carried out on the collected soil before it was air-dried in a large container. This was followed by <4mm sieving, using a stainless steel test sieve to remove gravels and large non-soil particles. The artificially spiked heavy metal treatments were prepared based on the range of heavy metal concentration proposed by theCanadian Council of Ministers of the Environment(CCME, 1999) and Department of Environment, Malaysia(DOE, 2009) with cadmium nitrate tetrahydrate [$Cd(NO_3)_2.4H_2O$], lead (II) nitrate [$Pb(NO_3)_2$], zinc sulfate heptahydrate [$ZnSO_4.7H_2O$] and copper (II) sulfate [$CuSO_4$] added to the soilfor a contamination level of 15 mg/kg for Cd, 140 mg/kg for Pb, 250mg/kg for Zn and 20 mg/kg for Cu, respectively. The amended soil was then continuously stirred and incubated for a week to ensure the homogeneity of the desired spiked heavy metal treatment is obtained. An initial uniform height of Vetiver(*V. zizanoides*), Imperata (*I. cylindrical*) and Pennisetum (*P. purpureum*) saplings were then planted in the plastic pots (0.1m x 0.12m) that were filled with 2 kilograms of soil, for all the treatments. All plants were watered evenlywith 50ml of tap wateronce a day and their growth performance observed throughout the 60-day of experiment.

Preparation of samples and chemical analysis: Freshly harvested plants were washed in running water and rinsed thoroughly with deionized water to remove any adhering soil particles before separating them into roots and shoots (tillers). Fresh weights of plant samples were determined before the samples were oven-dried for 72 hours at 70°C until it achieved a constant weight. Then the dry weight of the plant samples was determined before it washomogenized in a mortarand pestle and digested with hydrochloric acid (HCl), hydrogen peroxide (H_2O_2) and nitric acid (HNO_3). Approximately, 0.5g of the homogenized dried root and shoot samples underwent acid digestion according to Method 3050B (US EPA, 1996) followed by the Method 7000B (US EPA, 2007) for the elemental analysis using the Perkin-Elmer AAnalyst 400 flame atomic absorption spectrometry.Soil samples were also air-dried for 72 hours until it reached a constant weight before it was analyzed following similar analytical procedures.

Statistical analysis and data interpretation: The experimental data were analyzed by one-way analysis of variance (ANOVA) to evaluate the growth performance and metal accumulation in all the three tropical grasses. Further statistical validity test for significant differences among treatment means, was carried out using Fisher's least significant difference (LSD) testsat the level of significance $p \leq 0.05$.The ability for heavy metal accumulation and translocation upwards in these grasses were determined by calculatingthe tolerance index (TI), biological concentration factor (BCF), biological accumulation coefficient (BAC), translocation factor (TF) and metal uptake efficacy, using the following equations: TI = Dry matter content in heavy metal treatment divided by dry matter content in control;BCF = Concentration ofheavy metal in root divided by concentrationof heavy metal in soil; BAC = Concentrationof heavy metal in shoot divided by concentrationof heavy metal in soil; TF = Concentration of heavy metal in shoot divided by concentrationof heavy metal in root; and metal uptake efficacy (%) = Total concentration of heavy metal in shoot divided by total concentration of heavy metal removed from the soil.

RESULTS AND DISCUSSION

Physico-chemical properties of soil: Preliminary soil analyses (Table 1) showed that thecolour and texture of the growth media wasdull reddish brownsand with 92.79% sand, 5.56% silt and 1.65% clay. The soil had an ideal bulk density of1.54 g/cm^3 for plant growth,while its porosity (41.76%) constituted almost half of the soil composition to provide sufficient air and water for good growth of plant (BOPRC, 2014). The soil saturation level wasrelativelydry (12.56%) with a high water retention,where the percentage of soil porosity was almost the same as the soil field capacity (40.68%)indicating that most of the pore spaces in the soil were filled with water.

Soil pH was significantly ($p<0.05$) affected by the spiked heavy metal treatments in all the treatments inVetiver while only Cd and Zn spiked treatments were observed in Imperata and Zn treatment in Pennisetum, respectively (Figure 1).Cd, Pb and Cu spiked treatments did not affect soil pH in Pennisetum whilst Pb and Cu in Imperata.The acidic soil pH (5.04 ± 0.07) showed significant fluctuations between the range of3.69 and 6.67 in all of the spiked metal treatments.The optimum soil pH for the bioavailability and uptake of essential elements in plants has been reported to be between 5.5 and 7.5 (Moody, 2006). The changes insoil pH observed could be related to the proton ion (H^+) concentration in the soil, whereby a pH reduction would mean more protons are present and vice versa. This is related to the availability of heavy metals in the soil treatments which wouldsubsequently influence the growth performance of these grasses. It is known for example, that the uptake and accumulation of nitrate and sulfate in the roots are accompanied with proton uptake into the roots as well. If these processes are slowed down or inhibited, it would mean that there would be more protons in the soil (Tischner, 2000; Sorgonà *et al.*, 2011).Soil pH has a stronginfluence on the availability of plantnutrients and can affect the soil-plant interaction with regard to heavy metal accumulation (Husson, 2013).

Responses of plant growth: The relative growth of all the three grasses, in terms of plant heightincreased continuously throughout the study, with a significant decrease ($p<0.05$) observed in the case of Pennisetumin all the spiked heavy metal treatments when compared with control (Table 2). In Imperata, only Pb and Cu spiked treatments showed significant decrease in growth ($p<0.05$), while no significant difference ($p>0.05$) in plant height was found in all Vetiver treatments when compared with the control.These experiments showed that the application of spiked heavy metals in the soil did not affectplant height over dry matterin Vetiveral though the opposite was recorded in Pennisetum. Ovecka and Takac (2014) reported recently that the presence of spiked heavy metals in the soil cancontributeto reduce plantgrowth and this was observed in both Imperata and Pennisetum. However, the growth of Vetiverwas not significantly affected, suggesting that the grass washighly adaptable and tolerant toextreme environmental conditions of contaminated spiked heavy metals (Danh *et al.*, 2009).The largeincrease in plant heightobserved in the case of Imperata (44.89 ± 17.23 cm)could be due to requirement by the plant for Cu as a micronutrient.

The yield,in terms of dry matter content (g/pot) of roots and shoots,in all thetreated grasses showed a different picture (Table 3). Although plant height in Vetiver was not significantly affected, both Vetiver and Pennisetum grown underspikedheavy metal treatments exhibited a significantdecrease ($p<0.05$) in total yield of dry matter content when compared with the controls, despite the dry matter of shoot did not significantly decrease in Vetiver, but did in the case of Pennisetum. The roots of Vetiver and shoots of Pennisetum recorded significant reduction ($p<0.05$)in dry matter content. Among the three grasses, Vetiver yielded considerably higher root and shoot dry matter content.The application of spiked heavy metals to the soils significantly reduced root growth in Vetiverand shoot growth in Pennisetum, while not significantly affecting shoot growth in Vetiver and root growth in both Imperata and Pennisetum. However, spiked Cu treatment increased shoot growth in Imperata.

Dry matter content per pot producedwas used to estimate the tolerance index (TI) of the spiked heavy metal treatments in all three grasses. TI acts as an indicator to determine the capability of a plant to growin heavy metal contaminated soils. Imperata was the only grass that displayed a significant difference ($p<0.05$) in TI, among the three grasses. All of the spiked heavy metal treatments in Imperata exhibited relatively higher TI than both Vetiver and Pennisetum, regardless of the plant height and dry matter content recorded. As a result of the high TI, Cu-spiked (2.202), Cd-spiked (1.699) and Zn-spiked (1.303) treatments, Imperata showed good tolerance ability of growing under spiked heavy metal conditions, compared to both Vetiver and Pennisetum.

Accumulation of heavy metals: As shown in Tables 4–7, the accumulation of heavy metals in the roots and shoots of all three grasses werevariable.Cd accumulation in all the three grasses was significantly higher ($p<0.05$) in the Cd-spiked treatments than in the other treatments.All grasses recorded higher accumulation of Cd in the Cd-spiked treatments (76.45 – 93.08 mg/kg) compared to the other treatments (0.07 – 8.00 mg/kg).The Cd accumulated in bothroots and shoots of Cd-spiked treatments also significantly higher ($p<0.05$) than other treatments irrespective of the type of grass.Between roots and shoots, Cd accumulations were greater in the roots than in the shoots.The accumulation of Cd in the different type of grasses studied was in the order of Vetiver>Pennisetum>Imperata for all the treatments.

Similarly, the total accumulation of Pb was significantly higher ($p<0.05$) in all of the Pb-spiked treatments than in the other treatments (Table 5). All three grasses exhibited higher accumulation of Pb in the Pb-spiked treatments (103.20 – 340.70 mg/kg) compared to the other treatments (2.44 – 20.55 mg/kg). The accumulation of Pb in both roots and shoots of Pb-spiked treatments was significantly higher ($p<0.05$) than other treatments.Between roots and shoots, Pb accumulated more in roots irrespective of the type of grass. The trend for Pb accumulation was in the following order of Imperata>Pennisetum>Vetiver for all treatments.

All three grasses recorded significantly increased ($p<0.05$) total accumulation of Zn in the Zn-spiked treatments compared to the other treatments (Table 6).A higher accumulation of Znwas observed in Zn-spiked treatments (393.10 – 1284.00 mg/kg) compared to the other treatments (78.40 – 413.00 mg/kg).Zn accumulated in both roots and shoots of Zn-spiked treatmentswere significantly higher ($p<0.05$) than in other treatments. Unlike for Cd and Pb, in both Imperata and Pennisetum, there was a higher accumulation of Zn in the shoots than roots. Accumulation of Zn in all the three grasses was in the following order of Vetiver>Pennisetum>Imperata.

With regard to Cu accumulation, Cu-spiked treatments in all three grasses showed significantly higher ($p<0.05$) total Cu accumulation compared to the other treatments (Table 7). Higher accumulation of Cu was found in Cu-spiked treatments (22.84 – 49.80 mg/kg) compared to the other treatments (1.45 – 12.90 mg/kg).Significantly greater ($p<0.05$) Cu accumulation was observed in the roots whereas no significant differences ($p>0.05$) were observed in shoots of the Cu-spiked treatments. Between roots and shoots, Cu accumulation in the roots was relatively greater than in the shoots of all three grasses. The accumulation trend for Cu was in the following order of Pennisetum> Imperata> Vetiver.

It can be seen from the above results that Vetiver accumulated the highest amount of Cd (93.08 ± 3.81 mg/kg) and Zn (1284.00 ± 234.83 mg/kg) in the spiked heavy metal treatments, compared to Imperata and Pennisetum. All three grasses showeda similar inclinationin the order of heavy metal accumulation, with Zn >Pb> Cd > Cu regardless of the total amount of spiked heavy metal put into the soil. The high concentration of heavy metal accumulation found in the roots and shoots of all grasses could be attributed to the method of application ofthe spiked heavy metals.Theuse of direct pot assays for spiked heavy metalsinstead of field-site applicationis a possible cause for the high concentration of heavy metal accumulation found in the roots and shoots of these plants.

Translocation and efficacy of heavy metals: The association of the different heavy metals accumulated from the soils into the roots and shoots for all the three grasses, in terms of BCF, BAC, TF and efficacy (%) are presented in Tables 4–7.

In all the three grasses, relatively more heavy metals were accumulated in the roots than shoots, where it was observed that the root and soil concentration ratio (BCF) was >1 suggesting that heavy metal translocation from the soil to root was substantially higher and the roots acted as the sink for heavy metal accumulation. All the three grasses recorded remarkably higher BCF (> 1) in the accumulation of Cd (2.947 – 5.977) when grown under Cd-spiked treatments. Zn-spiked treatments in Vetiver (3.532) exhibited appreciably higher accumulation of Zn, followed byPb-spiked treatments in Imperata (2.075) and Pennisetum (1.824) that resulted in relatively higher concentration of Pb in the roots compared to the shoots.Comparatively, all the three grasses, showed BAC values< 1,suggesting that the translocation pathway of heavy metals from soils into the shoots may have been inhibited. The accumulation of both Cd and Pb in Vetiver exhibited the highest BAC in the Cd-spiked (0.229) and Pb-spiked (0.176) treatments compared to the other treatments.

Considering the relatively lower accumulation of heavy metals in the shoots than root, in all the three grasses, TF was assessed to gauge the capability of the plant to translocate heavy metals from the roots to the shoots.Zn-spiked treatments of both Imperata (1.265) and Pennisetum (1.201) recorded relatively higher TF for the accumulation of Zn.Although the TF value was < 1, Vetivershowed reasonably higher TF in the spiked heavy metal treatments than in other treatments for both the accumulation of Cd (0.038) and Pb (0.317), respectively. A higher TF was recordedfor the accumulation of both Cd (0.358 – 2.800) and Zn (0.349 – 1.265)despite the high TI observed in the spiked heavy metal treatments of Imperata.

The efficacy (%) of heavy metal accumulation was calculated in order to evaluate the potential and efficiency of metal translocation and bioaccumulation inside the plant, from roots to shoots. Theaccumulation efficacy revealed that the spiked heavy metal treatments for Vetiver accumulated reasonably higher Cd (3.69%) and Pb (23.84%) than for other treatments. Between the different grasses, Imperata accumulated relatively lower Cd (26.36%), Pb (14.75%), Zn (55.85%) and Cu (7.74%) compared to Pennisetum that recorded Cd (42.34%), Pb (19.28%), Zn (54.56%) and Cu (9.14%).Theaccumulation

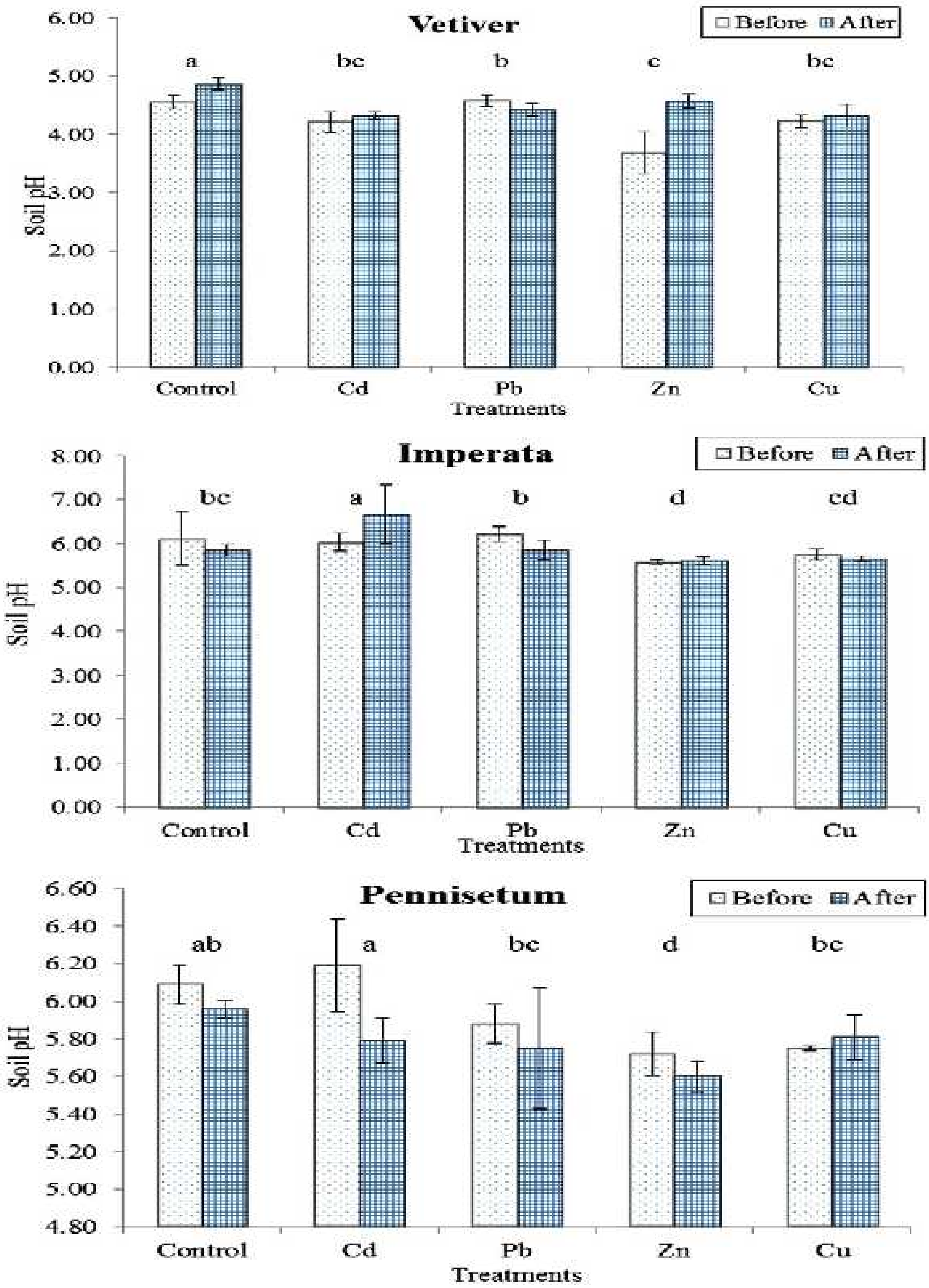

Figure 1. Changes in soil pH of Vetiver, Imperata and Pennisetum grasses as influenced by different types of spiked heavy metal treatments. Vertical bars represent standard deviation and same letters are not significantly different for each treatment means at 0.05 levels of probability.

efficacy of Vetiver was 4.56 – 9.09% higher for Pb and 12.27 – 13.67% higher for Cuwhen compared to the other grasses, while the efficacy for Cd accumulation in Pennisetum was 15.98 – 38.65% higher.A 1.29 – 24.62% higher efficacy for Zn was recorded in Imperata compared to other grasses.

The amount of metal content present in the spiked heavy metal treatments can be considered to be similar to that of a contaminated soil, following recentreports in the literature. The concentrations of Cd (15.30 mg/kg), Pb (143.30 mg/kg), Zn (258.90 mg/kg) and Cu(22.40 mg/kg) present were above the national and international guidelines for soil contamination permissible levels.Studies by the DOE (2009) observed that,for Malaysian soils, typical concentration range for naturally occurring heavy metals are as follows: Cd (0.09 –14.40 mg/kg), Pb (0.18 – 36.00 mg/kg), Zn (6.90 – 54.30 mg/kg) and Cu (4.00 – 19.80 mg/kg). On the other hand,the soil quality guidelines put forward by the Canadian Council of Ministers of the Environment, has set the allowable limits for heavy metal contamination to range from 1.4 – 10.0 mg/kg (Cd), 70.0 –140.0 mg/kg (Pb), 200.0 – 360.0 mg/kg (Zn) and 63.0 – 91.0 mg/kg (Cu) for both agricultural and urban residential soils (CCME, 1999).

The TF and heavy metal accumulation efficacy (%) results are vital to estimate the phytoremediation potential of a plant species. Malik *et al.* (2010) and Nazir *et al.* (2011) suggested that a plant would be suitable for phytoremediation when the BCF, BAC and TF values are>1. In thisstudy,the TF and efficacy (%)recorded Vetiverhad the best capability to accumulate higher Pb and Cu than the other two grasses. Nevertheless, Imperata exhibited remarkably higher TF and efficacy (%) for the accumulation of Zn whereas Pennisetum showed greater ability for Cd accumulation. However, none of the three grasses tested in this study satisfied the conditions that require all the BCF, BAC and TF values to be > 1.

Despite the low accumulation of heavy metal found in the shoots, all three grasses recorded high BCF values>1. All the heavy metals greatly accumulated in the roots irrespective of the type of heavy metals. Phytostabilization and phytoextraction are two different categories of phytoremediation which involve the application of different functions and characteristics of plants used to remove heavy metals from contaminated soil (Douchiche *et al.*, 2012). The primary mechanism involved in phytostabilization

Table 1. Physical and chemical parameters of the growth media soil.

Characteristics (Units)		Mean ± SD
Soil texture		
Sand (%)		92.79
Very coarse sand (%)	0.62	
Coarse sand (%)	46.59	
Medium coarse sand (%)	20.51	
Fine sand (%)	18.38	
Very fine sand (%)	6.69	
Silt (%)		5.56
Clay (%)		1.65
Soil physical		
Bulk density (g/cm^3)		1.54 ± 0.03
Porosity (%)		41.76 ± 0.95
Colour (Munsell colour charts)		2.5YR 5/4 (Dull reddish brown)
Soil biology		
Water content (%)		5.11 ±0.12
Field capacity (%)		40.68 ± 1.93
Saturation level (%)		12.56
Condition		Dry
Soil chemistry		
pH		5.04 ± 0.07
Metal contents (mg/kg)		
Cd		2.37 ± 1.44
Pb		28.66 ± 10.73
Zn		186.24 ± 56.57
Cu		11.22 ± 4.24

SD = Standard deviation

Table 2. Plant height (cm) of Vetiver, Imperata and Pennisetum grasses as influenced by different types of spiked heavy metal treatments

Treatment	Plant height (cm)		
	Vetiver	Imperata	Pennisetum
Control	64.86 a ± 20.61	33.08 b ± 6.34	72.89 a ± 19.48
Cd	73.81 a ± 24.92	29.07 bc ± 2.11	30.48 d ± 0.16
Pb	72.33 a ± 25.04	24.62 c ± 1.83	40.36 bc ± 6.97
Zn	61.23 a ± 20.19	26.11 bc ± 2.25	32.75 cd ± 0.66
Cu	60.03 a ± 22.63	44.89 a ± 17.23	48.71 b ± 10.54

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

Table 3. Dry matter content (g/pot) and tolerance index (TI) of Vetiver, Imperata and Pennisetum grasses as influenced by different types of spiked heavy metal treatments

Treatment	Dry matter content (g/pot)											
	Vetiver				Imperata				Pennisetum			
	Root	Shoot	Total	TI	Root	Shoot	Total	TI	Root	Shoot	Total	TI
Control	27.06 a ± 1.39	28.13 a ± 2.57	55.19 a ± 2.46		1.17 a ± 0.77	2.86 bc ± 0.35	4.03 c ± 0.10		5.33 a ± 1.43	17.00 a ± 3.27	22.33 a ± 0.37	
Cd	13.68 bc ± 1.99	23.23 a ± 4.00	36.91 bc ± 1.68	0.669 a	2.45 a ± 1.07	4.32 b ± 1.71	6.77 ab ± 0.23	1.699 ab	3.80 a ± 1.38	3.81 bc ± 0.31	7.61 e ± 0.16	0.354 a
Pb	15.40 b ± 2.76	24.01 a ± 3.38	39.41 b ± 3.87	0.712 a	2.01 a ± 0.98	1.84 c ± 0.44	3.85 c ± 0.12	0.9589 b	3.40 a ± 1.46	7.80 bc ± 4.25	11.20 c ± 0.48	0.528 a
Zn	15.22 bc ± 0.03	22.52 a ± 1.02	37.74 bc ± 0.83	0.686 a	1.07 a ± 0.66	3.87 bc ± 0.87	4.94 bc ± 0.15	1.303 bc	7.64 a ± 0.92	2.72 bc ± 0.83	10.36 d ± 0.88	0.511 a
Cu	12.14 bc ± 1.99	21.23 a ± 2.11	33.37 bc ± 3.76	0.604 a	1.78 a ± 0.74	6.83 a ± 1.14	8.61 a ± 0.15	2.202 a	5.18 a ± 0.45	8.57 b ± 4.45	13.75 b ± 0.70	0.632 a

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

Table 4. Metal accumulation of cadmium (Cd) and its biological concentration factor (BCF), biological accumulation coefficient (BAC), translocation factor (TF) and metal uptake efficacy (%) of Vetiver, Imperata and Pennisetum grasses as influenced by different types of spiked heavy metal treatments.

Treatment	Concentration of Cd (mg/kg)			BCF	BAC	TF	Efficacy (%)
	Root	Shoot	Total				
Vetiver							
Control	0.18 b ± 0.04	ND	0.18 b ± 0.04	0.076 b	0.000	0.000	0.00
Cd	89.65 a ± 4.31	3.43 ± 0.70	93.08 a ± 3.81	5.977 a	0.229	0.038	3.69
Pb	ND	ND	ND	0.000	0.000	0.000	0.00
Zn	0.36 b ± 0.11	ND	0.36 b ± 0.11	0.152 b	0.000	0.000	0.00
Cu	0.07 b ± 0.01	ND	0.07 b ± 0.01	0.030 b	0.000	0.000	0.00
Imperata							
Control	4.40 b ± 1.11	3.60 b ± 0.96	8.00 b ± 2.03	1.857 ab	1.519 a	0.818 b	45.00 b
Cd	56.30 a ± 14.89	20.15 a ± 5.16	76.45 a ± 10.93	3.753 a	1.343 ab	0.358 b	26.36 b
Pb	1.15 b ± 0.35	0.65 b ± 0.21	1.80 b ± 0.05	0.485 b	0.274 c	0.565 b	36.11 b
Zn	0.25 b ± 0.07	0.70 b ± 0.28	0.95 b ± 0.19	0.105 b	0.295 c	2.800 a	73.68 a
Cu	0.35 b ± 0.09	0.30 b ± 0.01	0.65 b ± 0.07	0.148 b	0.127 c	0.857 b	46.15 ab
Pennisetum							
Control	5.35 b ± 1.32	2.50 b ± 0.91	7.85 b ± 1.81	2.257 ab	1.055 b	0.467 bc	31.85 a
Cd	44.20 a ± 3.68	32.45 a ± 5.30	76.65 a ± 7.43	2.947 a	2.163 a	0.734 ab	42.34 a
Pb	1.80 b ± 0.28	0.90 b ± 0.14	2.70 b ± 1.56	0.759 b	0.380 c	0.500 bc	33.33 a
Zn	0.70 b ± 0.03	0.90 b ± 0.05	1.60 b ± 0.07	0.295 b	0.380 c	1.286 ab	56.25 a
Cu	0.75 b ± 0.09	1.05 b ± 0.21	1.80 b ± 0.37	0.316 b	0.443 c	1.400 a	58.33 a

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

Table 5. Metal accumulation of lead (Pb) and its biological concentration factor (BCF), biological accumulation coefficient (BAC), translocation factor (TF) and metal uptake efficacy (%) of Vetiver, Imperata and Pennisetum grasses as influenced by different types of spiked heavy metal treatments

Treatment	Concentration of Pb (mg/kg)			BCF	BAC	TF	Efficacy (%)
	Root	Shoot	Total				
Vetiver							
Control	2.44 b ± 0.17	ND	2.44 b ± 0.17	0.085 b	0.000	0.000	0.00
Cd	4.92 b ± 2.32	ND	4.92 b ± 2.32	0.172 ab	0.000	0.000	0.00
Pb	77.60 a ± 59.96	24.60 a ± 0.11	103.20 a ± 58.65	0.554 a	0.176 a	0.317 a	23.84 a
Zn	5.62 b ± 0.99	ND	5.62 b ± 0.99	0.196 ab	0.000	0.000	0.00
Cu	3.51 b ± 0.52	0.39 b ± 0.06	3.90 b ± 0.43	0.122 b	0.014 b	0.111 b	10.00 b
Imperata							
Control	9.65 b ± 3.40	3.95 b ± 1.76	13.60 b ± 6.65	0.337 b	0.138 ab	0.409 ab	29.04 a
Cd	11.15 b ± 4.27	7.75 b ± 2.58	18.90 b ± 2.33	0.389 b	0.270 ab	0.695 ab	41.01 a
Pb	290.45 a ± 21.85	50.25 a ± 3.75	340.70 a ± 11.87	2.075 a	0.359 ab	0.173 b	14.75 a
Zn	0.90 b ± 0.19	1.95 b ± 0.34	2.85 b ± 1.33	0.031 b	0.068 b	2.170 b	68.42 a
Cu	4.25 b ± 0.21	ND	4.25 b ± 0.21	0.148 b	0.000	0.000	0.00
Pennisetum							
Control	8.00 b ± 3.82	3.50 b ± 0.85	11.50 b ± 1.54	0.279 b	0.122 b	0.438 b	30.43 a
Cd	15.40 b ± 6.93	5.15 b ± 0.78	20.55 b ± 4.81	0.537 b	0.180 ab	0.334 b	25.06 a
Pb	255.40 a ± 14.85	61.00 a ± 4.81	316.40 a ± 8.48	1.824 a	0.436 a	0.239 b	19.28 a
Zn	4.70 b ± 0.71	7.30 b ± 2.18	12.00 b ± 5.52	0.164 b	0.255 ab	1.553 ab	60.83 a
Cu	2.75 b ± 1.34	5.00 b ± 2.93	7.75 b ± 3.46	0.096 b	0.174 b	1.818 a	64.52 a

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

Table 6. Metal accumulation of zinc (Zn) and its biological concentration factor (BCF), biological accumulation coefficient (BAC), translocation factor (TF) and metal uptake efficacy (%) of Vetiver, Imperata and Pennisetum grasses as influenced by different types of spiked heavy metal treatments.

Treatment	Concentration of Zn (mg/kg)			BCF	BAC	TF	Efficacy (%)
	Root	Shoot	Total				
Vetiver							
Control	185.00 b ± 35.79	76.10 b ± 49.26	261.10 b ± 32.57	0.993 b	0.409 b	0.411 b	29.15 a
Cd	272.00 b ± 55.15	77.60 b ± 16.55	349.60 b ± 60.43	1.460 b	0.417 b	0.285 b	22.20 a
Pb	211.00 b ± 123.45	144.50 b ± 12.02	355.50 b ± 109. 89	1.133 b	0.776 ab	0.685 ab	40.65 a
Zn	883.00 a ± 391.74	401.00 a ± 100.41	1284.00 a ± 234.83	3.532 a	1.604 a	0.454 b	31.23 a
Cu	191.00 b ± 86.27	222.00 ab ± 134.76	413.00 b ± 218.49	1.026 b	1.192 ab	1.162 a	53.75 a
Imperata							
Control	122.40 ab ± 21.92	56.60 b ± 38.33	179.00 bc ± 11.84	0.657 a	0.304 b	0.462 ab	31.62 b
Cd	81.95 b ± 21.43	28.60 b ± 6.08	110.55 c ± 17.52	0.440 a	0.154 b	0.349 b	25.87 b

Pb	56.80 b ± 11.74	40.15 b ± 4.03	96.95 c ± 9.04	0.305 a	0.216 b	0.707 ab	41.41 ab
Zn	173.55 a ± 38.40	219.55 a ± 10.11	393.10 a ± 24.33	0.694 a	0.878 a	1.265 ab	55.85 a
Cu	160.40 ab ± 3.82	61.45 b ± 4.31	221.85 b ± 1.74	0.861 a	0.330 b	0.383 b	27.70 b
Pennisetum							
Control	85.25 c ± 2.33	39.70 c ± 10.89	121.95 c ± 5.47	0.458 c	0.213 c	0.466 b	32.55 b
Cd	48.45 d ± 8.13	29.95 c ± 5.73	78.40 c ± 4.45	0.260 d	0.161 c	0.618 ab	38.20 b
Pb	69.95 cd ± 7.57	35.20 c ± 0.57	105.15 c ± 6.23	0.376 cd	0.189 c	0.503 b	33.48 b
Zn	196.60 a ± 7.64	236.05 a ± 0.49	432.65 a ± 4.82	0.786 a	0.944 a	1.201 a	54.56 a
Cu	128.00 b ± 0.14	95.50 b ± 14.04	223.50 b ± 16.91	0.687 ab	0.513 b	0.746 ab	42.73 b

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

Table 7. Metal accumulation of copper (Cu) and its biological concentration factor (BCF), biological accumulation coefficient (BAC), translocation factor (TF) and metal uptake efficacy (%) of Vetiver, Imperata and Pennisetum grasses as influenced by different types of spiked heavy metal treatments.

Treatment	Concentration of Cu (mg/kg)			BCF	BAC	TF	Efficacy (%)
	Root	Shoot	Total				
Vetiver							
Control	4.13 b ± 1.57	3.57 a ± 1.64	7.70 b ± 1.78	0.368 c	0.318 a	0.864 ab	46.36 a
Cd	9.70 b ± 1.27	1.89 a ± 0.67	11.59 b ± 0.93	0.865 ab	0.168 a	0.195 b	16.31 a
Pb	3.69 b ± 1.50	5.49 a ± 3.16	9.18 b ± 0.66	0.329 c	0.489 a	1.488 a	59.80 a
Zn	4.23 b ± 1.62	3.75 a ± 1.53	7.98 b ± 1.91	0.377c	0.334 a	0.887 ab	46.99 a
Cu	17.95 a ± 8.98	4.89 a ± 2.21	22.84 a ± 5.77	0.898 a	0.245 a	0.272 b	21.41 a
Imperata							
Control	6.60 b ± 1.13	4.20 a ± 1.25	10.80 b ± 2.12	0.588 ab	0.374 a	0.636 a	38.89 ab
Cd	1.45 b ± 0.21	ND	1.45 b ± 0.21	0.129 b	0.000	0.000	0.00
Pb	2.20 b ± 0.99	ND	2.20 b ± 0.99	0.196 b	0.000	0.000	0.00
Zn	2.10 b ± 1.55	2.10 a ± 1.69	4.20 b ± 0.74	0.187 b	0.187 a	1.000 a	50.00 a
Cu	45.90 a ± 19.66	3.85 a ± 1.20	49.75 a ± 13.46	2.295 a	0.193 a	0.084 a	7.74 b
Pennisetum							
Control	8.75 b ± 4.74	4.15 a ± 0.49	12.90b ± 3.62	0.780 ab	0.370 a	0.474 b	32.17 ab
Cd	3.45 b ± 2.05	4.25 a ± 1.20	7.70 b ± 1.47	0.307 ab	0.379 a	1.232 ab	55.19 ab
Pb	1.85 b ± 0.07	0.70 a ± 0.57	2.55 b ± 1.04	0.165 ab	0.062 a	0.378 b	27.45 ab
Zn	0.40 b ± 0.18	2.25 a ± 1.48	2.65 b ± 0.58	0.036 c	0.201 a	5.625 a	84.91 a
Cu	45.25 a ± 21.43	4.55 a ± 2.31	49.80 a ± 14.75	2.263 a	0.228 a	0.101 b	9.14 b

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

is the immobilization of heavy metal ions in the soil by storing them at root level without aiming to remove the heavy metals from the soil (Ali *et al.*, 2013). On the other hand, phytoextraction mainly relies on the efficiency of heavy metal translocation from the roots to shoots after the accumulation of metals in the roots of the plant. Phytoextraction involves the harvesting of above ground biomass (shoots) for the removal of heavy metals from contaminated soil (Lone *et al.*, 2008).

A plant is suitable for phytostabilization if its BCF > 1,even if it has a low TF. However plants with TF > 1 and relatively high efficacy (%) are more favorable for phytoextraction. All the three grasses studied can be used for phytostabilization in Cd contaminated soils, whilst Vetiver demonstrated promising phytostabilization traits for the accumulation of Zn. Both Imperata and Pennisetum showed good phytostabilization properties forPb and Cu.The study also showed that both Imperata and Pennisetumcan be utilized for Znphytoextraction, based on their remarkably high TF and accumulation efficacy (%) values.

Conclusions: The trend for heavy metal accumulation in all the three grasses varied andwas in the order of Zn >Pb> Cd > Cu. Vetiver accumulated appreciably higher total concentrations of Cd and Zn than both Imperata and Pennisetum. All three grasses accumulated relatively higher heavy metal concentrations in the roots than shoots except for Zn accumulation in both Imperata and Pennisetum. As a result of BCF values>1, the accumulation of Cd, Pb and Cuin all three grasses highlighted that the roots acted as the sinkfor heavy metals accumulation. The study indicated that different promising potential for phytostablization was found in Vetiver for Cd and Zn; in Imperata for Cd, Pb and Cu; and in Pennisetum for Cd, Pb and Cu. Both Imperata and Pennisetum also exhibited as good Zn phytoextraction properties when grown in contaminated soil.

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