

# Phyto-evaluation of Cd-Pb Using Tropical Plants in Soil-Leachate Conditions

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**ABSTRACT:** Sources of soil contamination can exist in various types of conditions including in the form of semifluids. In this study, 3 different types of tropical plants, Acacia (*Acacia mangium* Willd), Mucuna (*Mucuna bracteata* DC. ex Kurz) and Vetiver (*Vetiveria zizanioides* L. Nash), were tested under different levels of soil-leachate conditions. The relative growth rate, metal tolerance, and phytoassessment of cadmium (Cd) and lead (Pb) accumulation in the roots and shoots were determined using flame atomic absorption spectrometry. Tolerance index, translocation factor, metal accumulation ratio, and percentage metal efficacy were applied to assess the metal translocation ability among all the 3 types of plants. Significantly higher ( $P < .05$ ) accumulation of Cd and Pb was exhibited in the roots and shoots of all 3 plants growing under the soil-leachate conditions. However, negative growth performance and plant withering were observed in both Acacia and Mucuna with increased application of higher soil-leachate levels. Vetiver accumulated remarkably higher total concentration of Cd (116.16–141.51 mg/kg) and Pb (156.37–365.27 mg/kg) compared with both Acacia and Mucuna. The overall accumulation trend of Cd and Pb in the 3 plants growing under the soil-leachate conditions was in the order of Vetiver > Acacia > Mucuna. The findings of the study suggest that Vetiver has great potential as Cd and Pb phytoremediator in soil-leachate conditions.

**KEYWORDS:** Metal accumulation, Acacia, Mucuna, Vetiver, soil-leachate conditions

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## Introduction

Leachate from landfills is produced as a by-product of the infiltration of precipitation and the continuous process of biochemical breakdown that often take place during the course of natural disintegration and degradation of waste materials underneath soil covers. The discharge of leachate consists of a myriad composition of organic compounds, inorganic ions, and heavy metals that are potentially detrimental to the environment.<sup>1,2</sup> Heavy metals such as zinc (Zn), copper (Cu), iron (Fe), and nickel (Ni) are commonly referred to as trace metals and are essentially required by all living organisms in low concentrations for growth and development. However, other heavy metals such as lead (Pb), cadmium (Cd), mercury (Hg), and chromium (Cr) are nonessential metals that are not required for the growth of living organisms and are toxic at a certain concentration.<sup>3</sup>

Generally, the heavy metals found in a landfill leachate consist of Cd, Cu, Cr, Pb, Ni, and Zn.<sup>4</sup> Among these metals, Cd and Pb are highly hazardous, even at a very low level of concentration compared with other heavy metals. Naturally occurring Cd and Pb in soils are often undetectable and extremely poisonous. Both Cd and Pb cannot be degraded or destroyed in the biological environment and are persistent in soils for a long period of time.<sup>5,6</sup> Moreover, both metals can be easily bioaccumulated from one organism to another via the food chain. Furthermore, due to its harmful characteristics, both Cd and

Pb are categorized among the 126 chemical pollutants of the Toxic and Priority Pollutants.<sup>7</sup>

The use of various tropical plants for heavy metal phytoremediation has been expansively studied over the years.<sup>8–13</sup> Recent reports by previous works<sup>14–16</sup> have revealed the phytoextraction properties of Cd and Pb in a few selected tropical plant species growing under conventional contaminated soil-based culture. However, all these studies were limited regarding the phytoassessment findings using different tropical plants growing under soil-leachate culture conditions. As a result, this article reports the use of landfill leachate (treated leachate) as the source of polluted material to (1) assess its effects on plant growth performance, (2) evaluate Cd and Pb accumulation and its tolerance level, and (3) determine the viability and phytoremediation potential of 3 different tropical plants growing under the soil-leachate conditions. The 3 tropical plants, arbitrarily selected in this study, based on their fundamental fast growing, nonsusceptibility to pest resistance and minimal maintenance characteristics were Acacia, Mucuna, and Vetiver.

## Materials and Methods

### *Site description and plant sampling*

The study was conducted using pot experiments in the plant-house located in Rimba Ilmu, Institute of Biological Sciences,



**Table 1.** Experimental treatment variables.

TREATMENT	DETAIL OF SOIL AND TREATED LEACHATE, %
Control	100% soil
80S+20L	80% soil+20% treated leachate
60S+40L	60% soil+40% treated leachate
50S+50L	50% soil+50% treated leachate
40S+60L	40% soil+60% treated leachate
20S+80L	20% soil+80% treated leachate
100L	100% treated leachate

Faculty of Science, University of Malaya, Kuala Lumpur, under natural light conditions with the average room temperature of between 27.5°C and 35.5°C. Three tropical plant species, *Acacia* (*Acacia mangium* Willd), *Mucuna* (*Mucuna bracteata* DC. ex Kurz), and *Vetiver* (*Vetiveria zizanioides* L. Nash), were placed under 6 different levels of soil-leachate treatments (Table 1). The saplings of *Acacia* as well as *Mucuna* and *Vetiver* were obtained from the Lentang Seed and Planting Material Center, Forestry Department of Peninsular Malaysia and Humibox Malaysia, respectively. Fresh and healthy plant saplings with a uniform height of 40 to 45 cm were selected for the study. Plant growth parameters such as height, leaf number, and percentage plant survivorship were continuously monitored throughout the 75-day period of the study.

#### *Growth media preparation and experimental design*

The treated leachate was collected from the closed Taman Beringin landfill, Jinjang, Kuala Lumpur, Malaysia. The preliminary composition of leachate characteristics is shown in Table 2. The toxicity of the landfill leachate recorded higher concentration levels of As, Cd, Fe, and Pb, compared with the national and international maximum permissible effluent discharge standards. Top soil (0-20 cm) was collected from a field situated in University of Malaya, Kuala Lumpur, at 3° 7' N latitude and 101° 39' E longitude. All collected soils were air-dried for a week followed by a <4mm sieving to remove gravels and large nonsoil particles. Each plant was grown in plastic pots (0.18m diameter×0.16m depth) containing 3 kg of growth media (mixture of soil-leachate) samples. All the treatments were conducted under the completely randomized design with 3 replications.

#### *Samples and chemical analysis*

All plant species were uprooted at the end of a 75-day experimental period and brought to the laboratory and washed in running water, followed by deionized water to remove any adhering soil particles, before the plants were sectioned into

parts of roots and shoots. All the plant materials were oven-dried for 72 hours at 70°C to obtain a constant dry matter yield before it was homogenized in a mortar and pestle. Approximately, 0.5 g of the homogenized dried root and shoot samples underwent acid digestion with hydrochloric acid (HCl), nitric acid (HNO<sub>3</sub>), and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) according to the Method 3050B<sup>23</sup> followed by Method 7000B<sup>24</sup> for total recoverable elemental analysis of both Cd and Pb using the PerkinElmer AAnalyst 400 (PerkinElmer, Waltham, MA 02451, USA) flame atomic absorption spectrometer. The highly precise chemical analysis technique was controlled using the BAM Germany (BRM#12-mixed sandy soil) certified reference material with the Cd (93.46%) and Pb (108.25%) rate of metal recovery.

#### *Statistical and data analysis*

The growth performance was evaluated using the root-shoot (R/S) ratio, tolerance index (TI), and relative growth rate (RGR) formula,<sup>25-27</sup> whereas the ability for metal accumulation and translocation upward in the plant species was assessed by determining the translocation factor (TF), metal accumulation ratio (MAR), and percentage of metal uptake efficacy as follows:

$$\text{R/S ratio} = \frac{\text{Dry matter yield in root}}{\text{Dry matter yield in shoot}}$$

$$\text{TI} = \frac{\text{Total dry matter yield in treatment}}{\text{Total dry matter yield in control}}$$

$$\text{RGR} = \frac{\left[ \ln \left( \frac{\text{Final biomass of treatment}}{\text{Initial biomass of treatment}} \right) \right]}{\text{Days of growth}}$$

$$\text{TF} = \frac{\text{Metal concentration accumulated in shoot}}{\text{Metal concentration accumulated in root}}$$

$$\text{MAR} = \frac{\left[ \begin{array}{l} \text{Metal concentration accumulated in shoot} \\ \times \text{Dry matter yield in shoot} \end{array} \right]}{\left[ \begin{array}{l} \text{Metal concentration accumulated in root} \\ \times \text{Dry matter yield in root} \end{array} \right]}$$

Metal uptake

$$\text{efficacy (\%)} = \left[ \frac{\text{Metal concentration accumulated in shoot}}{\text{Total metal concentration removed from the growth media}} \right] \times 100$$

All experimental data were analyzed by performing the 1-way analysis of variance and further statistical validity test for significant differences among treatment means was conducted by employing the Fisher least significant difference tests at 95% level of confidence with the aid of Microsoft Excel Office 365 versions 2016 software.

**Table 2.** Characteristics of treated leachate compared with national and international maximum permissible effluent discharge standards.

PARAMETER	TREATED LEACHATE	MALAYSIA		THAILAND <sup>c</sup>	SINGAPORE <sup>d</sup>	JAPAN <sup>e</sup>	US <sup>f,g</sup>
		A <sup>a</sup>	B <sup>b</sup>				
pH at 25°C	7.8	6.0–9.0	5.5–9.0	5.5–9.0	6.0–9.0	5.0–9.0	6.0–9.0
Temperature, °C	28.6	40.0	40.0	40.0	45.0	NA	NA
Al, mg/L	0.008	10.0	15.0	NA	NA	NA	NA
As, mg/L	0.202	0.05	0.1	0.25	0.01–0.1	0.1	1.1–5.0
<b>Cd</b> , mg/L	0.609	0.01	0.02	0.03	0.003–0.1	0.03	1.0
Cr, mg/L	0.097	0.2	1.0	0.2–0.75	0.05–1.0	0.5	1.1
Cu, mg/L	0.055	0.2	1.0	2.0	0.1	3.0	NA
Fe, mg/L	6.545	1.0	5.0	NA	1.0–10.0	10	NA
Mn, mg/L	0.844	0.2	1.0	5.0	0.5–5.0	10	NA
Ni, mg/L	0.294	0.2	1.0	1.0	0.1–1.0	NA	NA
<b>Pb</b> , mg/L	0.897	0.1	0.5	0.2	0.1–1.0	0.1	5.0
Zn, mg/L	0.004	2.0	2.0	5.0	0.5–1.0	2.0	0.5
Se, mg/L	0.359	NA	NA	0.02	0.5–0.01	NA	1.0
Mg, mg/L	58.771	NA	NA	NA	150–200	NA	NA
Ca, mg/L	348.009	NA	NA	NA	150–200	NA	NA
K, mg/L	628.967	NA	NA	NA	NA	NA	NA
Na, mg/L	727.371	NA	NA	NA	NA	NA	NA

Abbreviation: NA, not available.

<sup>a</sup>Malaysia DOE.<sup>17</sup>

<sup>b</sup>Malaysia DOE.<sup>17</sup>

<sup>c</sup>Thailand Ministry of Science.<sup>18</sup>

<sup>d</sup>Singapore NEA.<sup>19</sup>

<sup>e</sup>Japan Ministry of Environment.<sup>20</sup>

<sup>f</sup>US EPA.<sup>21</sup>

<sup>g</sup>US EPA.<sup>22</sup>

Bold are the key elements (types of heavy metals).

## Results and Discussion

### *Effects on plant growth*

During the 75-day experimental period, all the 3 tropical plants recorded different growth trends. Table 3 shows that a significantly lower ( $P < .05$ ) leaf number was recorded in all soil-leachate treatments of *Mucuna* compared with the control. All soil-leachate treatments with the exception of the 80S + 20L for *Mucuna* showed significantly reduced ( $P < .05$ ) percentage of plant survivorship and plant height compared with the control. The results indicate that the application of soil-leachate had adverse effects on the growth of *Mucuna*. Recent studies by Nwaichi and Wegwu<sup>28</sup> and Azeez et al<sup>29</sup> reported similar effects in *Mucuna* (*Mucuna pruriens*) species regarding its growth rate and phyto-accumulative ability. However, no significant differences ( $P > .05$ ) were observed in all the soil-leachate treatments regarding plant height and leaf number between *Acacia* and the control. Similarly, *Vetiver* showed no significant difference ( $P > .05$ )

in terms of plant height and percentage survivorship in the treatments grown in soil-leachate conditions compared with the control. Between the 3 plant species, *Vetiver* exhibited appreciably higher plant height, leaf number, and percentage survivorship than both *Acacia* and *Mucuna*. Truong et al<sup>30</sup> and Danh et al<sup>31</sup> had earlier reported that *Vetiver* has high tolerance ability to survive under a wide range of contaminated conditions without affecting its growth. Nevertheless, only selected soil-leachate treatments (60S + 40L, 50S + 50L, 40S + 60L, and 100L) in *Vetiver* displayed significantly lower ( $P < .05$ ) leaf number compared with the control. All the 3 types of plant species showed progressive growth performance regarding plant height, leaf number, and percentage survivorship, particularly in the soil-leachate 80S + 20L treatment. The findings indicated that *Vetiver* was able to grow under both hydroponic and soil-leachate conditions as was reported by Chen et al<sup>32</sup> and recently by Truong and Danh,<sup>33</sup> whereas both *Acacia* and *Mucuna* were only able to survive under 20% soil-leachate conditions.

**Table 3.** Relative growth rate (g/d), plant height (cm), leaf number, and plant survivorship (%) of Acacia, Mucuna, and Vetiver as influenced by different levels of soil-leachate treatments.

TREATMENT	PLANT HEIGHT, CM	LEAF NUMBER	PLANT SURVIVORSHIP, %	RGR, G/D
<i>Acacia</i>				
Control	45.97 ± 1.88 abc	9.29 ± 3.42 ab	100.00 ± 0.00 a	0.01075 a
80S+20L	47.63 ± 11.12 ab	9.19 ± 3.4 ab	100.00 ± 0.00 a	0.00606 b
60S+40L	43.05 ± 8.40 bc	11.24 ± 5.28 ab	24.29 ± 10.95 b	-0.00107 c
50S+50L	43.10 ± 12.75 bc	10.33 ± 4.22 ab	22.86 ± 16.57 b	0.00225 bc
40S+60L	40.72 ± 10.02 c	7.52 ± 3.48 b	21.43 ± 15.47 b	0.00290 bc
20S+80L	46.62 ± 9.58 abc	7.86 ± 3.74 ab	23.57 ± 17.22 b	-0.00006 c
100L	49.71 ± 6.72 a	12.71 ± 3.96 a	22.14 ± 15.98 b	0.00323 bc
<i>Mucuna</i>				
Control	91.21 ± 13.62 a	27.00 ± 14.60 a	100.00 ± 0.00 a	0.02337 a
80S+20L	74.00 ± 24.16 a	20.05 ± 10.05 b	85.71 ± 10.23 a	0.00501 bcd
60S+40L	28.21 ± 12.46 b	2.76 ± 1.24 c	35.71 ± 22.61 b	0.00353 d
50S+50L	24.04 ± 16.62 b	2.48 ± 1.51 c	31.86 ± 17.55 b	0.00721 bc
40S+60L	32.10 ± 10.50 b	1.05 ± 0.85 c	23.86 ± 15.69 b	0.00729 bc
20S+80L	33.36 ± 15.63 b	1.62 ± 0.56 c	28.57 ± 19.29 b	0.00771 b
100L	35.52 ± 19.25 b	0.95 ± 0.42 c	19.00 ± 14.27 b	0.00459 cd
<i>Vetiver</i>				
Control	81.43 ± 4.29 ab	25.95 ± 9.34 ab	100.00 ± 0.00 a	0.01600 ab
80S+20L	93.71 ± 3.92 a	22.48 ± 3.25 abc	100.00 ± 0.00 a	0.01753 a
60S+40L	90.83 ± 12.05 ab	13.19 ± 2.54 d	98.57 ± 14.29 a	0.01161 c
50S+50L	75.95 ± 6.38 ab	16.86 ± 10.21 cd	97.14 ± 27.11 a	0.01439 abc
40S+60L	82.07 ± 7.36 a	13.81 ± 7.28 d	98.29 ± 20.57 a	0.01413 abc
20S+80L	74.99 ± 13.51 ab	28.29 ± 14.53 a	100.00 ± 0.00 a	0.01276 bc
100L	53.83 ± 10.32 b	16.19 ± 9.85 cd	100.00 ± 0.00 a	0.01238 c

Mean ± standard deviations followed by same letters are not significantly different for each treatment means at .05 levels of probability.

The overall RGR for Acacia and Mucuna was significantly decreased ( $P < .05$ ) in all the treatments compared with the control. The decrease in RGR may possibly be due to the accumulation of the metals and toxicity effects in the plants. Moreover, due to the high number of withered plants, both the 60S+40L (-0.00107 g/d) and 20S+80L (-0.00006 g/d) treatments in Acacia recorded a negative RGR compared with the other treatments. However, only 60S+40L (0.01161 g/d) and 100L (0.01238 g/d) soil-leachate treatments in Vetiver demonstrated significantly lower ( $P < .05$ ) RGR compared with the control. Nonetheless, among all the 3 plants, Vetiver (0.01161-0.01600 g/d) exhibited a reasonably higher RGR than both Acacia (0.01238-0.01075 g/d) and Mucuna (0.00353-0.02337 g/d). The effects of plant growth parameters such as

plant height, leaf number, and percentage survivorship contributed to the overall RGR of the plant.

Dry matter yield was significantly affected ( $P < .05$ ) by the soil-leachate treatment variables (Table 4). The lowest dry matter yield was observed in all of the roots and shoots of soil-leachate treatments in Mucuna as compared with the control. All the 3 plants recorded significantly lower ( $P < .05$ ) total dry matter yield in the soil-leachate treatment compared with the controls. The 80S+20L treatments in the shoots of both Acacia ( $7.76 \pm 1.37$  g/pot) and Vetiver ( $14.89 \pm 1.83$  g/pot), respectively, showed no significant differences ( $P > .05$ ) in terms of dry matter yield compared with the control. However, the opposite was observed in the other different soil-leachate level treatments. Among the 3 plant species, Vetiver recorded an appreciably

**Table 4.** Dry matter yield (g/pot area), root-shoot (R/S) ratio, and tolerance index (TI) of Acacia, Mucuna, and Vetiver as influenced by different levels of soil-leachate treatments.

TREATMENT	DRY MATTER YIELD (G/POT AREA)			R/S RATIO	TI
	ROOT	SHOOT	TOTAL		
<i>Acacia</i>					
Control	4.40±0.52 a	9.00±0.69 a	13.40±0.18 a	0.489 b	
80S+20L	3.16±0.59 b	7.76±1.37 ab	10.92±1.88 b	0.408 b	0.815 a
60S+40L	1.57±0.32 d	3.49±0.41 c	5.06±0.68 e	0.451 b	0.378 c
50S+50L	2.17±0.08 cd	4.69±0.50 c	6.86±0.53 cde	0.464 b	0.512 bc
40S+60L	2.40±0.68 bcd	5.20±1.63 bc	7.60±2.06 cd	0.462 b	0.567 bc
20S+80L	2.45±0.22 bcd	3.46±1.20 c	5.90±1.31 cde	0.709 a	0.441 bc
100L	2.68±0.63 bc	5.14±0.49 bc	7.82±0.84 c	0.522 ab	0.584 b
<i>Mucuna</i>					
Control	32.49±12.31 a	11.34±0.97 a	43.83±11.35 a	2.865 a	
80S+20L	1.56±0.29 b	2.55±0.33 b	4.11±0.43 b	0.610 b	0.094 a
60S+40L	1.40±0.25 b	2.66±0.57 b	4.06±0.32 b	0.525 b	0.093 a
50S+50L	1.13±0.17 b	2.98±0.31 b	4.11±0.27 b	0.381 b	0.094 a
40S+60L	1.17±0.39 b	3.30±0.35 b	4.47±0.06 b	0.354 b	0.102 a
20S+80L	0.88±0.08 b	3.47±0.92 b	4.35±1.00 b	0.254 b	0.099 a
100L	0.73±0.16 b	3.01±0.11 b	3.74±0.17 b	0.242 b	0.085 a
<i>Vetiver</i>					
Control	7.68±1.55 a	18.89±4.56 a	26.57±3.29 a	0.407 d	
80S+20L	7.10±1.82 a	14.89±1.83 a	21.99±3.39 b	0.476 cd	0.827 a
60S+40L	8.13±1.98 a	9.32±0.53 b	17.45±2.05 bc	0.872 abcd	0.657 a
50S+50L	7.82±2.66 a	7.87±1.28 b	15.69±3.95 c	0.994 abc	0.590 a
40S+60L	8.42±1.72 a	7.82±0.68 b	16.24±1.40 c	1.077 ab	0.611 a
20S+80L	8.57±2.03 a	10.20±1.61 b	18.77±3.52 bc	0.840 abcd	0.706 a
100L	9.88±2.18 a	7.99±2.11 b	17.87±2.13 bc	1.237 a	0.673 a

Mean ± standard deviations followed by the same letters are not significantly different for each treatment means at .05 levels of probability.

higher yield of dry matter yield than both Acacia and Mucuna. Nevertheless, the root-shoot (R/S) ratios of both Mucuna and Vetiver exhibited significant differences ( $P < .05$ ) under all the soil-leachate treatments compared with the control, regarding the TI that employed to evaluate the tolerance ability of a plant species to grow under soil-leachate conditions. Vetiver demonstrated higher TI than both Acacia and Mucuna whereby a  $TI \geq 1$  represents high tolerance proficiency. The results showed that Vetiver did not exhibit adverse growth effects and is able to withstand soil-leachate conditions compared with both Acacia and Mucuna. Hence, the findings demonstrate that Vetiver can act as a potential phytoremediator under the contaminated soil-leachate conditions.

#### *Distribution of Cd and Pb in the plants*

Both Cd and Pb accumulation in the roots and shoots of all the 3 types of plant species is shown in Tables 5 and 6. All 3 plants recorded significantly higher ( $P < .05$ ) Cd uptake in its roots and shoots and total metal accumulations under the soil-leachate treatments compared with the controls. Between the roots and shoots, Cd accumulation was considerably greater in the roots than in the shoots. Similarly, Pb uptake was significantly greater ( $P < .05$ ) in the roots and total metal accumulation under the soil-leachate treatments of both Mucuna and Vetiver than in the control. A significantly higher ( $P < .05$ ) accumulation of Pb was recorded in the shoots of all the



**Table 5.** Concentration of Cd (mg/kg) in the roots and shoots of Acacia, Mucuna, and Vetiver as influenced by different levels of soil-leachate treatments.

TREATMENT	CONCENTRATION OF CD, MG/KG		
	ROOT	SHOOT	TOTAL
<i>Acacia</i>			
Control	16.50±3.46 c	3.60±1.44 b	20.10±3.10 b
80S+20L	66.83±5.01 b	17.55±5.03 a	84.39±7.94 a
60S+40L	72.93±9.46 ab	16.60±4.44 a	89.53±13.58 a
50S+50L	83.80±9.78 ab	16.01±1.96 a	99.81±8.56 a
40S+60L	73.10±11.63 ab	16.25±4.55 a	89.35±9.17 a
20S+80L	84.93±8.41 ab	15.55±0.82 a	100.48±8.78 a
100L	90.20±15.10 a	13.59±2.18 a	103.79±17.28 a
<i>Mucuna</i>			
Control	4.18±1.94 c	0.91±0.61 b	5.08±2.37 b
80S+20L	20.95±2.60 b	7.11±0.48 a	28.07±2.94 a
60S+40L	24.97±7.51 ab	6.58±1.49 a	31.55±7.87 a
50S+50L	26.73±5.35 ab	5.57±1.11 a	32.31±5.36 a
40S+60L	23.35±2.17 ab	5.21±1.81 a	28.56±3.48 a
20S+80L	29.03±4.05 ab	5.75±1.07 a	34.78±4.15 a
100L	30.67±6.22 a	5.62±1.06 a	36.29±7.27 a
<i>Vetiver</i>			
Control	10.11±2.17 b	5.27±3.07 b	14.77±3.80 b
80S+20L	89.12±8.65 a	27.04±5.84 a	116.16±11.56 a
60S+40L	104.03±7.50 a	23.99±6.90 a	128.02±11.39 a
50S+50L	114.13±24.83 a	24.50±4.20 a	138.64±25.10 a
40S+60L	122.04±21.56 a	19.43±6.13 a	141.47±26.66 a
20S+80L	118.63±19.81 a	22.88±4.62 a	141.51±16.45 a
100L	120.93±20.46 a	19.03±3.61 a	139.97±18.61 a

Mean±standard deviations followed by the same letters are not significantly different for each treatment means at .05 levels of probability.

soil-leachate treatments in Mucuna and Vetiver with the exception of the 100L leachate treatment. However, 80S+20L, 60S+40L, and 50S+50L treatments caused a significant increase ( $P < .05$ ) in Pb uptake in the roots and in total metal accumulation of Acacia compared with the control. Nonetheless, only the 80S+20L treatment brought about a significantly larger ( $P < .05$ ) accumulation of Pb in the shoots of Acacia. The findings are contrary to the observations made by Majid et al<sup>34</sup>, Justin et al,<sup>35</sup> and Maiti et al<sup>36</sup> who reported that Acacia is a potential heavy metal phytoremediator in both roots and shoots. The possible reason for the substantial reduction in metal accumulation in the roots and shoots of Acacia in this study is due to the presence of high levels of soil-leachate conditions.

Comparatively, between roots and shoots, all plant species accumulated higher amounts of Pb in the roots than in the shoots. The accumulation trend for both Cd and Pb in different plants was in the order of Vetiver > Acacia > Mucuna for all of the soil-leachate treatments. The shoots of 80S+20L treatment in Vetiver recorded the highest amount of Cd (27.04±5.84 mg/kg) and Pb (165.24±26.54 mg/kg) accumulation compared with both Acacia and Mucuna. The substantial reduction in both Cd and Pb accumulation in Acacia and Mucuna compared with Vetiver was likely due to the plant withering during the experimental period. The results indicated that the concentrations of both Cd and Pb accumulated in the shoots of all the 3 plants decreased progressively as a result of the increased application of soil-leachate levels.

**Table 6.** Concentration of Pb (mg/kg) in the roots and shoots of Acacia, Mucuna, and Vetiver as influenced by different levels of soil-leachate treatments.

TREATMENT	CONCENTRATION OF PB, MG/KG		
	ROOT	SHOOT	TOTAL
<i>Acacia</i>			
Control	99.33±16.52 d	23.03±11.55 b	122.37±17.74 d
80S+20L	218.60±38.25 abc	90.33±17.60 a	308.93±21.30 abc
60S+40L	269.20±86.59 ab	56.10±11.11 ab	325.30±86.08 ab
50S+50L	281.03±74.57 a	49.47±5.70 b	330.50±71.31 a
40S+60L	178.87±49.41 bcd	40.80±23.11 b	219.67±72.02 abcd
20S+80L	146.17±30.55 cd	43.60±26.44 b	189.77±54.35 d
100L	144.13±14.26 cd	46.43±28.38 b	190.57±42.49 d
<i>Mucuna</i>			
Control	26.97±7.39 c	13.67±1.05 d	40.63±7.47 c
80S+20L	50.43±13.20 b	26.87±4.23 a	77.30±10.06 b
60S+40L	49.30±7.84 b	26.40±4.25 a	75.70±7.82 b
50S+50L	56.07±12.72 ab	25.73±3.56 a	81.80±16.13 b
40S+60L	52.83±6.92 ab	22.93±2.99 bc	75.77±6.14 b
20S+80L	56.33±12.97 ab	24.33±3.36 ab	80.67±14.09 b
100L	72.73±13.59 a	21.07±1.37 c	93.80±12.22 a
<i>Vetiver</i>			
Control	49.57±17.04 e	32.97±7.74 d	82.53±24.51 e
80S+20L	200.03±11.07 a	165.24±26.54 a	365.27±35.27 a
60S+40L	170.33±30.07 abc	138.73±32.13 a	309.07±2.24 b
50S+50L	180.90±22.68 ab	94.77±8.92 b	275.67±19.02 bc
40S+60L	137.53±20.68 cd	92.07±7.51 bc	229.60±23.31c
20S+80L	105.60±14.54 d	69.23±13.80 bc	174.83±15.17 d
100L	95.87±28.85 d	60.50±5.31 cd	156.37±34.10 d

Mean±standard deviations followed by the same letters are not significantly different for each treatment means at .05 levels of probability.

### *Metal translocation in plant*

The association of Cd and Pb accumulated from the soil-leachate treatments into the roots and shoots in all the 3 plants is presented in terms of TF, MAR, and percentage of metal uptake efficacy, as shown in Table 7. Considering the relatively lower accumulation of Cd and Pb in the shoots than the roots in all the 3 plants, TF was used to assess the capability of the plant to translocate metal from the roots to the shoots. The 80S+20L (0.324) and 100L (0.299) treatments in Mucuna recorded significant differences ( $P<.05$ ) of TF values compared with the control for the accumulation of Cd and Pb, respectively. Nevertheless, no significant differences ( $P>.05$ ) of TF values were observed in Acacia for both Cd and Pb

accumulation. However, Cd accumulation in all soil-leachate treatments of Vetiver showed significantly lower ( $P<.05$ ) TF values compared with the control. The plant response to stressful conditions, caused by both Cd and Pb present in the soil-leachate conditions, may have affected the translocation of metals from the soil to the above ground parts of the plants (shoots) and hence influence the overall TF values. With the relatively lower TF values, the findings suggest that both Cd and Pb accumulation favored translocation from the soil-leachate source into the roots than shoots in all 3 tropical plants. Although the TF values were <1, Vetiver (0.531-0.852) exhibited appreciably higher TF in the accumulation of Pb than both Acacia (0.188-0.433) and Mucuna (0.299-0.566).

**Table 7.** Metal accumulation of cadmium (Cd) and lead (Pb) in its translocation factor (TF), metal accumulation ratio (MAR), and metal uptake efficacy (%) of Acacia, Mucuna, and Vetiver as influenced by different levels of soil-leachate treatments.

TREATMENT	ACCUMULATION OF CD			ACCUMULATION OF PB		
	TF	MAR	EFFICACY, %	TF	MAR	EFFICACY, %
<i>Acacia</i>						
Control	0.230 a	0.471 ab	18.16 a	0.239 a	0.489 b	18.63 a
80S+20L	0.263 a	0.645 a	20.61 a	0.433 a	1.064 a	29.59 a
60S+40L	0.226 a	0.501 ab	18.36 a	0.229 a	0.509 ab	18.22 a
50S+50L	0.194 a	0.419 ab	16.18 a	0.188 a	0.407 b	15.63 a
40S+60L	0.231 a	0.499 ab	18.43 a	0.218 a	0.471 b	17.73 a
20S+80L	0.184 a	0.260 b	15.53 a	0.284 a	0.400 b	21.32 a
100L	0.151 a	0.289 b	13.10 a	0.312 a	0.479 b	23.07 a
<i>Mucuna</i>						
Control	0.229 bc	0.080 b	17.85 a	0.530 ab	0.185 c	34.28 ab
80S+20L	0.342 a	0.559 a	25.44 a	0.566 a	0.926 b	35.41 a
60S+40L	0.277 b	0.527 a	21.49 a	0.546 ab	1.037 b	35.01 a
50S+50L	0.215 bc	0.568 a	17.55 a	0.466 ab	1.230 ab	31.74 ab
40S+60L	0.222 bc	0.626 a	18.01 a	0.441 b	1.244 ab	30.40 b
20S+80L	0.201 c	0.793 a	16.64 a	0.447 b	1.762 a	30.62 b
100L	0.184 c	0.758 a	15.52 a	0.299 c	1.231 b	22.83 c
<i>Vetiver</i>						
Control	0.503 a	1.237 a	32.52 a	0.690 a	1.424 a	40.62 a
80S+20L	0.304 b	0.639 b	23.21 ab	0.824 a	1.308 ab	45.05 a
60S+40L	0.231 b	0.265 bc	18.60 b	0.852 a	0.664 abc	44.84 a
50S+50L	0.221 b	0.222 bc	17.97 b	0.531 a	0.447 c	34.51 a
40S+60L	0.157 b	0.146 c	13.55 b	0.679 a	0.511 bc	40.28 a
20S+80L	0.200 b	0.238 bc	16.48 b	0.671 a	0.634 abc	39.56 a
100L	0.162 b	0.131 c	13.86 b	0.661 a	0.395 c	39.47 a

Mean followed by the same letters are not significantly different for each treatment means at .05 levels of probability.

The MAR and percentage of metal uptake efficacy were calculated to evaluate the potential and efficiency of the overall metal translocation and bioaccumulation in the plants. The MAR revealed that the accumulation of both Cd and Pb was significantly greater ( $P < .05$ ) in all the soil-leachate treatments of Mucuna than the control. The results indicated that the application of high levels of soil-leachate materials can enhance metal accumulation in Mucuna. Conversely, all soil-leachate treatments of Cd accumulation together with 50S+50L, 40S+60L, and 100L treatments of Pb accumulation in Vetiver recorded a significant decrease ( $P < .05$ ) in MAR compared with the control. Furthermore, a significantly higher ( $P < .05$ ) MAR for Pb accumulation was solely observed in the 80S+20L treatment of Acacia among other treatments. However, no significant difference ( $P > .05$ ) in percentage

Cd efficacy was recorded between all soil-leachate treatments and the control in both Acacia and Mucuna. Similarly, Acacia and Vetiver exhibited no significant differences ( $P > .05$ ) in percentage Pb metal efficacy among all soil-leachate treatments and the control. Despite plant withering in Acacia and Mucuna, both plants demonstrated reasonably high MAR and percentage of metal efficacy in the accumulation of Cd and Pb. These findings reveal that the uptake of both Cd and Pb may not be strictly inhibited by the plant growth rate over time and its capability to bioaccumulate before the plants gradually withered due the presence of excessive amounts of soil-leachate materials.<sup>37,38</sup> Among the different plants, Vetiver (13.55%-32.52% of Cd and 34.51%-45.05% of Pb) recorded remarkably higher percentages for both Cd and Pb metal efficacy than both Mucuna (15.52%-25.44% of Cd and



22.83%–35.41% of Pb) and Acacia (13.10%–20.61% of Cd and 15.63%–29.59% of Pb), respectively. The positive characteristics of Vetiver with fast growth, good tolerance, and its ability to withstand high concentration levels of soil-leachate conditions were similarly reported in previous works<sup>30,31,39,40</sup> and Vetiver remained to be the most promising species compared with the other tropical plants studied for the use in soil-leachate phytoremediation.

## Conclusions

The trend for both Cd and Pb accumulation, under soil-leachate conditions, in all the 3 tropical plants studied was in the order of Vetiver > Acacia > Mucuna. All 3 plants accumulated remarkably higher concentrations of both Cd and Pb in the roots and shoots. However, Vetiver exhibited the greatest potential for phytoremediation under soil-leachate contaminated conditions owing to its good tolerance ability to withstand soil-leachate and high percentage metal efficacy for both Cd and Pb. Vetiver was also fast growing and showed high dry matter yields production compared with both Acacia and Mucuna. In short, Vetiver would be a suitable plant species to be used for practical application and consideration on heavy metal phytoremediation and/or landfill cover.

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## Author Contributions

CCN and MMR conceived and designed the research study. CCN performed the field work and collect the experimental data. CCN, ANB, MMR and MRA analyzed the data. CCN and ANB wrote the manuscript. ANB, MMR, MRA and NZM provided their inputs for improving the manuscript quality. All authors read and approved the final manuscript.

## REFERENCES

- Renou S, Givaudan JG, Poulain S, Dirassouyan F, Moulin P. Landfill leachate treatment: review and opportunity. *J Hazard Mater.* 2008;150:468–493.
- Foo KY, Hameed BH. An overview of landfill leachate treatment via activated carbon adsorption process. *J Hazard Mater.* 2009;171:54–60.
- Kabata-Pendias A. *Trace Elements in Soils and Plants.* New York, NY: CRC Press; 2010.
- Kjeldsen P, Barlaz MA, Rooker AP, Baun A, Ledin A, Christensen TH. Present and long-term composition of MSW landfill leachate: a review. *Crit Rev Environ Sci Technol.* 2002;32:297–336.
- Memon AR, Schröder P. Implications of metal accumulation mechanisms to phytoremediation. *Environ Sci Pollut R.* 2009;16:162–175.
- Ali H, Khan E, Anwar M. Phytoremediation of heavy metals—concepts and applications. *Chemosphere.* 2013;91:869–881.
- US EPA. *Priority Pollutant List—Federal Water Pollution Control: Clean Water Act 33.* Washington, DC: United States Environment Protection Agency; 2014.
- Tsao D. *Phytoremediation.* Berlin, Germany; Heidelberg, Germany: Springer-Verlag; 2003.
- Ashraf M, Ozturk M, Ahmad MSA. *Plant Adaptation and Phytoremediation.* Dordrecht, The Netherlands: Springer; 2010.
- Golubev IA. *Handbook of Phytoremediation.* New York, NY: Nova Science; 2011.
- Furini A. *Plants and Heavy Metals: Springer Briefs in Biometals.* Dordrecht, The Netherlands: Springer; 2012.
- Ng CC, Rahman MM, Boyce AN, Abas MR. Heavy metals phyto-assessment in commonly grown vegetables: water spinach (*I. aquatica*) and okra (*A. esculentus*). *SpringerPlus.* 2016;5:469.
- Ng CC, Law SH, Boyce AN, Rahman MM, Abas MR. Phyto-assessment of soil heavy metal accumulation in tropical grasses. *J Anim Plant Sci.* 2016;26:686–696.
- Adie GU, Osibanjo O. Accumulation of lead and cadmium by four tropical forage weeds found in the premises of an automobile battery manufacturing company in Nigeria. *Toxicol Environ Chem.* 2010;92:39–49.
- Zeng X, Gao G, Yang J, Yuan J, Yang Z. The integrated response of torpedograss (*Panicum repens*) to Cd–Pb co-exposures. *Ecol Eng.* 2015;82:428–431.
- Wu Z, Wu W, Zhou S, Wu S. Mycorrhizal inoculation affects Pb and Cd accumulation and translocation in Pakchoi (*Brassica chinensis* L.). *Pedosphere.* 2016;26:13–26.
- Malaysia DOE. *Environmental Quality Act 1974; Environmental Quality (Industrial effluent) Regulations 2009: Standard A and B.* Putrajaya, Malaysia: Department of Environment; 2009.
- Thailand Ministry of Science. *Technology and Environment, Enhancement and Conservation of the National Environmental Quality Act 1992.* Bangkok, Thailand: Ministry of Science, Technology and Environment; 1992.
- Singapore NEA. *Environmental Protection and Management (Trade effluent) Regulations 2008: Maximum Concentrations of Metals in Trade Effluent.* Singapore: National Environment Agency; 2008.
- Japan Ministry of Environment. *Water Pollution Control Law—Uniform National Effluent Standards.* <https://www.env.go.jp/en/water/wq/nes.html>. Accessed November 22, 2016.
- US EPA. *Effluent Limitations Guidelines, Pretreatment Standards, and New Source Performance Standards for the Landfills Point Source Category.* Washington, DC: United States Environment Protection Agency; 2000.
- US EPA. *Maximum Concentration of Contaminants for the Toxicity Characteristic Leaching Procedure.* Washington, DC: United States Environment Protection Agency; 2004.
- US EPA. *Method 3050B Acid Digestion of Sediments, Sludges and Soils.* Washington, DC: United States Environment Protection Agency; 1996.
- US EPA. *Method 7000B Flame Atomic Absorption Spectrophotometry.* Washington, DC: United States Environment Protection Agency; 2007.
- Watson DJ. The physiological basis of variation in yield. *Adv Agron.* 1952;4:101–145.
- Hunt R. Relative growth rates. In: Hunt R, ed. *Basic Growth Analysis.* Dordrecht, The Netherlands: Springer; 1990:25–34.
- Poorter H, Garier E. Ecological significance of inherent variation in relative growth rate and its components. In: Pugnaire F, Valladares F, eds. *Handbook of Functional Plant Ecology.* London, England: Taylor & Francis; 1999:81–120.
- Nwaichi EO, Wegwu MO. Nutrient profile of a contaminated soil phytoremediated by *Centrosema pubescens* and *Mucuna pruriens*. *Bioremediat J.* 2012;16:212–217.
- Azeez JO, Hassan OA, Adesodun JK, Arowolo TA. Soil metal sorption characteristics and its influence on the comparative effectiveness of EDTA and legume intercrop on the phytoremediative abilities of Maize (*Zea mays*), Mucuna (*Mucuna pruriens*), Okra (*Abelmoschus esculentus*), and Kenaf (*Hibiscus cannabinus*). *Soil Sediment Contam.* 2013;22:930–957.
- Truong P, Tan Van T, Pinners E. *Vetiver System Applications Technical Reference Manual.* The Vetiver Network International; 2008.
- Danh LT, Truong P, Mammucari R, Tran T, Foster N. Vetiver grass, *Vetiveria zizanioides*: a choice plant for phytoremediation of heavy metals and organic wastes. *Int J Phytoremediation.* 2009;11:664–691.
- Chen KF, Yeh TY, Lin CF. Phytoextraction of Cu, Zn, and Pb enhanced by chelators with Vetiver (*Vetiveria zizanioides*): hydroponic and pot experiments. *ISRN Ecol.* 2012;2012:729693.
- Truong P, Danh LT. *The Vetiver System for Improving Water Quality: Prevention and Treatment of Contaminated Water and Land.* The Vetiver Network International; 2015.
- Majid NM, Islam MM, Justin V, Abdu A, Ahmadpour P. Evaluation of heavy metal uptake and translocation by *Acacia mangium* as a phytoremediator of copper contaminated soil. *Afr J Biotechnol.* 2011;10:8373–8379.
- Justin V, Majid NM, Islam MM, Abdu A. Assessment of heavy metal uptake and translocation in *Acacia mangium* for phytoremediation of cadmium-contaminated soil. *J Food Agric Environ.* 2011;9:588–592.
- Maiti SK, Kumar A, Ahirwal J. Bioaccumulation of metals in timber and edible fruit trees growing on reclaimed coal mine overburden dumps. *Int J Min Reclam Env.* 2016;30:231–244.
- Shahandeh H, Hossner LR. Plant screening for chromium phytoremediation. *Int J Phytorem.* 2000;2:31–51.
- Merkel N, Schultze-Kraft R, Infante C. Assessment of tropical grasses and legumes for phytoremediation of petroleum-contaminated soils. *Water Air Soil Poll.* 2005;165:195–209.
- Ng CC, Boyce AN, Rahman MM, Abas MR. Effects of different soil amendments on mixed heavy metals contamination in Vetiver grass. *Bull Environ Contam Toxicol.* 2016;97:695–701.
- Ng CC, Boyce AN, Rahman MM, Abas MR. Tolerance threshold and phyto-assessment of Cadmium and Lead in Vetiver grass, *Vetiveria zizanioides* (Linn.) Nash. *Chiang Mai J Sci.* 2017;44:1367–1378.