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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 soilleachate 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 (116.16-141.51mg/kg) and Pb (156.37-365.27mg/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.^{1,2} 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.3

Generally, the heavy metals found in a landfill leachate consist of Cd, Cu, Cr, Pb, Ni, and Zn.⁴ 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.^{5,6} 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

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Pb are categorized among the 126 chemical pollutants of the Toxic and Priority Pollutants.7

The use of various tropical plants for heavy metal phytoremediation has been expansively studied over the years.8-13 Recent reports by previous works¹⁴⁻¹⁶ 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 soilleachate 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 planthouse located in Rimba Ilmu, Institute of Biological Sciences,

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Table 1. Experimental treatment variables.

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 $(0.18 \text{ m diameter} \times 0.16 \text{ m depth})$ 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 ovendried for 72hours 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 (HNO₃), and hydrogen peroxide (H₂O₂) according to the Method 3050B23 followed by Method 7000B24 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, $25-27$ 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:

R/S ratio = Dry matter yield in root / Dry matter yield in shoot TI = Total dry matter yield in treatment / Total dry matter yield in control $RGR =$ ln Final biomass of treatment ln Initial biomass $=\left(\ln \left(\text{Final biomass of treatment} \right) \right)$ (Initial biomass of treatment) Days of growth / $TF = Metal concentration accumulated in shoot /$ I L I \mathbf{r} L \overline{a} Metal concentration accumulate d in root $MAR =$ Metal concentration accumulated in shoot $=\left[\times \text{Dry matter yield in shoot}\right]$ Metal concentration accumulated in root \mathbf{r} L \overline{a} I

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 Dry matter yield in root

 \overline{a}

Metal uptake

 $\text{efficacy}(\%) = [\text{Meta}$ concentration accumulated in shoot / Total metal concentration removed from the growth media] \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.

Abbreviation: NA, not available.

aMalaysia DOE.¹⁷

bMalaysia DOE.17 ^cThailand Ministry of Science.¹⁸

dSingapore NEA.¹⁹

eJapan Ministry of Environment.²⁰

f US EPA.21

gUS EPA.22

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 Wegwu²⁸ and Azeez et al²⁹ 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 al³⁰and Danh et al³¹ 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 al³² and recently by Truong and Danh,³³ 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.

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- 0.01600g/d) exhibited a reasonably higher RGR than both Acacia (0.01238-0.01075g/d) and Mucuna (0.00353- 0.02337g/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 soilleachate 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 $(7.76 \pm 1.37 \text{ g}/\text{pot})$ and Vetiver $(14.89 \pm 1.83 \text{ g}/\text{pot})$, 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.

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 ≥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

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 al³⁴, Justin et al,³⁵ and Maiti et al³⁶ 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 $(27.04 \pm 5.84 \,\text{mg/kg})$ and Pb $(165.24 \pm 26.54 \,\text{mg/kg})$ 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.

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.

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 soilleachate 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 soilleachate materials.37,38 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^{30,31,39,40} and Vetiver remained to be the most promising species compared with the other tropical plants studied for the use in soilleachate 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 soilleachate 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.

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