Phytoextraction of Copper and Lead from Spiked Soil using Acalypha wilkesiana (Copper leaf) and Polyscias fruticosa (Aralia)

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Abstract

Soil contaminated with heavy metals industrially or anthropogenically is a major global concern. This study aimed at investigating the potential of Acalypha wilkesiana and Polyscias fruticosa plants to remove copper (Cu) and lead (Pb) from soil spiked with solutions of Cu and Pb. Information on the ability of both plants to uptake and accumulate Cu and Pb from spiked soil is insufficient. Plant cuttings were grown in a 2 kg soil spiked with heavy metals and kept at the greenhouse for the period of 240 days. Root, stem and leaf of the plants were analyzed for Cu and Pb using Atomic Absorption Spectrophotometer (AAS). Cu accumulation (2.58 -542.66 mg/kg) was appreciably higher than Pb accumulation (26.24 - 46.44 mg/kg) in A. wilkesiana, while Cu accumulation ranged 4.54 - 384.19 mg/kg was found to be lower than the Pb accumulation ranged 1.22-759.48 mg/kg in P. fruticosa. The maximum accumulation of Cu and Pb in the shoot were found to be 52.50 and 82.93 mg/kg in A. wilkesiana, while 38.26 and 123.98 mg/kg in P. fruticosa. Accumulation of Cu and Pb by both plants were correlated using the indices bioaccumulation, transfer factor and translocation index. The result is encouraging and suggests that both plants could be applied for phytoextraction of Cu and Pb from heavy metal spiked soil.

Keywords: Phytoextraction; Spiked soil; Copper; Lead; Acalypha wilkesiana; Polyscias fruticosa.

INTRODUCTION

Soils that are polluted with heavy metals concentration causes an environmental issue which requires a potent and low-cost solution. Contaminated soil can be restored by applying techniques such as physical, chemical, bioremediation and phytoremediation. "In recent years, efforts have focused on the remediation strategies that are less expensive and less destructive than current approaches. Phytoextraction emerged as an intense research effort for more efficient and less hazardous techniques to remediate contaminated soils. It comprises the removal of metals by plants through uptake and accumulation into body parts" (Rahman *et al.*, 2013). Pb contamination can be reduced by the use of phytoremediation method which is an alternative to other physical and chemical remediation techniques (Pas-Alberto and Sigua, 2013). Cu is an essential metal for plant growth in which if it's accumulation in the shoots is greater than 20 mg/kg it can cause a harmful effect (Borkert *et al.*, 2008). Activities in the urban areas such as traffic, metal industries and housing increases Cu concentration. The primary principle of phytoremediation is considered to be the use of plants together with companion soil microbes to lower the quantity or harmful effects of pollutants in the environments (Durumin Iya et al., 2018). Phytoremediation of contaminated environment is cost effective, socially acceptable and eco-friendly technique for the recovery of deprived site (Grobelak et al., 2010).

Plants that are used for an efficient phytoremediation techniques should tolerate and accumulate the element of interest, grow fast, easy propagation, good root system and produces high biomass (Rodriguez-Vila *et al.*, 2015). It was reported that *P. fruticosa* has the potential to remove some organic compounds such as octane (C_8) from indoor air (Reshma *et al.*, 2017). A pot experiment on *A. wilkesiana* plant was conducted to investigate the accumulation and translocation of heavy metals from a spiked soil (Durumin Iya *et al.*, 2018). Available information on the potential of *P. fruticosa* and *A. wilkesiana* to remove Cu and Pb from contaminated (spiked soil) soil is inadequate. In this study, *P. fruticosa* (Ming Aralia) of Araliaceae family was selected for its ability to produce good root system and more biomass. *A. wilkesiana* plant (Copper leaf) from the family Euphorbiacece was also selected due to its potential to grow fast and good root system.

The objective of the study was (i) to investigate the survival ability and growth of both plants in spiked soil (ii) to determine the potential of *A. wilkesiana* and *P. fruticosa* using a pot experiment to remove Cu and Pb from soil spiked with heavy metals. Several issues were addressed in the experiment such as translocation index of the metals, shoot/root concentration ratio of the metals and removal percentage of the metals. The novelty of work is the ability of the two plants to extract Cu and Pb from the spiked soil to the root and subsequently translocate it to the stem and leaves in a usual plant growth condition at the greenhouse.

METHODOLOGY

Experimental Set Up

The soil for this research was collected from Kuching area, Sarawak Malaysia. It was dried in a room, homogenized and bigger particles were removed. A total of 48 polyethylene bags were filled with approximately 2.0 kg of the top soil before spiking with heavy metals solution.

Physical and Chemical Characteristics of top soil

The following soil parameters were measured before and after spiking the top soil sample with heavy metals solution. Soil pH was measured with a pH meter (ThermoOrion model-420) using a 1:2.5 soil: water ratio (Malik *et al.*, 2010), while EC was measured with conductivity meter (Farahat *et al.*, 2017). The total nitrogen in soil sample was determined according to the Kjeldahl method (Nelson and Sommers, 1972). The cationic exchange capacity was measured by the ammonium acetate method (Luo *et al.*, 2010). Available phosphorous, potassium and soil moisture contents were determined following the procedure described by Fati (2011).

Soil Sample Spiking

A pot experiment was carried out according to the procedure outlined by Singh *et al.* (2010). A stock solution of 1000 mg/L of Pb and Cu were prepared. Each 2.0 kg of soil sample was spiked and mixed thoroughly with a 10.0 mL of 100.0 mg/L solution at a greenhouse.

Plant Cuttings

P. fruticosa and A. wilkesiana cuttings (about 5.0 cm each) were made from young healthy plants and kept in a room for 2 hours to dry up, and then about 2.0 cm from the bottom of the cuttings were scraped with a sterilized blade to enable the growth of roots. Several cuttings were made and planted on an uncontaminated sand for two weeks and the best germinating plants were selected for pot experiment.

Planting of plants cuttings

Selected young plants were transplanted into each poly bag (one plant per poly bag). The control poly bags contains unspiked soil with planted plant. The experimental set up was carried out in the growth chamber at green house of faculty of Resources Science and Technology, Universiti Malaysia Sarawak (N 01° 33′ 03.6″ E 110° 45′ 56.5″). The plant condition of growth at greenhouse were as follows: 16 hours light at 22 °C and 8 hours dark at 18 °C, humidity 59%, netted and received water when needed. The same treatment was applied to all the plants. The pots experimental designs were set and plants were planted on 25th October, 2017, while harvesting of the plant started on December, 2017 until June, 2018. To avoid leaching from the spiked soil contents, each polybag was placed on a small plastic tray and any leached liquid was returned back to the polybag.

Soil Sample Analysis

A soil analysis for heavy metals was performed according to procedure used by Hseu (2004) with minor modification. Approximately, 1.0 gram of soil sample in a crucible was heated to 550 °C for 4 hours in a muffle furnace model STUART/FX105-27. A volume of 1.0 mL distilled water and 1.0 mL of concentrated HNO₃ were added to the sample and heated to dryness on a hot plate model Thermo-scientific 5P131320-33 in a fume cupboard. After drying, crucible was heated in a furnace for 15 minutes at 400 °C. A volume of 1.0 mL distilled water was added followed by 2.0 mL of concentrated HCl and evaporated to dryness on hot plate. A volume of 2.0 mL concentrated HCl was then added to crucible and filtered through 0.45 µm filter paper. It was then transferred quantitatively into 100 mL capacity volumetric flask and was made to the mark with distilled water. Several soil properties were analyzed such as cation exchange capacity (Aprile and Lorandi, 2012), total nitrogen (AOAC, 1990), soil moisture, pH , electric conductivity, phosphorus and potassium content (Reynolds, 1971).

Plant organ anlysis

Prior to Atomic Absorption Spectrophotometer (AAS) analyses, plants were divided into root, stem and leaf, washed and dried in an oven for 48 hours at 60 °C (Ugolini *et al.*, 2013). The sample was grind and analysed according to the procedure described by Soon (1998) with minor modification. Exactly 1.0 g of ground plant parts in a crucible was ashes in a muffle furnace for 4 hours at 480 °C. After cooling, 1.0 mL of distilled water and 2.0 mL of concentrated HNO₃ acid was added. The sample was evaporated to dryness on a hot plate. After cooling, it was then dissolved in 2 mL of concentrated HNO₃ acid by heating on a hot plate at approximately 100 °C. The sample was filtered through microporous membrane 47 mm and 0.45 µm filter paper into 50 mL volumetric flask and made it up to the mark with distilled water. Plants and soil samples were analysed for Cu and Pb metals using Atomic Absorption Spectrophotometer (AAS) model Optima 8300 series.

Analysis of Data

Data obtained was analyzed to estimate the capability of the plants for Cu and Pb removal. The Cu and Pb uptake from root to the shoots (stem + leaf) was evaluated using the transfer factor from equation (1) (Lubben and Sauerbeck, 1991). TF= SC + (RC/TC) (1)

where, SC - metal concentration in shoots, RC - metal concentration in root and TC - metal concentration in soil. The potential of the plants to translocate Cu and Pb from the roots to the shoots was obtained by translocation index (TI) using equation (2) (Paiva *et al.*, 2002). % TI = (SMC/TPA)*100 (2)

where, SMC - metal accumulation in the shoots and TPA -total metal accumulation in the whole plant (shoots + roots). Percentage removal of Cu and Pb metals was calculated using equation (3) (Rahman *et al.*, 2013).

% R= TCM/IMC

where, TC is concentration of metal in plant and IMC - the initial status of metal present in soil. Shoot to root concentration ratio for both plants was calculated using equation (4) (Santos *et al.*, 2010).

SR=SC/RC

(4)

(3)

A bioaccumulation coefficient was calculated as the ratio of metal concentration in plants to the metal concentration in the soil (Hseu, 2004). BCF = (Metal in plant_root/Metal in soil) (5)

RESULTS AND DISCUSSION

The top soil pH was found to be 7.06, which is neutral (Table 1). The raise in the soil pH value will decrease the soluble forms of trace metals in the soil sample which potentially restrict heavy metal uptake by plants tissues. Moreover, leaching from biogenic components are reduced or stopped by the raise in soil pH level (Placek et al., 2016). The CEC value obtained for the soil sample was low (Table 1). Thus, the higher the CEC of the soil, the greater the sorption and immobilization of the metals (Bhargava *et al.*, 2012). However, some soil properties are more important than others, CEC is more relevant than the pH value in affecting the translocation of some heavy metals to the above ground tissue (Luo, *et al.*, 2010).

Table 1: Some soil parameters analyzed					
Soil properties	Concentration				
pH	7.06				
CEC	0.6 mmol/kg				
Moisture content	97%				
Electric conductivity	0.20 μs/cm				
Nitrogen	110 mg/kg				
Phosphorous	8.01 mg/kg				
Potassium	96.5 mg/kg				
Copper	49.32±0.08 mg/kg				
Lead	37.07±0.16 mg/kg				

The physical symptoms of plant toxicity from the effect heavy metals differs among the plant species and metals tested. In general, *A. wilkesiana* and *P. fruticosa* plants appeared to be healthy and tolerate the contaminated soil condition of excess metals. The plants did not show any sign of chlorosis, purple spots and browning, wrinkling, decrease in growth, necrosis and leaf death.

Accumulation of Cu and Pb content in plants organs

Prior to planting of young plants into spiked soil, concentration of Cu and Pb was determined after the soil was spiked with solution of heavy metals, Cu and Pb mean values were 353.97 ± 0.39 and 84.47 ± 0.06 mg/kg, respectively. Both plants survived and grown in heavy metals spiked soils. The uptake and accumulation of Cu and Pb in the roots of the plants were found higher compared to their stem and leaves (Table 2). It was reported that Cu concentration in the roots of *Brassica juncea* was found higher than the shoots grown on Cu amended soil (Ariyakanon and Winaipanich, 2006). Durumin Iya *et al.* (2018), recorded a higher concentration of Cu in the root of *A. wilkesiana* compared to the stem and leaf. The higher concentration of Cu observed in the root could be because of its continue Cu observed in the root could be due to its continues movement from soil to roots indicating the affinity of roots to accumulate higher amount of Cu and Pb in the roots (Table 2) and shoots (Table 3) of *A. wilkesiana* were found to be high on 180 and 240 harvesting days, respectively.

	Cu							
Plants type	Soil	Root	Stem	Leaf	Soil	Root	Stem	Leaf
A.wilkesian								
а								
Control A	49.32±0.08	1.86 ± 0.01	1.05 ± 0.02	0.96 ± 0.01	46.40±0.21	6.23±0.03	2.02±0.01	1.62 ± 0.01
60 days	162.69±7.96	292.44±5.66	22.14±0.64	13.17±0.71	3.37±0.25	26.24±1.19	30.66±0.85	27.36±1.07
120 days	163.47±1.22	362.41 ± 0.44	2.58 ± 0.29	10.89 ± 1.10	8.35±0.23	36.22±1.19	41.18±1.55	30.98 ± 0.34
180 days	103.54 ± 1.05	542.66±2.26	3.26 ± 0.05	53.24±2.36	11.44 ± 0.52	34.32±1.50	38.84±1.51	36.88±1.23
240 days	83.81±0.75	461.92±2.18	2.68 ± 0.03	44.98±1.57	14.75 ± 0.49	42.76±1.22	46.44±2.18	36.49±1.49
P. fruticosa								
Control B	37.07±0.16	1.64 ± 0.03	1.27±0.03	0.89 ± 0.01	35.03±0.15	5.97±0.03	2.33±0.03	1.51±0.02
60 days	197.14±17.05	42.62±0.55	19.88±0.92	6.71±0.25	16.25±0.29	3.09±0.25	1.63±0.17	1.22 ± 0.17
120 days	153.09±1.80	36.88±0.96	7.08±0.12	4.54±3.32	7.94±6.34	8.94±7.27	14.50±13.63	32.85±5.85
180 days	143.75 ± 0.74	44.19±1.69	6.04±1.39	8.60 ± 1.74	58.40±16.49	11.07±2.78	19.90±14.75	61.71±17.29
240 days	115.26±1.02	69.58±0.85	21.87±0.69	16.39±0.98	75.91±2.33	21.75±3.51	27.61±2.65	96.37±3.89
Control A = unspiked soil planted with A. wilkesiana Control B = unspiked soil planted with P. fruticosa plant								

Table 2: Mean (n=3) values of Cu and Pb Accumulation in Plants Roots, Stem, Leaves and Soil (mg/kg)

	Translocation Index TI%		Shoot/metals	Concentration	Total plant	
			(SMC) Cu Pb		Accumulation (TPA)	
	Cu	Pb			Cu	Pb
A.wilkesiana						
Control A	2.01±0.03	3.64 ± 0.02	3.87±0.04	9.87±0.05	51.9	36.9
60 days	35.31±1.35	57.93±1.92	327.75±7.01	84.17±3.11	10.8	68.8
120 days	13.47±1.39	72.16±1.89	375.88±1.83	108.38±3.08	3.6	66.6
180 days	56.50 ± 2.41	75.72±2.74	599.16±4.67	110.04 ± 4.24	9.4	68.8
240 days	47.66±1.60	82.93±3.67	509.58±3.78	125.69±4.89	9.4	65.9
P. fruticosa						
Control B	2.16±0.04	3.84 ± 0.05	3.80±0.07	9.81±0.08	56.8	39.1
60 days	26.59±1.17	2.85±1.67	69.21±1.72	5.94±0.59	38.4	47.9
120 days	11.62±3.44	47.35±19.48	48.50 ± 4.40	56.29±26.75	23.9	84.1
180 days	14.64±3.13	81.61±32.04	398.83±73.82	92.68±34.82	3.8	88.1
240 days	38.26±1.67	123.98±6.54	107.84±2.52	145.73±10.05	35.5	85.1

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Table 3: Cu and Pb Shoot Concentration, Total Plant Accumulation and Translocation Index

Furthermore, P. fruticosa recorded a high concentration of Cu and Pb in its roots on 180 and 240 days. The inconcistency may be due to the competition between heavy metals translocation system during uptake process (Israr et al., 2011). Regardless of the harvesting days, Cu and Pb concentration in root of A. wilkesiana was 7 - 9 folds higher than shoots, conversely, Pb concentration in the shoots was found to be 2 folds more than roots. Both accumulations of Cu and Pb in the root and shoot (stem + leaf) of the plants were higher than that of control plant parts. P. fruticosa recorded Cu accumulation in the roots 2 folds higher than shoots and Pb accumulation in the shoots 5 - 8 folds than roots except on 60 harvesting day, where roots concentration is higher than shoots. Shoots and roots ratio was obtained in order to assess the translocation index (TI) of the metal within the plant. Shoot and root concentration ratio for both plants studied for Cu was < 1 suggesting that Cu movement from the root to the shoot was low (Table 4). Conversely, shoot to root concentration ratio for both plants for Pb was > 1. Therefore, Pb movement from root to the shoot was high except for 60 harvesting day (Table 4). The high threshold values of shoot/root ratio (>1) implies that plant shoots(stem + leaf) acts as an easy way for Pb uptake. Considering A. wilkesiana, TI for Cu and Pb ranged from 3.58±0.76 to 10.77±0.19 mg/kg and 65.98±0.75 to 68.82±0.62 mg/kg, respectively. The values of TI obtained for Cu and Pb from P. fruticosa ranged 3.67±0.04 to 38.42 ± 0.68 mg/kg, and 47.98 ± 0.58 to 88.06 ± 0.92 mg/kg (Table 3). The TI value for Pb from both plants were found higher compared to TI value of Cu from both plants. Furthermore, the values obtained for transfer factor (TF) showed the plants ability to move Cu and Pb from soil to root and shoots are higher for both metals in A. wilkesiana than in P. fruticosa (Table 4). In a similar study by Rahman et al. (2013) reported that the total removed Cu and Pb concentration varied depending on the type of plant species. High uptake values for Pb (21.6 mg/kg) and Cu (21.5 mg/kg) were recorded for sunflower, while uptake of Cu by Indian mustard and amaranth were 18.10 mg/kg and 21.4 mg/kg, respectively. The Pb uptake by Indian mustard and amaranth were 14.3 mg/kg and 16.9 mg/kg, respectively (Rahman et al., 2013). The A. wilkesiana was observed to have high bioaccumulation efficiency for both Cu and Pb compared to P. fruticosa (Table 5). P. fruticosa was found more efficient for the removal of Cu and Pb on 180 and 120 days, respectively. The bioaccumulation factor for both metals from A. wilkesiana were found to be 2 to severals folds higher when compared to the values in P. fruticosa. The results indicated that higher accumulation by plant is an important trend to raise the efficiency of phytoremediation.

	Transfer Factor (TF	Shoot/Root concentration		Removal (%)		
Plant Type	Cu	Pb	Cu	Pb	Cu	Pb
A.wilkesiana						
Control A	2.05±0.25	3.77±0.82	1.08 ± 0.09	0.62 ± 0.01	ND	ND
60 days	2.01±0.88	24.98±12.44	0.12 ± 0.24	2.21±1.61	6.65	2.27
120 days	2.29±1.50	12.98±13.39	0.04 ± 3.16	1.99±1.59	7.62	2.92
180 days	5.79±4.45	9.62±8.15	0.10 ± 1.07	2.21±1.83	12.15	2.97
240 days	6.08±5.04	8.52±9.98	0.10 ± 0.73	1.94±3.01	10.33	3.39
P. fruticosa						
Control B	2.20±0.78	4.01±0.92	1.32 ± 0.01	0.64 ± 0.02	ND	ND
60 days	0.35 ± 0.10	0.37±2.03	0.62 ±2.13	0.92±1.36	1.4	0.16
120 days	0.32±2.40	7.09±4.22	0.32±3.58	5.29±2.68	0.98	1.52
180 days	2.77±99.76	0.16±0.21	0.04 ± 0.04	7.37±11.53	8.09	2.50
240 days	0.94±2.47	0.19±4.31	0.55±1.96	1.10±1.86	2.19	3.93

Table 4: Transfer Factor (TF), Shoot - Root concentration ratio and Removal (%) of Cu and Pb

ND = not detected

The growth promoting microorganism was not used or no soil amendments has been made to promote biomass production and this may increase the level of heavy metal accumulation ability of the individual plants. In this experiment, both plants recorded a high accumulation of Cu and Pb, although *A. wilkesiana* has more good root system and broader leaves than *P. fruticosa*.

Table 5: Bioaccumulation factor for copper (Cu) and lead (Pb) grown on spiked soil and cultivated on different harvesting period

	Bioaccumulation factor							
	Си				Pb			
Plant type	60	120	180	240	60	120	180	240
	days	days	days	days	days	days	days	days
A. wilkesiana	1.79	2.22	5.24	5.51	7.79	4.34	3.00	2.89
P. fruticosa	0.22	0.24	2.67	0.60	0.19	1.13	0.019	0.029

Soil sample with Pb concentration greater than 40.20 mg/kg are considered to be contaminated by Pb metal. The Canadian Council of Ministers of the Environment interim soil assessment criterion for Cu has set 30 mg/kg as a miximum reference value (Rahman *et al.*, 2013). The SC/RC ratio values obtained are important to show the ability of a plants to be used for phytoremediation purposes. One of the standard way for identifying a hyperaccumulator plant is the concentration of the metal in the leaf. A hyperaccumulator plant should accumulate more than 1000 mg/kg of Cu, Pb and Ni, while the accumulation for Cd should be more than 100 mg/kg (Rahman *et al.*, 2013). However, according to the standard presented above neither *A. wilkesiana* nor *P. fruticosa* plants will be considered as hyperaccumulator for Cu or Pb. Moreover, both plants have not achieved a threshold value of leaf-metal concentration of 100 mg/kg for Cu or Pb possibly due to the use of polyethylene bags which may affect the rooting system of the plants to spread more into the soil. Another possible reason may be due to the use of more than one heavy metals in the solution spiked into the soil and this lead to metal uptake competition within the plants (Israr *et al.*, 2011).

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The SC/RC ratio (>1) indicated that roots translocation of Pb and dividing it into partitions from root to shoot had positive control on plant species (Table 5). High biomass were observed for A. wilkesiana plant which make phytoextraction process become easy. Both plants accumulated higher concentration of Cu in the roots compared to shoots(stem + leaf) and this may be due to the high metal concentrations available in the soil (Durumin Iva et al., 2018). In contrast, concentration of Pb accumulated by the two plants are higher in the shoots (stem + leaf) when compared to the roots. Pb is well known to behave as immobile metal in the soil and it has a low solubility in the soil matrix. Furthermore, several plants hold Pb in the roots via absorption and precipitation while its movement to the stem, leaves and fruit is slow (Cui et al., 2004). A. wilkesiana was expected to accumulate more Cu than Pb because of the important role of Cu in a plant (Maimon et al., 2009). Eventhough, Pb is not movable easily to above root biomass, the two plants were able to accumulate high amount of Pb in the shoots. The concentration of Cu and Pb accumulated by A. wilkesiana and P. fruticosa were within the range 40-600 mg/kg for Cu and 5-150 mg/kg for Pb (Table 4). The uptake of Pb to shoots by both plants was appreciably more than Cu uptake to shoot. A positive relationship between the plant growth and their potential to extract Cu and Pb was observed. The decontamination of Cu and Pb is mirrored by the means of removal index (R) and both plants were successful in reducing the concentration of Cu and Pb. A percentage removal of Pb was observed for both plants which ranged between 2.27-3.39% and 0.16 -3.93% for A. wilkesiana and P. fruticosa, respectively. Percentage removal of Cu was found higher in both plants with values ranged between 6.65-12.15% and 0.98-8.09% for A. wilkesiana and P. fruticosa, respectively.

CONCLUSION

This study was carried out to determine the use of A. wilkesiana and P. fruticosa for phytoextraction of copper and lead from heavy metals spiked soil. Present findings revealed that both plants were able to accumulate Cu and Pb in its organs on all the harvesting days. High concentration of Cu accumulated in the root of A. wilkesiana and P. fruticosa were 542.66±2.26 and 384.19±70.69 mg/kg, respectively. Highest quantity translocated to the stem and leaf were observed from Pb with concentration 96.37±3.89 (leaf) and 46.44±2.18 (stem) mg/kg from P. fruticosa and A. wilkesiana, respectively. The experiment also revealed that uptake and translocation of Cu does not follow a specific pattern, it was inconsistent. While in case of Pb there was consistency in its uptake and translocation except in very few plants organs. The dry weight of both plants increased with the increased in the accumulation of Cu and Pb, non of the two heavy metals showed an antigonistic effect on the plants organs through out the period. A. wilkesiana removed more Cu (12.15%) and P. fruticosa removed more of Pb (3.93%). This study suggests a further experiment on using a single metal spiked soil, soil amendments or the use of growth promoting bacteria to enhance the removal capacity of the plants. Therefore the results obtained from this study are encouraging and indicated that A. wilkesiana and P. fruticosa are promising plants species for the removal of Cu and Pb from spiked soil owing to its survival, growth, uptake and transfer to aboveground plant parts.

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Conflict of Interest

The authors want to declare that there is no conflict of interests.

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