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Full Length Research Article

THE USE OF ACALYPHA WILKESIANA PLANT FOR PHYTOREMEDIATION OF HEAVY METALS FROM LANDFILL SOIL

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ABSTRACT
The effective elimination of heavy metals from a polluted environment is of utmost significance, particularly in cases where soil contamination is induced by the discharge of landfill leachate. The contamination of landfill soil with heavy metals has been one of the major concerns for decades. Therefore, identifying potential plants for phytoremediation of landfill soil is unavoidable. This research aimed to analyze soil of an abandoned landfill from Matang Malaysia and to conduct phytoremediation of landfill metal-contaminated soil using the *Acalypha wilkesiana* plant. Analyses shows that Cd, Pb, Cu, As, Mn, Ni, and Cr were higher than the Environmental Quality Standard of Malaysia. While Zn was found less than the standard, Fe was not detected by the plant. The removal of heavy metal by *A. wilkesiana* was higher compared to unexposed plants. The highest removal occurred on Mn, Cr, Cu, and Ni with concentrations 10.93, 1.50, 1.30, and 1.10 mg/kg, respectively, which was achieved in 6 months harvesting period. The results also revealed that the bio-concentration and translocation factors of Cu, Cd, Ni, and As were above 1, which means the plant has the ability to accumulate and transfer metals to the shoot of the plant. The plant has the capability to be used in the phytoremediation of heavy metals from landfill soil.

Keywords: Landfill soil, Phytoremediation, Heavy metals, *Acalypha wilkesiana*

INTRODUCTION
The presence of contaminated and derelict landfill is one of the subjects of concern everywhere in the world especially in developed countries. The standard of such landfills is not in accordance with the management; therefore, the soil becomes the way for contaminated leachate to pass through (Barasarathi et al., 2021). The presence of landfill leachate in the environment and its impact is a subject of serious concern to the government and environmentalists (Emenike et al. 2016). Leachate is a liquid that comes out of landfill soil to the bottom due to an increase in the diffuse level of liquid from either penetration of water or degradation of solid waste. Heavy metals are the contaminants found in landfill leachate; their toxic impacts make the very important to the ecosystem (Emenike et al., 2017). Environmental contamination with heavy metals is a serious issue which is associated with waste disposal and management. Environmental contamination is a worldwide fact that concern with the entire ecosystem (Alvarez-Mateos et al., 2019). Generation and disposal of waste into the environment is one of the issues that cause environmental contamination with heavy metals. Some years ago, Malaysian landfills contained inadequate leachate collection systems and no bottom liners, which caused a serious metal pollution from landfill leachate (Barasarathi et al., 2021), while some were abandoned. Therefore to remove or reduce the type of soil contamination that can occur, the use of sustainable treatment technology that can decontaminate the heavy metals polluted soil should be employed. The approach of using plants is necessary for the reduction of heavy metals from landfill soil because the soil prevents the movement of leachate from the landfill. Phytoremediation is the application of plants species to remove the contaminants or their toxic effects from the soil, water and air. Plants can be used to reduce heavy metals, radionuclides, and organic pollutants. This technology is efficient and environmentally friendly, and it is a solar-driven technique in contaminated soil treatment (Durumin Iya et al., 2018).

A. wilkesiana (copper leaf) is a specie from Euphorbiaceae family. This specie is abundant in Asia, Africa and America. The plant can grow fast with a with a combination of different colors which depends on cultivation (Durumin Iya et al., 2018).

The use of plants (Phytoremediation) has been applied to remove or reduce some certain amount of heavy metals from polluted environment, but limited research were conducted on the capacity of *A. wilkesiana* to remediate landfill soil. Therefore, the present research was conducted based on this reason. The objectives of the research were to assess heavy metals concentration from contaminated soil and determine the absorption and transfer of heavy metals within the plant parts. The novelty of the study is the capability of plants to absorb and transfer heavy metals within the experimental period in the greenhouse condition.

MATERIALS AND METHODS
Field Experimental Setup
This experiment was performed by a series of field studies to ensure and maintain a proper and good propagation condition for the plants at green house of UNIMAS. The study was focused on the capability of the plants to uptake and accumulates heavy metals and hydrocarbons from landfill sites at Matang, Kuching.

Soil and Plant Samples Collection
Matang old landfill site was located about 16.00 km from UNIMAS with GPS position of N 01 ^{0}34' 56.6'' and E 110 ^{0} 14' 39.2''. Unwanted materials such as broken bottles, grasses, debris and plastics were removed from the surface of the soil. The soil sample was collected within a depth of 7-15 cm. The materials used for the sampling such as scoops and containers were plastics. The samples were kept in a cooler box during transportation to the laboratory. Five (5) different locations with a distance of 17.5 m from each other were selected. At each location, three different sampling points were chosen and the samples were collected. Plant samples were collected within the university plant nursery

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with the help of greenhouse staff. Several healthy plant cuttings of *A. wilkesiana* were made.

Pot Experiment
A total of fifteen (15) soil samples were allowed to dry in a room and mixed thoroughly to ensure homogeneity. The heavy metals were determined from the landfill and control soil. One hundred and seventy (170) plant cuttings were planted on uncontaminated soil for three (3) weeks. After propagation, the healthy ones were selected for this experiment. Fourty eight (48) polythene bags were filled with approximately 500 g of dried landfill soil sample and each was transplanted with one (1) *A. wilkesiana* plant. Another forty-eight (48) polythene bags were filled with 500 g of control soil and one (1) *A. wilkesiana* plant was transplanted in each bag. Eight polythene bags (8) were used for exposed soil for a period of six (6) months of harvesting period. A set of 8 replicates (polythene bags with one plant) for control was established on 1, 2, 3, 4, 5, and 6 months of harvesting periods.

Determination of Heavy Metals in Plants and Landfill Soil
Initial concentrations of heavy metals were determined from the landfill and control soil using An atomic Absorption Spectrophotometer (AAS). On each harvesting day, plants were removed from the soil, washed and separated into three tissues (root, stem and leaf), then dried in an oven at 65 ^{0}C for 72 hours. Digestion and analysis of heavy metals from the sample was conducted.

Procedure
The soil and plant samples were treated with an acid digestion technique, using HCl and HNO_3 in a ratio of 3:1 respectively as described by Machado et al. (2017), with some modifications. Two grams of the samples were taken in a crucible, adding 9 mL HCl followed by slow addition of 3 mL HNO_3 with the help of a 25 mL measuring cylinder. A blank was prepared without soil or plant samples. The contents were heated on a hot plate at about 90 ^{0}C until it dried. Distilled deionized water (50 mL) was added and filtered with a micron filter. The digested sample was made up to the 100 mL mark of the 100 mL volumetric flask. The digested extract of landfill soil, control soil, exposed plants and unexposed plants was analyzed using an atomic absorption spectrophotometer (AAS).

Stabilization and Extraction potential of the plant
The phytostabilisation and phytoextraction capacity of *A. wilkesiana* plant was determined by applying Bioconcentration Factor (BCF), Translocation Factor (TF), and Extraction Coefficient (EC), and the following equations 1, 2 and 3, were used respectively (Durumin-Iya et al., 2018)

$$BCF = \frac{metal\ in\ plant\ root}{metal\ in\ soil} \quad (1)$$
$$TF = \frac{metal\ in\ plant\ shool}{metal\ is\ root} \quad (2)$$
$$EC = \frac{metal\ in\ plant\ shoot}{metal\ in\ soil} \quad (3)$$

It was reported that, if BCF>1, the plant is suitable for phyto-extraction and if BCF>TF the plant is suitable for phyto-stabilisation (Durumin-Iya et al., 2018).

Statistical Data Analysis
The data reported in this research were averaged values and standard deviation (SD) of eight replicates. Statistical analysis of the data was carried out using one-way analysis of variance (ANOVA). The differences in the concentration within plant parts and within harvesting time were considered statistically significant value at $p > 0.05$. And also, correlation matrix was used to analyze the total concentration of metals in the plant. The statistical analysis was carried out using Microsoft office 2019.

RESULTS AND DISCUSSION
Plant survival and growth on Landfill Soil
Results indicated that, they have the capacity to survive in landfill soil, this may be due to the protective mechanism for survival which may involve increased in the secondary plant's metabolites such as lignin, which is stimulated in heavy metals stress conditions. These secondary metabolites in the plants acquire homeostatic mechanisms that tolerate them to keep acceptable concentrations of essential nutrients and metal ions in the cellular compartments and to reduce the damaging effects of an excess of nonessential ones (Haider and Azmat, 2012).

Metals in Landfills Soil and Plants
Landfill soil can be polluted by accumulating heavy metals and metalloids through different sources such as disposal of high metal wastes, leaded gasoline, paints, animal manures, sewage sludges, pesticides and domestic wastse. Table 1.0 shows the concentrations of heavy metals in landfill soils detected from Matang, Kuching. The concentration of heavy metals in Matang landfill soil has been determined before planting the plant cuttings. The landfill soil samples used were analysed for heavy metals quantity during each harvesting period.
The results of heavy metals concentrations from landfill leachate are shown in Figure 1.0. The at findings indicates that Cr, Mn, Co, Ni, Pb, Fe, Cu, Zn, As, and Cd were detected from landfill soil samples. The presence of these heavy metals indicates the disposal of a variety of waste at the site in which some of these heavy metals were detected and reported in considerable amounts by Al Raisi et al. (2014). The level of Pb in the landfill soil (3.51 mg/kg) shows the disposal of lead batteries, lead-based paints, plastics, and pipes in the site. The detection of Ni (3.21 mg/kg) in excess can be caused by the disposal of batteries at the site. The concentration levels of Cr and Mn were 3.83 and 11.62 mg/kg, respectively, and the presence in soil samples indicates the disposal of considerable amounts of steel on the site. Cu with a concentration 5.74 mg/kg could be from paints, blades, bottle caps, insecticides, pharmaceuticals and also cosmetics. The presence of Zn (3.81 mg/kg) can be attributed to the disposal of batteries, fluorescent lamps, food wastes and burning tyres at the site (Al Raisi et al., 2014).

The uptake and accumulation of heavy metals such as Co, Cr, Cu, Cd, Fe, Mn, Ni, Pb, As and Zn by *A. wilkesiana* parts was investigated. The plants were able to uptake heavy metals from the landfill soil and accumulated them in the root, then translocated them to the stem and leaf. Generally, accumulation of heavy metals increased with the increasing harvesting period. The plants have significantly transferred Ni, Mn and Cr to the leaf. *A. wilkesiana* accumulated Cd and Co in the root. However, it did not accumulate Fe from landfill soil. The plants accumulated and transferred heavy metals via the pattern 6 months > 5 months >4 months > 3 months >2 months >1months.

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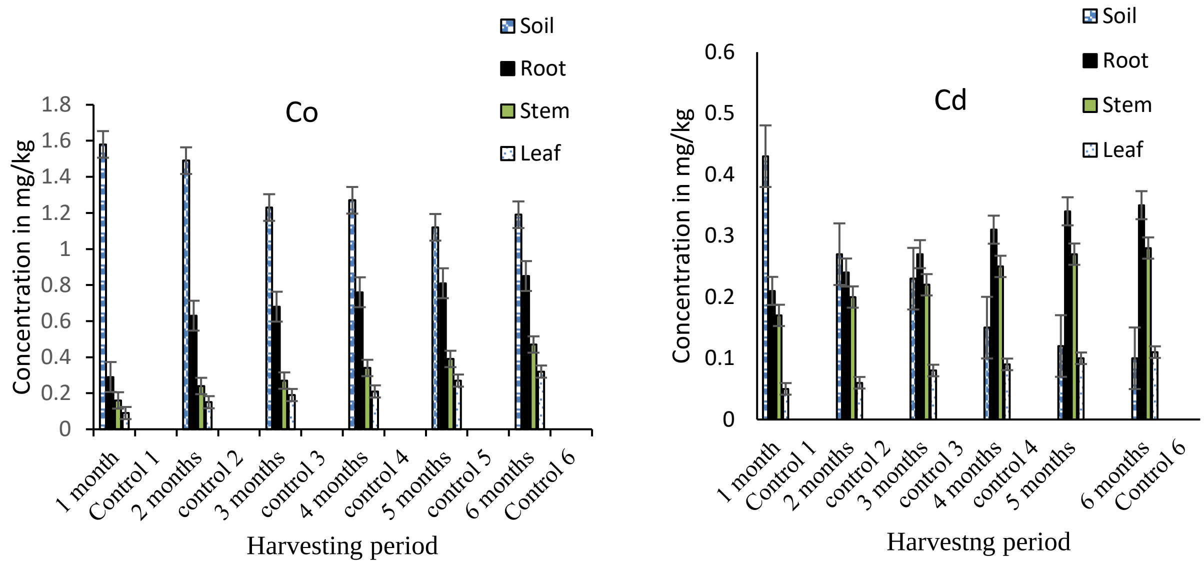

Figure 1.0: The concentrations (mg/kg) of Co and Cd from exposed soil and plant part and control soil and plant part at each harvesting period

Accumulation of Co by *A. wilkesiana* was high in the root on 6 months harvesting period with value 0.85 ±0.09 mg/kg as shown in Figure 1.0. The pattern of Co accumulation and translocation is in the oder root > stem > leaf. A low accumulation (0.17±0.07 mg/kg) was detected in *A. wilkesiana* leaf on 2 months harvesting period. It was reported in a similar study by Galal and Shehata (2015) that the accumulation of heavy metals in *Plantago major* L. was higher in the root than in the leaf. The present study agreed with the study conducted on *Festuca rubra* L and *Juncus sp.* L., where both plants accumulated more Co in the root than in the leaf (Lago-Vila et al., 2015). The plant accumulated Cd with appreciable concentrations as shown in Figure 1.0. A ranged of values from 0.33-0.35, 0.24-0.28 and 0.08 - 0.11 mg/kg of Cd were accumulated in plant root, stem and leaf, respectively. Accordingly, a high concentration of Cd was found in root on 6 months harvesting period. The pattern of Co and Cd accumulations and translocations is similar. Both elements were found high in plant roots followed by stem and leaf. The high concentration of Cd in plant root could be as a result of A. wilkesiana heavy metals tolerance ability which could have limited the movement of metals from root to shoot (Barasarathi et al*.,* 2021).

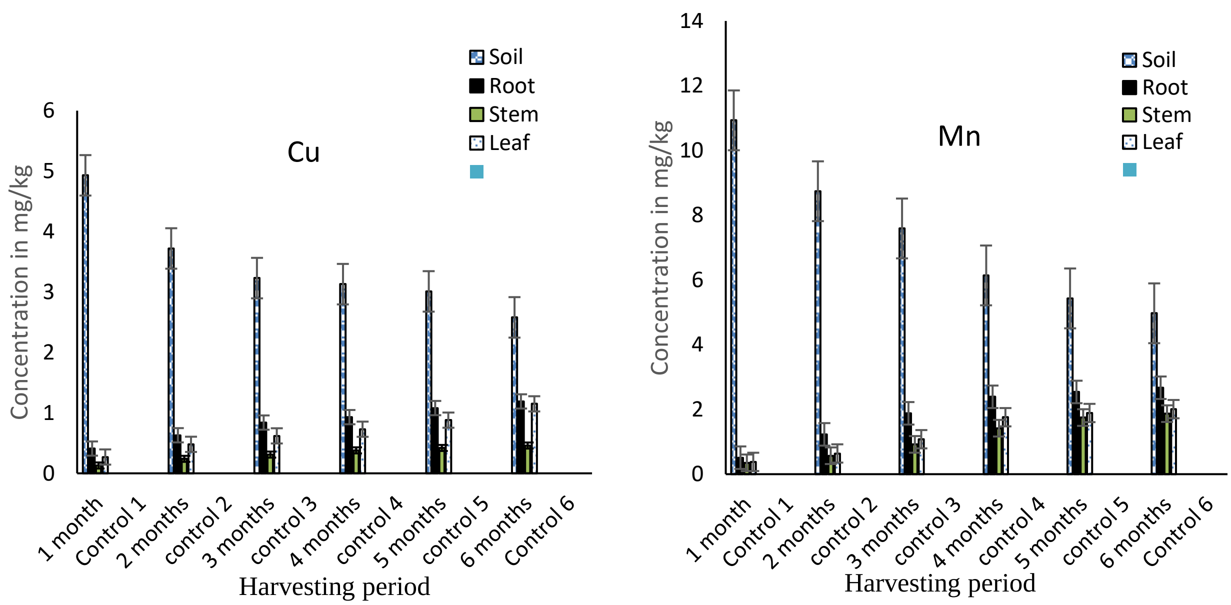

Figure 2.0: The concentrations (mg/kg) of Cu and Mn from exposed soil and plant part and control soil and plant part on each harvesting

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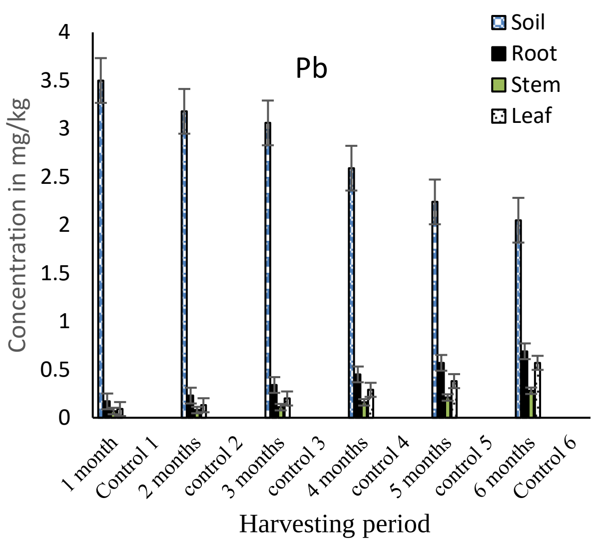
period

A significant Cu concentration was detected in the root of *A. wilkesiana* with quantity ranged between 0.86 - 1.19 mg/kg (Figure 2.0), and translocated to the leaf with a trend root > leaf > stem. Most plant species developed a defence process that is against the metal's toxicity caused by high Cu concentration. The plants form a complex with citric acid, phytochelatins and metallothionein. Consequently, most plant species that are tolerant to Cu clearly show a plan typical for excluders, while the mechanism of the intensive quantity of Cu by plants is not found in large numbers (Karczewska et al., 2015). There was a signifacant accumulation of Mn in the plant root, stem and leaf with values ranging from 0.67 -2.67, 0.4-1.83 and 1.01-2.01 mg/kg, respectively. The concentration was noticed to be higher in the roots compared to the stem and leaves, so the pattern of accumulation was root > leaf > stem. The plants accumulated Mn in the root, stem and leaf with higher concentrations compared to other heavy metals accumulated by the plants.



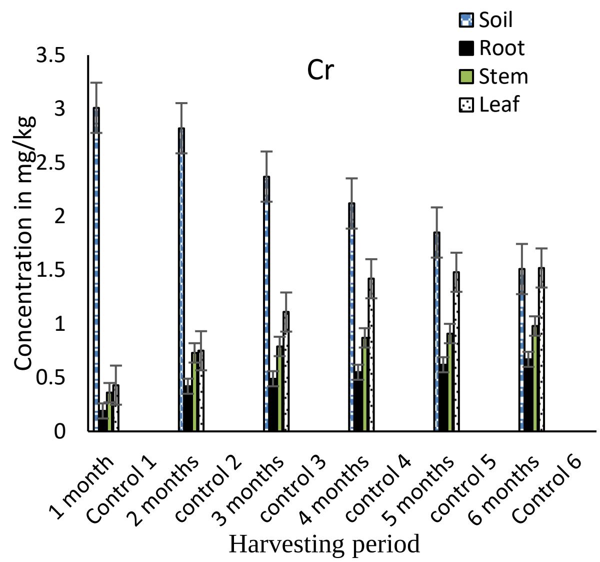


Figure 3.0: The concentrations (mg/kg) of Pb and Cr from exposed soil and plant part and control soil and plant part at each harvesting period

Figure 3.0 indicates the concentration of Pb in *A. wilkesiana* parts. High accumulation of Pb occurred in the root with concentration 0.69±0.01 mg/kg on 6 month harvesting period. A low Pb accumulation was detected in the stem on 1 month harvesting period. The accumulation followed the trend root > leaf > stem. The high concentration of Pb in plant roots detected here might be due to the belief that the uptake of heavy metals from landfill soil is from the roots to other parts of the plants (Barasarathi et al., 2021). These results agreed with those results of the research conducted on *Medicago sativum* and reported that high Pb concentration was found in the roots of *M. sativum* compared to other plant parts (Coupe et al., 2013). Accumulation of Cr was found to be high in the leaf as shown in Figure 3.0. The plant uptake and accumulated Cr ranged from 0.42-0.67 mg/kg in its roots, and translocated it to the leaf via the stem. Higher concentrations were detected in the leaf more than the root and stem. Increase in Cr uptake and translocation within the parts of the plant follow the trend leaf > stem > root. Cr accumulation in the leaves had ranged from 4.67 to 15 µg/g. High concentration of Cr found in the leaves may be due to the ability of the plant to tolerate heavy metals.

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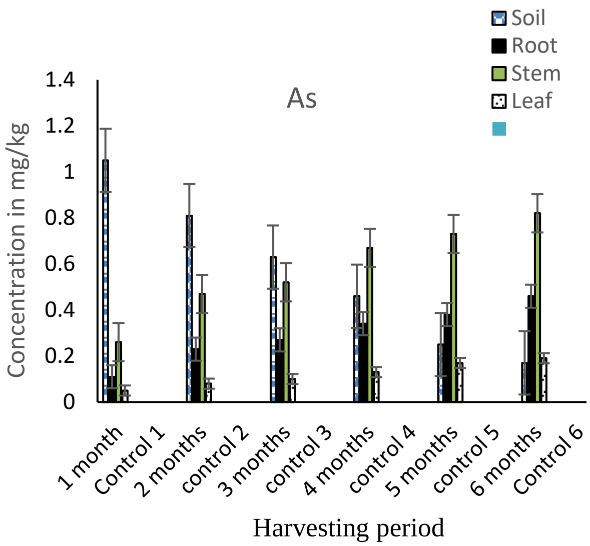

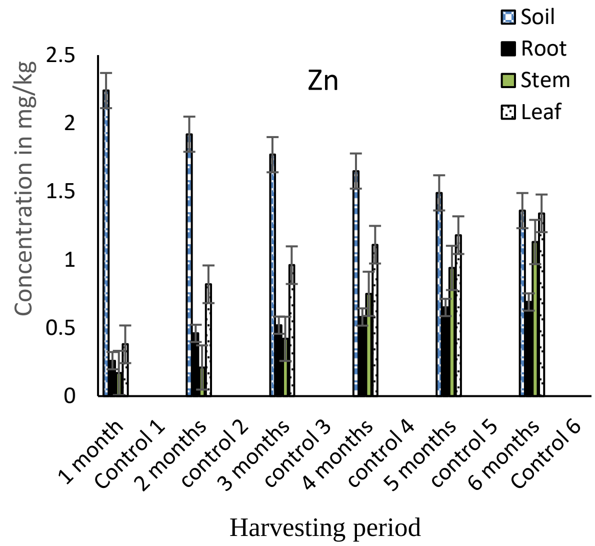

Figure 4.0: The concentrations (mg/kg) of As and Zn from exposed soil and plant part and control soil and plant part on each harvesting period

Figure 4.0 shows accumulation of As in the parts of the plant for the period of 6 months harvesting period. Arsenic was accumulated in the root with a concentration ranging from 0.23 – 0.46 mg/kg. The concentration was found high in the stem compared to root and leaf and the pattern of accumulation followed the trend stem > root > leaf A high translocation of As occurred from the root to the leaf. Accordingly, some studies discovered that a low concentration of As will arouse plant growth and hyper accumulators were found capable of accumulating high quantity of As and translocating it to the leaves and fruits without any effect (Rajoo et al*.,* 2016). Accumulation of Zn in plant root, stem and leaf ranged from 0.43 – 0.69 mg/kg are presented on Figure 4.0. Results obtained show a high Zn concentration in the leaves of the plant compared to other part and the accumulation pattern follow the trend leaf > root > stem. The highest Zn concentration occurred in the leaves on 6 months harvesting period. Zn accumulation in *A. wilkesiana* parts shows a tolerance ability to Zn toxicity, which could be the reason why the plant was capable to accumulate and translocate the metal to different parts. It was reported that the tolerance of metal toxicity by the plant involves two mechanisms which are exclusion and accumulation of the metals (Barasarathi et al*.*, 2021).

The concentration of Ni accumulated was ranged 0.74 – 0.97 mg/kg and presented in Figure 5.0. A high Ni concentration was observed in the leaf of the plant compared to root and stem. The accumulation follow the trend leaves > root > stem. Ni uptake by plant root depends on its concentration, plant chemical processes, the soil pH or solution, presence of other metals and organic matter contents. Nickel is supplied to meristematic parts of plants by transfer from older leaf to younger leaf and to buds, fruits and seeds, via the phloem which is controlled by Ni ligand complexes" (Chen et al., 2009). Some plants such as terrestrial constantly absorp Ni via the roots through passive diffusion and active metal transfer. The strength of Ni absorption depends on its concentration in the nutrient medium. Ni is transferred to all root tissues through the apoplast and symplast (Kastori et al., 2022).

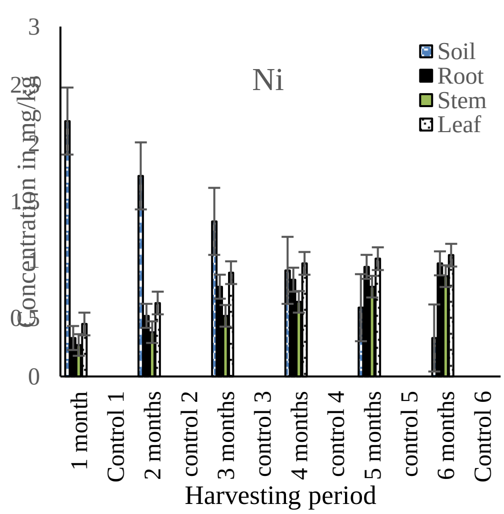

Figure 5.0: The concentrations (mg/kg) of Ni from exposed soil and plant part and control soil and plant part on each harvesting period

Bioconcentration and Translocation Factors

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The use of bioconcentration and translocation factors have proven appropriate tools to recognize the ability of the growing plants in terms of metal ions absorption (Khalid et al., 2017; Patek-Mohd et al., 2018; Zhang et al., 2017). Based on the biological concentration, the potential of this plant species for stabilization and extraction is assessed, and the BCF is used for this purpose. If the BCF ≥ 1, the plant has the ability to absorb and translocate the metals. The results presented in (Tables 2 and 3) show the values of BCF and TF of metals. *A. wilkesiana* has the ability to absorb and translocate metals to the above-ground part. But if the BCF < 1; therefore, the plant does not have the potential for extraction (Tables 2 and 3).

Table 2.0: Bio-concentration factor of heavy metals for six harvesting periods

Heavy metals	1 month	2 months	3 months	4 months	5 months	6 months
Co	0.18	1.3	0.55	2.8	0.72	4.5
Cr	0.06	0.3	0.21	0.6	0.34	1.3
Cd	0.49	1.7	1.17	2.3	2.83	3.5
Cu	0.08	0.2	3.85	0.3	0.36	0.5
Mn	0.05	0.3	0.25	0.3	0.47	0.5
Pb	0.05	0.1	0.10	0.2	0.25	0.3
Zn	0.12	0.3	0.29	0.4	0.44	0.5
As	0.10	0.3	0.43	0.7	1.31	3.5
Ni	0.15	0.7	0.58	1.3	0.42	2.9

Table 3.0: Translocation factor of heavy metals for three harvesting periods

Heavy metals	1 month	2 months	3 months	4 months	5 months	6 months
Co	0.86	0.7	0.68	0.7	0.81	0.9
Cr	4.16	4	3.88	4.3	3.85	3.7
Cd	1.43	1	1.15	1.1	1.09	1.1
Cu	0.98	1.2	1.11	1.1	1.20	1.4
Mn	1.06	1.4	1.06	1.6	1.43	1.4
Pb	0.59	1	0.91	1	1.04	1.3
Zn	1.50	2	1.94	2	1.98	2
As	2.82	2.4	2.30	2.4	2.37	2.2
Ni	2.18	1.8	1.83	2	1.89	2

Statistical Data Analysis

The total average concentration of metals was compared by using one-way analysis of variance (ANOVA) from Microsoft office 2019, and the results are presented in Tables 4A and 4B. *P*-value of 2.31066 * 10^{-5} was obtained for all the parameters which is less than the normal *p*-value of 0.05. The F-value was 9.1138 and F_{crit} 2.3928. Usually, the 2 the F score the lower the significant score value. The result shows that the F values (9.1138) and the p-value is less (2.31066E-05), which means the difference is statistically significant.

Table 4A: The sum, average and variance of heavy metals

Heavy metals	*Count*	*Sum*	*Average*	*Variance*
Co	3	1.64	0.546667	0.074633
Cr	3	0.74	0.246667	0.015233
Cu	3	2.8	0.933333	0.168433
Cd	3	3.17	1.056667	0.185033
Mn	3	6.51	2.17	0.1956
Ni	3	2.87	0.956667	0.008233

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Pb	3	1.54	0.513333	0.044433
Fe	3	0	0	0
As	3	1.47	0.49	0.0999
Zn	3	2.16	0.72	0.3667

Table 4B: The statistical analysis heavy metals concentrations

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	9.500066667	9	1.055563	9.113823	2.31066E-05	2.39281411
Within Groups	2.3164	20	0.11582			
Total	11.81646667	29				

Pearson's correlation matrix between the total concentration of metals accumulated by the plant was calculated in the form of matrices and used as a measure for similarity and inter-relationship among the metals. The correlation matrix ranges and the results of the correlation matrix determined were presented on Table 5A and 5B, respectively.

Table 5A: The correlation matrix between 1.00 and 0.00 range

S/N	Range	Correlation
1	0.90 to 1.00 (− 0.90 to −1.00)	very high positive (negative) correlation
2	70 to 0.90 (− 0.70 to − 0.90),	high positive (negative) correlation
3	0.50 to 0.70 (− 0.50 to −. 070)	moderate positive (negative) correlation
4	0.00 to 0.30 (0.00 to − 0.30)	low positive (negative) correlation and negligible correlation
(Mukaka, 2012)		

Table 5B: The correlation matrix between the metals

	Co	**Cr**	**Cu**	**Cd**	**Mn**	**Ni**	**Pb**	**As**	**Zn**
Co	1								
Cr	0.886268	1							
Cu	0.290018	-0.18623	1						
Cd	-0.92088	-0.99671	0.10602	1					
Mn	0.88559	0.569742	0.701342	-0.63445	1				
Ni	-0.14993	-0.59082	0.902719	0.523512	0.326437	1			
Pb	0.509089	0.05253	0.971367	-0.13326	0.850618	0.774655	1		
As	0.194563	0.626756	-0.88231	-0.56157	-0.28329	-0.99897	-0.74522	1	
Zn	-0.31554	-0.71916	0.816619	0.660502	0.161304	0.985497	0.656107	0.99217	1

A very high positive significant correlations occurred between Ni and Cu, Pb and Cu, Zn and Ni, Zn and As, while very high negative correlations were observed between Cd and Co, Cd and Cr, As and Ni. Other correlations between the metals were observed between high positive or negative and negligible correlation as presented in Table 5A.

CONCLUSION

The abundance of *A. wilkesiana* plant in almost everywhere in the world is worth exploring for its possible application in the phytoremediation of metals from landfill soil. The findings revealed that higher accumulation of Co, Cd, Cu, Mn and Pb was noticed at the root of the plant compared to a other metal. Leaves of the plant were observed to accumulate higher concentration of Cr, Ni and Zn, while As was high in the stem of the plant with a concentration 0.82 mg/kg during 6 months harvesting period. The study shows that the movement of metals from one stage to another stage (soil to root, root to stem and stem to leaves) did not follow a specific pattern, it depends on the concentration of metal in the soil as well as metal behavior. However, the plant was not good for phytoextraction of Fe, the results demonstrated that the plant was suitable for phytoremediation of heavy metals such as As, Co, Cd, Cu, Cr, Mn, Pb, Ni and Zn from landfill soil.

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Conflict of interest
There is no conflict of interest

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