Accumulation and Translocation of Heavy Metals by *Acalypha wilkesiana* Parts in the Phytoextraction of Contaminated Soil

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ABSTRACT

This study was to investigate survival, growth and accumulation potential of Acalypha wilkesiana *in phytoextraction of heavy metals contaminated soil.* Acalypha wilkesiana *was tested to evaluate its tolerance and phytoextraction capacity in soils contaminated with metals. It was tested under 10 mL of 100 mg/kg of As, Cr, Cd, Co, Cu, Fe, Mn, Ni, Pb, and Zn solution, along 240 days in greenhouse experiment with harvesting period of 60 days interval. Twenty four cuttings from* Acalypha wilkesiana *were subjected to the same treatment. Plants roots stem and leaves were dry-digested and analyzed using Atomic Absorption Spectrophotometer (AAS). Results show that Fe was the most accumulated metal followed by Cu, Mn, As and Zn with 5002.4, 542.7, 492.2, 396.7 and 308.2 mg/kg, respectively. The concentration of Cr, Ni, and Co was 101.2, 99.09, and 89.63mg/kg respectively. The highest concentration of Pb was 46.44 mg/kg, Cd was not detected by the plant. Bioconcentration Factor (BCF) of metals were above unity in root, stem, and leaf except for Fe which showed a value below the unity, and Pb shows highest BF value of 7.79. The Translocation Factor (TF) of Cr, Co, Fe, Ni, and Pb were higher, while that of As, Cu, Mn, and Zn were below the unity, Co showed the highest value of 15.93. Furthermore, Extraction Coefficient (EC) of Cr, Co, Ni, and Pb were greater than 1, while for remaining metals were lower than unity, the highest EC was observed from Pb with a value 17.21.*

Keywords: Acalypha wilkesiana*; heavy metals; bioconcentration factor; translocation factor; extraction coefficient*

ABSTRAK

Penelitian ini bertujuan untuk menyelidiki kelangsungan hidup, pertumbuhan dan potensi akumulasi Acalypha wilkesiana *dalam fitoekstraksi tanah terkontaminasi logam berat.* Acalypha wilkesiana *diuji untuk mengevaluasi toleransi dan kapasitas fitoekstraksi pada tanah yang terkontaminasi dengan logam. Pengujian menggunakan 10 mL 100 mg/kg larutan As, Cr, Cd, Co, Cu, Fe, Mn, Ni, Pb, dan Zn, selama 240 hari dalam percobaan rumah kaca dengan periode panen dalam rentang 60 hari. Dua puluh empat stek dari* Acalypha wilkesiana *memperoleh perlakuan yang sama. Batang akar tanaman dan daun dikeringkan dan dianalisis menggunakan Spektrofotometer Serapan Atom (AAS). Hasil menunjukkan bahwa Fe adalah logam yang paling terakumulasi diikuti oleh Cu, Mn, As dan Zn dengan kadar masing-masing 5002,4; 542,7; 492,2; 396,7 dan 308,2 mg/kg,. Konsentrasi Cr, Ni, dan Co masing-masing adalah 101,2; 99,09 dan 89,63 mg/kg. Konsentrasi Pb tertinggi adalah 46,44 mg/kg, Cd tidak terdeteksi oleh tanaman. Faktor Biokonsentrasi (BCF) dari logam berada di atas satu dalam akar, batang, dan daun kecuali untuk Fe yang menunjukkan nilai di bawah satu, dan Pb menunjukkan nilai BF tertinggi 7,79. Faktor Translokasi (TF) Cr, Co, Fe, Ni, dan Pb lebih tinggi, sedangkan As, Cu, Mn, dan Zn berada di bawah satu, Co menunjukkan nilai tertinggi 15,93. Selanjutnya, Koefisien Ekstraksi (EC) dari Cr, Co, Ni, dan Pb lebih besar dari satu, sedangkan untuk logam yang tersisa lebih rendah dari satu, EC tertinggi diamati untuk Pb dengan nilai 17,21.*

Kata Kunci: Acalypha wilkesiana*; logam berat; faktor biokonsentrasi; faktor translokasi; koefisien ekstraksi*

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INTRODUCTION

Presently, the main concern of researchers is the existence of toxic pollutants (such as heavy metals, dyes from textile industries) in water, soil and the entire environment due to an increase in industrial and anthropogenic activities which have adverse effects of different forms on human, animal and plant life [1-2]. Industries and agriculture sectors such as, mining, petroleum and its product, photographic, battery, printing and electroplating factories are responsible for the release of most environmental contaminants [3-5]. Sources of water are polluted by some agricultural and industrial actions which contain substances that are non-degradable and continue to exist in the environment such as heavy metals, suspended solids, microbes, organic compounds, and oils. These substances affect the ecosystem through the food chain and become a problem to the environmental world over; they are called toxic which causes a variety of diseases [6-7].

Most heavy metals and metalloids are harmful, and they cause unsuitable results and harsh problems even when their concentrations are very low. Heavy metals can also remove and replace important metals in the plant coloration and interrupt the protein catalytic activities. There are two groups of heavy metals, essential (such as copper and zinc) and non-important ones (such as arsenic, chromium, lead, mercury). The toxicity of heavy metals causes a variety of health effects to human [8]. Phytoremediation fundamentally refers to the use of plants and associated soil microbes to reduce the concentrations or toxic effects of contaminants in the environments. It can be used to remove heavy metals and radionuclides as well as for organic pollutants such as polynuclear aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and pesticides. This method is efficient, environmental friendly, and it can be considered as a solar-driven technique in the treatment of contaminated soil [8].

The aquatic plant was found useful in the removal of Pb and Zn ions from wastewater [9]. *Acalypha wilkesiana* (copper leaf) is a plant from the family Euphorbiaceae. The plants can be found the world over especially in the tropics of Africa, America, and Asia. It is a fast-growing shrub which gives a splash of color in the landscape with bronze-red to muted red; the leaves are like the shape of a heart with a color combination of green, purple, yellow, orange, pink or white depending on cultivation [10]. Phytoremediation has been applied to remove or reduce the concentration of contaminants from the polluted area, but few studies were carried out on the potential of *A. wilkesiana* to remediate a several heavy metal contaminated soil, it is on these bases that present study was carried out. Therefore, the objectives of this experiment were to determine survival potential of *A. wilkesiana* in a several heavy metals contaminated soil and evaluate the accumulation and translocation of the metals within the plant tissues. The novelty of the present experiment is the ability of *A. wilkesiana* to survive, grow, uptake, accumulate and translocate several metals within a given time interval in a normal growth condition at the greenhouse.

EXPERIMENTAL SECTION

This experiment was carried out from 20th February 2017 to 20th December 2017 at greenhouse FRST, Universiti Malaysia Sarawak. Moreover, there is currently ongoing research on this plant and another plant at Universiti Malaysia Sarawak.

Instrumentation

Stainless steel mesh BS 410-1 model, polyethylene bag, scoop, weighing balance, research materials: Ney VULCAN D-550 series muffle furnace, FAVORIT HS 5434 series hot plate, and Perkin Elmer AAS model Optima 8300 series.

Procedure

The procedures for soil and plant tissue analysis were fully explained with a reference.

Data analysis

Mean, standard deviation, standard error was calculated. Differences in the concentration between harvesting time were considered statistically significant at p-value < 0.05.

Soil sample

The soil was collected from Kota Samarahan, the Sarawak area. It was allowed to be dried; larger particles were removed and homogenized. A total of 32 polyethylene bags were filled with approximately 2.0 kg of the soil before spiking them with the solution containing standard heavy metals.

Plant

A. wilkesiana rose cuttings (about 6.5 cm) were made and kept for 2 h 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. These cuttings were used for pot experiment.

Pot experiment

A pot experiment was carried out according to the procedure outlined by Singh et al. [11]. Each 2.0 kg of soil was spiked and mixed thoroughly with a 50 mg/kg solution containing heavy metals (As, Cd, Cu, Co, Cr,

Fe, Mn, Ni, Pb and Zn) at a greenhouse of faculty of Resources Science and Technology, Universiti Malaysia Sarawak (N 01° 33' 03.6'' E 110° 45' 56.5''). There were four sets of plants (A, B, C, and D) and a control, where each set has eight replicates. Polyethylene bags (pots) containing the planted plants and the control were put in the growth chamber. The growth conditions at greenhouse were as follows: 16 h light at 22 °C and 8 h dark at 18 °C, netted and received water daily.

The pots experimental designs were set and plants were planted on 20th February 2017, while harvesting of the plant started on April 2017 until December 2017. Plants were then removed from the pots, and the roots and shoots were then separated. Roots of the plants were washed with distilled water to remove attached soil. Washed plant tissue (roots, stem, and leaves) were put in an oven at 70 °C for 3 days. The dried weights of roots, stem, and leaves were recorded. Dried plant tissues were used for further preparation and analyses.

Quality assurance/quality control

The glassware and plastic containers were washed with tap and distilled water, then immersed in 2.0 N HNO_3 solutions before use. All the chemicals used for atomic absorption spectrophotometer (AAS) analysis were of analytical grade and certified standards. Five replicates analysis showed a good precision within the results.

Soil and plant analyses

Soil analyses. Before heavy metals analyses, a dried soil sample was grounded and sieved through 2 mm stainless steel mesh BS 410-1 model, to remove plant material and other particles. The experiment was performed according to the procedure outlined by Hseu [12] with several modifications. Dried soil samples were dry-digested (dry ashing), and 1.0 g of the soil in a crucible was heated to ash at 550 °C for 4 h in a Ney VULCAN D-550 series muffle furnace. After cooling, 1.0 mL of deionized water and 1.0 mL of concentrated nitric acid was added. The sample was then evaporated to dryness on a FAVORIT HS 5434 series hot plate, and heated in a muffle furnace to 400 °C for 15 min. The sample was immediately added with 1.0 mL of deionized water and 2.0 mL of concentrated hydrochloric acid and then evaporated to dryness on a hot plate. The sample was then added with 10.0 mL deionized water, filtered with Whatman 125 mm and 0.45-micron filter in 50 mL volumetric flask. Deionized water was added up to the 50 mL mark of volumetric flask.

Plant tissues analyses. Analysis of plant tissues was carried out according to the method described by Soon [13]]. Prior to heavy metals analyses, dried plant tissues were digested, and 0.5 g of briefly grounded plant tissues was ashed in Ney VULCAN D-550 series muffle furnace at 480 °C for 4 h. After cooling, 1.0 mL of deionized water and 1.0 mL of concentrated nitric acid were added. The plant tissue samples were evaporated to dryness on a FAVORIT HS 5434 series hot plate. After cooling, the samples were dissolved in 2.0 mL of 20% (v/v) nitric acid by heating on a hot plate at approximately 100 °C. Samples were filtered through Whatman 125 mm and additionally filtered through 0.45 µm filter paper into 10 mL volumetric flask, deionized water was then added to the 10 mL mark of the flask. Samples were analyzed on a Perkin Elmer AAS model Optima 8300 series. As, Cd, Cu, Co, Cr, Fe, Mn, Ni, Pb, and Zn were analyzed at the wavelength of 193.7, 228.8, 324.8, 240.7, 357.9, 248.3, 279.5, 232.0, 217.0 and 213.9 nm, respectively.

Phytoextraction and phytostabilization potential of the plant. Three indices were used to assess the phytoextraction and phytostabilization potential of heavy metals in the plant parts such as Bioconcentration Factor (BCF), Translocation Factor (TF) And Extraction Coefficient (EC) calculated using the equations 1, 2 and 3, respectively [14]

$$BCF = \frac{\text{metal in plant root}}{\text{metal in soil}} \quad (1)$$

$$TF = \frac{\text{metal in plant shoot}}{\text{metal in root}} \quad (2)$$

$$EC = \frac{\text{metal in shoot}}{\text{metal in soil}} \quad (3)$$

Plants are suitable for phytoextraction if they have high BCF values greater than 1, and suitable for phytostabilization if BCF value is high and TF value is low [14].

Statistical analysis

All data reported were averaged values, standard deviation (SD) and standard error of five good replicates selected from eight independent replicates. Statistical analysis of the data was carried out using one-way analysis of variance (ANOVA). Differences in the concentration between harvesting time were considered statistically significant at p-value < 0.05. The statistical analysis was accomplished by IBM SPSS Statistics version 24.

RESULT AND DISCUSSION

Plant Survival and Growth

Survival of *A. wilkesiana* throughout the experiment was greater than 95 % and this plant species adapted to the contaminated soil environment. However, no adverse effect or signed of yellowish leaves or stunted growth was observed on the plant throughout the experimental period. This plant was capable of accumulating eight heavy metals at first harvest out of the ten heavy metals spiked. Cd was not detected in any plant part, while the accumulation of Fe was found to be higher compared to other heavy metals.

Bioconcentration Factor (BCF), Translocation Factor (TF) and Extraction Coefficient (EC)

The BCF, TF and EC values are presented in Fig. 1, 2 and 3, respectively. The BCF threshold of > 1 was achieved with higher values Pb (BCF = 7.79), Pb (BCF = 4.33), Cu (BCF = 5.24) and Cu (BCF = 5.54) on 60, 120, 180 and 240 experimental days, respectively compared to other metals. At day 240, all heavy metals showed a BCF value of > 1 except Fe which showed the maximum BCF value of 0.092. Plants with BCF values less than 1 are considered unsuitable for phytoremediation [15].

The TF threshold of > 1 was also observed in Co, Cr, Ni, and Pb at all experimental days except Fe on 240 experimental days. As, Cu, Mn, and Zn were failed to be efficiently translocated to shoot compared to other elements. The highest TF value was observed at different experimental days, Co (TF = 15.93), Co (TF = 7.51), Pb (TF = 2.21) and Pb (TF = 1.99). Any metal with TF values > 1 shows that the plant has high efficiency to translocate particular metals from roots to the shoots, and a higher amount of metal in the root with < 1 TF value shows an ability of the plant to harmonize between metal accumulation and translocation [16]

The EC threshold of > 1 was achieved by Pb, Ni, and Co in all the experimental days. The maximum EC was attained by Pb on 60 and 120 days with a value of 17.21 and 8.65, respectively which followed by Co on 180 and 240 days with a value of 11.06 and 7.88, respectively. EC value > 1 showed a higher metal availability in the soil, wider metal distribution and thus increase metal accumulation in plant tissues [11]. The other heavy metals show EC < 1.

Accumulation of Heavy Metals in the Root, Stem, and Leaf

The accumulation of different heavy metals (Co, Cr, Cu, Ni, Fe, Zn, Mn, As and Pb) in three different parts of *A. wilkesiana* were determined. Generally, higher concentrations of heavy metals were observed in root and stem compared to leaf part for all heavy metals except for Co which accumulated more in the leaf on 180 and 240 harvesting days. However, Fe concentrations were consistently higher in the root than in the other plant parts in all the experiment (harvesting) days. In this study, the accumulation of heavy metals in the leaves ranged 10.9–53.24, 2.92–87.87, 2.2–79.39, 40.81–20.3, 28.5–81.42, 3.73–93.77, 27.36–36.88 and 0.00–67.75 mg/kg for Cu, Co, Cr, Mn, Ni, Zn, Pb and As, respectively.

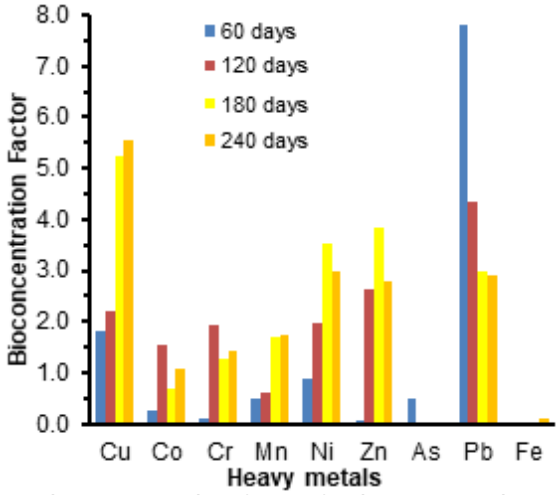

Fig 1. Bioconcentration factor for heavy metals on 60, 120, 180 and 240 harvesting days

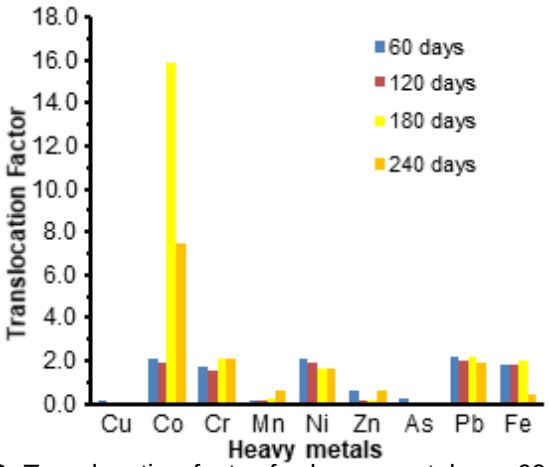

Fig 2. Translocation factor for heavy metals on 60, 120, 180 and 240 harvesting days

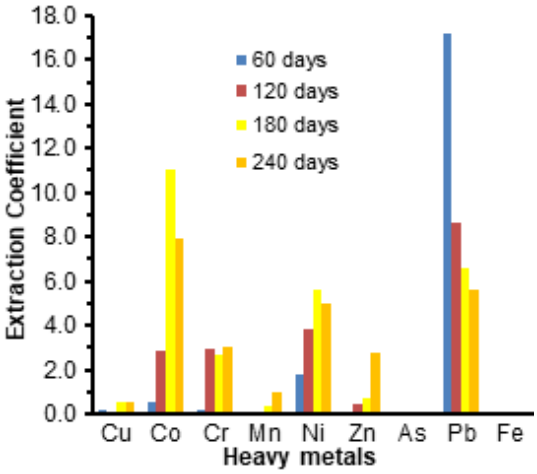

Fig 3. Extraction Coefficient for heavy metal on 60, 120, 180 and 240 harvesting days

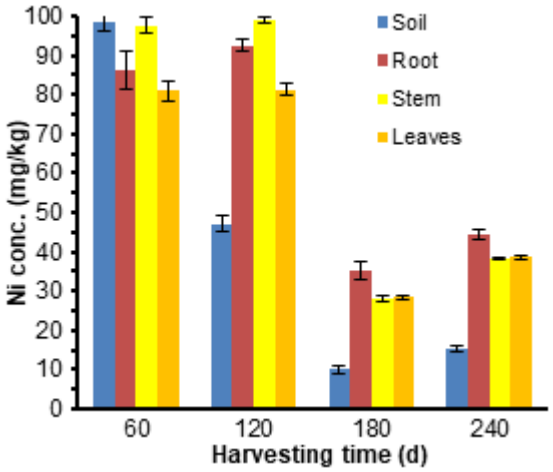

Fig 4. Ni concentration in soil and three plant parts of *A. wilkesiana*

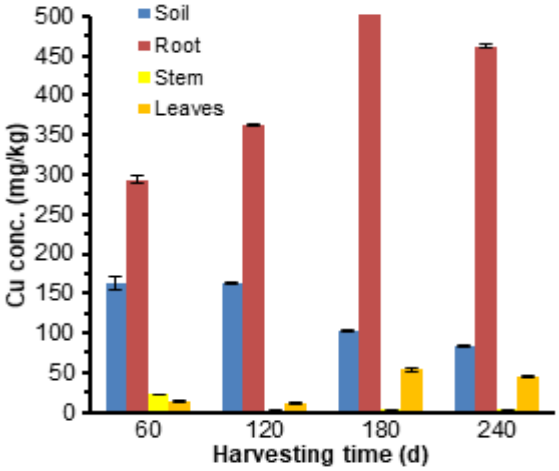

Fig 5. Cu concentration in soil and three plant parts of *A. wilkesiana*

A significantly higher accumulation of Ni was detected in the stem of *A. wilkesiana rose* with a mean value of 99.09 ± 0.92 mg/kg. Soil et al. [17] reported phytoremediation of Cd and Ni from contaminated soil by *Vetiveria zizanioides* L., and the accumulation of Ni was significantly observed more in the aerial parts (stem and leaf). However, the highest of Ni accumulation of *A. wilkesiana* were in the root and leaf with the concentration of 92.59 ± 1.64 and 81.42 ± 1.36 mg/kg, respectively. Ni accumulation was observed to be higher on 60 and 120 harvesting days, but the concentration decreases in the root, stem, and leaves after 180 and 240 days. This observation is contradicted with the study conducted by Das & Mazumdar [18] on vetiver *grass* where Ni was found to accumulate more in root than in shoot, with the accumulation rate in shoot/root from 1.05 to 10.32%. This showed that a small amount of Ni was translocated to the shoot [18]

The accumulation of Ni in the roots, stem and leaves of *A. wilkesiana* plants decreases from root to stem and stem to leaves on 180 and 240 harvesting days, while it was significantly observed to increase from root to stem then decreases from stem to leaves on 60 and 120 harvesting days. In 60 and 120 harvesting days, the increase was from 86.29 to 97.64 and 92.59 to 99.09 mg/kg, respectively. Moreover, the results indicated a relatively poor accumulation from stem to leaves on 180 and 240 harvesting days. The plant showed a great capability to uptake and accumulated Ni in its roots tissues more on 60 and 120 harvesting days. However, Ni concentration in the stem and leaves on all the harvesting days were greater than the concentrations of 0.1 to 5 mg/kg found in most plants [19]

Maximum mean values of Cu detected in the root of *A. wilkesiana* were 292.44 ± 5.66, 392.41 ± 0.44, 542.66 ± 2.26 and 461.92 ± 2.18 mg/kg for 60, 120, 180 and 240 harvesting days, respectively as shown in Fig. 5. The Cu uptake by the plant grown in a spiked soil was significantly higher in the root on the harvesting days. Regardless of the harvesting days, the concentration of Cu in the root was found to be four to ten folds higher than in the shoot. Olowu et al. [20] reported the concentration of heavy metals in root, stem, and leaf of *Acalypha indica* and *Panicum maximum jacq and* Cu was found to be in the range of 7.5 ± 5.6 mg/kg to 30±26 mg/kg dry biomass of the samples [20]. High Cu concentration was also detected in the whole plant of *Digitalis purpurea* (39 mg/kg) followed by *Phytolacca americana* (30 mg/kg) and *Mentha suaveolens* (28 mg/kg) [21]. Cu is also an important metal for the growth of plants, but when accumulation in the shoots of a plant is greater than 20 mg/kg it causes a harmful effect [22].

Outridge and Noller (1991) reported a mean value of 37.00 mg/kg from the shoots of *T. latifolia* plant grown on unpolluted site [23]. Cu accumulation in roots was found greater than the shoots in *B. juncea* grown on Cu amended soil [24]. In this experiment, the concentration of Cu was higher in roots 542.70 mg/kg than that of leaves 53.24 mg/kg and stem 22.14 mg/kg. Higher Cu accumulation in the roots could be because of its continues movement from soil to roots showing a natural attraction of roots to uptake large amount of Cu and was able to transport a little to the shoots. Xiong and Wang [25] reported that Cu accumulation in the stem of vegetable plants was observed to be controlled by its concentration in soil and it can go higher if the concentration in the soil is increased [25].

Accumulation of Co by *A. wilkesiana* in a soil spiked with Co, Cr, Cu, Cd, Pb, Mn, Ni, As, Fe and Zn heavy metals were found significantly higher in the leaf and stem parts as shown in Fig. 6. The concentration of Co in the plant parts increases with time, and higher accumulation was detected in the leaf of 240 harvesting days with a concentration of 87.87 ± 2.51 followed by the

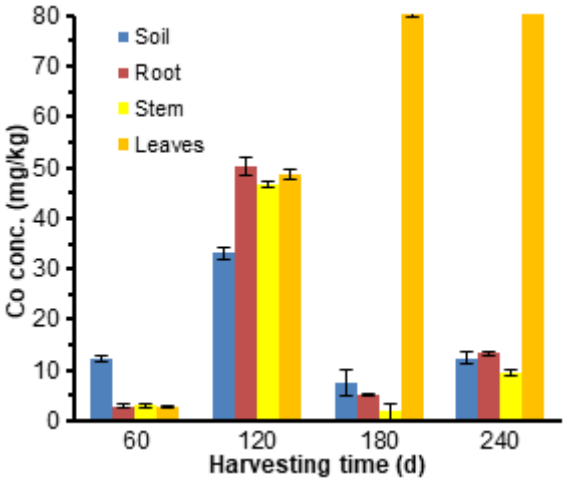

Fig 6. Co concentration in soil and three plant parts of *A. wilkesiana*

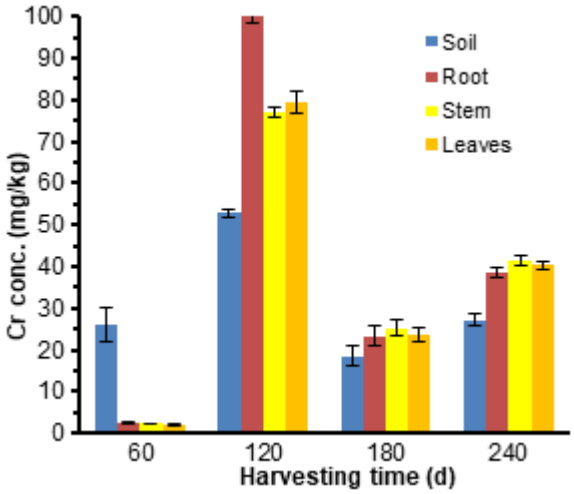

Fig 7. Cr concentration in soil and three plant parts of *A. wilkesiana*

stem at day 180 with concentration 81.63 ± 1.71 mg/kg. The accumulation was higher in the root of the plant at day 120 with a concentration of 50.19 ± 1.91 mg/kg. It was reported that *Plantago major* L. root accumulated 12.2 mg/kg, and its shoot accumulated 5.6 mg/kg [26], and this finding contradicts with the present study in which *A. wilkesiana* was able to accumulate more Co at the upper part of the plant, which shows that *A. wilkesiana* accumulated Co 18 fold than *P. major*.

The concentration of Co is high, and there is no toxicity data observed on this plant. Experiments have been carried out on how Co toxicity affects soil microorganisms; Lock et al. also reported that Co is relatively toxic to plants when higher concentrations have been dosed [27]. A. *wilkesiana* species accumulate more Co in the leaves (87.87 mg/kg) than in the stem and roots, except on 120 harvesting days, where it accumulated more Cr in the root (50.19 mg/kg) than in its stem and leaves. Li et al. reported that plants could uptake small concentration of Co, while its accumulation and translocation is based on the plant species that are under the experiment [28]. The uptake of Co by the roots involves active translocation through the cell membranes, though the molecular processes that are involved are still not available [29]. In turn, soil properties influence heavy metal availability for plants. Moreover, there is insufficient useful information obtained to measure the effect of soil properties on the toxicity of Co in different plant species.

Accumulation of Cr by *A. wilkesiana* planted in spiked soil was higher in the root compared to other parts of the plant (see Fig. 7). The concentration of Cr in *A. wilkesiana* increased from 60 to 240 harvesting days with significantly higher accumulation on day 120. Moreover, the highest accumulation of Cr was recorded in the root at day 120 with concentration 101.23 ± 2.92 mg/kg. Other higher accumulations were significantly observed on the stem and leaf at day 120 with concentrations of 76.93 ± 1.27 and 79.39 ± 2.57 mg/kg, respectively. In a study carried out on the removal of Cr using the *Sorghum* plant, Cr accumulated in the root (112 mg/kg) higher than in shoot (101 mg/kg) under equal treatment and condition [30]. This is in contrast to another study where *Portulaca oleracea* can accumulate Cr with concentration more than 1000 mg/kg which makes it behave as a hyper accumulator of Cr [31]. Reeves and Baker [32] suggested that for a plant to act as Cr accumulator, it should accumulate chromium in its tissue more than 1000 mg/kg [32].

A. wilkesiana cannot be considered as hyper accumulator because of the highest accumulation was nine folds lower than 1000 mg/kg. In general, plants have a low ability to absorb and translocate Cr, and different plants vary in their capacity to uptake and accumulate Cr in tissues [33]. It was reported that plants grown on contaminated soils after a long period of wastewater application which contains Cr, its amount of leaves rarely go greater than few µg/g. Even in plants grown on Cr rich serpentine soils, the average Cr content does go beyond 45 µg/g dry weight [33]. In this experiment, *A. wilkesiana* grown on soil spiked with several heavy metals was able to accumulate 79.39 mg/kg dry weight in its leaves. According to the results obtained from the present experiment, *A. wilkesiana* cannot be considered a hyper accumulator plant. It did accumulate Cr, mainly in Fig. 8 shows that Zn accumulation in *A. wilkesiana* was found to be significantly higher in the root at 120 harvesting days with a concentration of 308.25 ± 3.09 mg/kg. Study on *P. major* plant observed a significantly higher accumulation of Zn in the root with a concentration of 169.0 ± 8.8 mg/kg [26]. However, the highest accumulation of Zn in the stem and leaf of *A. wilkesiana*

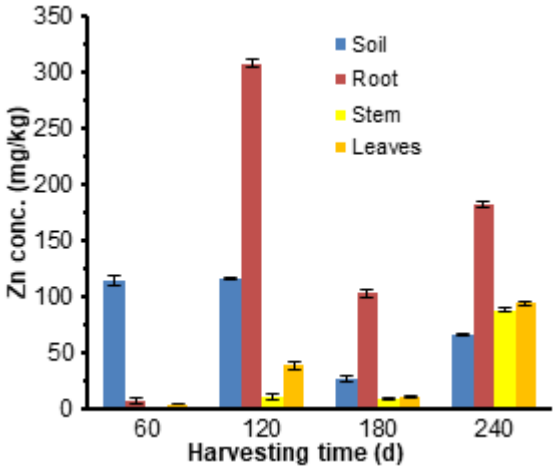

Fig 8. Zn concentration in soil and three plant parts of *A. wilkesiana*

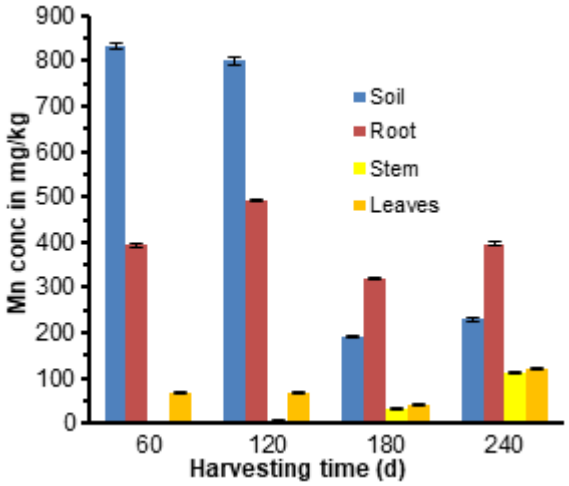

Fig 9. Mn concentration in soil and three plant parts of *A. wilkesiana*

were observed at 240 harvesting days with a concentration of 87.88±1.59 and 93.77 ± 1.71 mg/kg, respectively.

In this study, a solution of several heavy metals was used to spike the soil. Therefore, a decrease in the concentration of Zn in the plant part at different harvesting day could be caused by many heavy metals compete for the uptake by the plant [34]. Furthermore, noticeable higher accumulation of Zn compared to Cr accumulation by *A. wilkesiana* showed that Cr enhances the uptake of Zn. Zn amount was found more in the root (308.20 mg/kg) compared to the stem (87.88 mg/kg) and leaves (93.77 mg/kg) which is, however, higher than the permissible limits [35].

Zinc is an important metal to plants which have a mean value in the aboveground tissues of normal plants to be 66 mg/kg [22], while its toxic level is up to 230 mg/kg [36]. Zn accumulation from root to stem was found to be decreasing, while stem to leaves was observed to be increasing on all the harvesting days. Therefore, Zn accumulation was significantly higher in the roots, followed by the leaves, then the stem. Translocation of Zn in the stem and leaves of *A. wilkesiana* was much lower than the root. Sheel et al. reported that the uptake of Zn and Bioconcentration Factor capability of *Azolla* species increases with the increase in the concentration of Zn in the growing soil [37]. In contrary to the present experiment, Zn becomes more effective because it can easily translocate from root to the stem and leaves which is responsible for reducing the concentration of the important nutrients of plant and inhibits biochemical reaction [37]. Since Zn is mobile metal and bio-accessible, its higher concentration in the soil and plant can easily enter the food chain.

Fig. 9 shows the accumulation of Mn in *A. wilkesiana* at different harvesting time. A noticeable higher accumulation of Mn on 60, 120, 180 and 240 harvesting days was found in the root with concentrations 394.42 ± 4.54, 491.66 ± 1.79, 319.11 ± 1.23 and 396.17 ± 4.87 mg/kg, respectively. The accumulation decreases in the trend root > leaves > stem. The highest accumulation of Mn by *A. wilkesiana* occurred at 120 harvesting days, while the stem and leaf accumulation was observed to be highest at 240 harvesting days with a concentration of 111.51 ± 2.42 and 120.34 ± 1.49 mg/kg, respectively. In a similar study, Murtaza et al. reported an accumulation of Mn in vegetables ranged between 5.1 to 162.4 mg/kg [38]. In contrast to present study, Satphathy and Reddy reported accumulation of Mn in the stem, leaf, and root of *Brassica juncea* as 4552.3, 3003.4 and 2974.6 mg/kg, respectively [39] in which *B. juncea* accumulated Mn 18 fold that of *A. wilkesiana*.

The concentration of Mn in this experiment was found more in root 491.70 mg/kg followed by the leaves 120.34 mg/kg and stem 111.51 mg/kg. The higher Mn amount found in the leaves of Sugarcane and Chinese chestnut shows its capability in being mobile from root to leaves [40] because chlorophyll in leaves needs Mn during photosynthesis. Furthermore, an operational explanation was made by Baker that Mn concentration in the shoots of hyper accumulator plants is constantly higher than in roots, indicating plant ability to accumulate, transport and store Mn in the stem, leaves and fruit parts [32,41-42].

Fig. 10 shows the accumulation of Pb in three parts of *A. wilkesiana* with concentration ranged between 26.24 ± 1.19 to 42.76 ± 1.22, 30.57 ± 0.85 to 46.44 ± 2.18 and 27.36 ± 1.07 to 36.88 ± 1.23 mg/kg in root, stem, and leaves, respectively. A significantly higher accumulation of Pb occurred in the stem of the plant at 240 harvesting days with a concentration of 46.44 mg/kg, while the lowest accumulation of Pb was observed in the

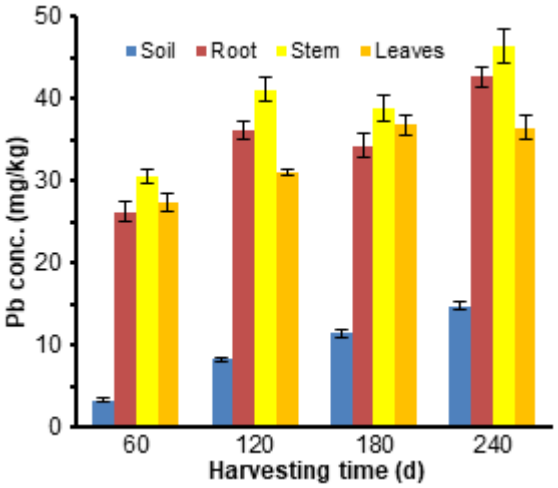

Fig 10. Pb concentration in soil and three plant parts of *A. wilkesiana*

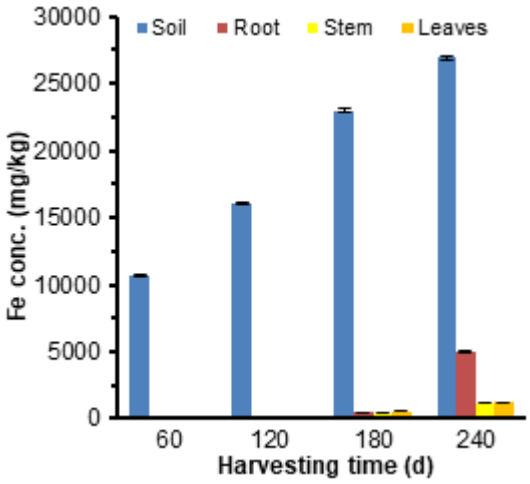

Fig 11. Fe concentration in soil and three plant parts of *A. wilkesiana*

root of the plant at day 60 with a concentration of 26.24 ± 1.19 mg/kg. A low Pb accumulation was also observed by Knapp et al. in which the uptake of Pb by carrots, tomatoes, and amaranth ranged between 1.6–1.9, 0.3–0.7 and 0.8–1.0 mg/kg, respectively [43].

In contrast, Yanqun et al. reported that Pb concentrations in shoots of *Elsholtzia polisa* and *Stellaria vestita Kurz* were 1015.4 and 3141.2 mg/kg, respectively [44]. Moreover, accumulation of Pb in plant roots ranged 629.3 mg/kg in *Crisium chlorolepis* to 7456.5 mg/kg in *S. vestita Kurz* [44]. Pb has lower solubility properties in the soil and shows strong barrier even if its highly accumulated at the root, its usually significantly translocated to the stem, leaf and other plant parts such as fruit and seed [17].

The experiments on Pb uptake in *A. wilkesiana* revealed that stem accumulated highest amount of Pb (46.44 mg/kg) followed by roots (42.76 mg/kg) both on 240 harvesting days. The accumulation of Pb was maximum at 240 days in roots, stem, and leaves. There was an increase of Pb concentration from the roots to the stem, and a decrease of Pb accumulation from stem to leaves was observed in all the harvesting days. The increase and decrease of Pb accumulation from root to stem and stem to leaves were slow and steady throughout the harvesting days. The trend of Pb translocation reveals that Pb was effectively translocated from roots to stem while from stem to leaves the translocation was relatively poor.

A. wilkesiana showed a significantly highest accumulation of Fe on day 240 of the experiment with a concentration of 5002.4 ± 15.21, 1169.4 ± 39.70 and 1251.0 ± 4.30 mg/kg for the root, stem, and leaf, respectively, as shown in Fig. 11. Furthermore, Fe accumulation by the plant parts ranged between 64.47 ± 0.55 to 5002.4 ± 15.21 mg/kg, with lowest and highest accumulation of Fe at 60 and 240 harvesting days, respectively. Moreover, an increase of Fe uptake by *A. wilkesiana* root from day 60 to day 240 ranged between 64.69 to 5002.4 mg/kg.

This present study agreed with the similar studies conducted by Nematian and Kazemeini in which they found that accumulation of Fe in root ranged 36.64–93266.00 mg/kg and it was 151.56 to 35722.80 mg/kg in the shoot of the plants, with the highest accumulation was in the root and shoot of *Centurea iberica* and *Carthamus oxyacantha,* respectively [45]. The highest Fe accumulation by *A. wilkesiana* root, stem, and leaf at 180 and 240 harvesting days were 486.4, 424.0, 542.02 mg/kg and 5002.4, 1169.4, 1251 mg/kg, respectively. Accordingly, Salsola soda root accumulated Fe (22645.3 mg/kg) and *Camphorosma monospoliacum* shoot accumulated Fe (10604.9 mg/kg) [46]. In contrast, concentrations of Fe accumulated by *A. wilkesiana* were below concentration reported in the study of Fe by the roots of *Salsola soda* and shoots of *Camphorosma monospeliacum* respectively [46].

Fe is needed for the functioning of different enzymes, especially those that take part in oxidation-reduction processes, for chlorophyll, reduction of nitrite/sulphate and N_2 fixation [33]. It was reported in another study that, the observed harmful symptoms of Cr are similar to those of Fe deficiency [33], which was related to the ability of Cr to displace other metals (such as Fe) from some important places and generates a low Fe accumulation [47]. In the present experiment, the toxic effect was not observed, in contrast, accumulation of Fe in the tissues of *A. wilkesiana* was found to be higher.

The accumulation of As observed by *A. wilkesiana* was only at 60 harvesting days (see Fig. 12). As accumulation trend is root > leaf > stem with concentration

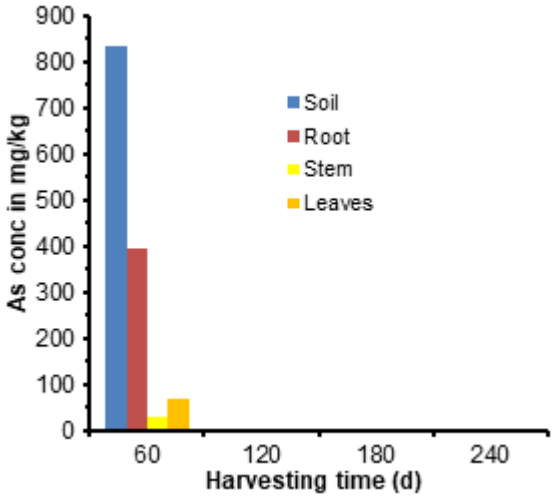

Fig 12. As concentration in soil and three plant parts of *A. wilkesiana*

of 396.74 ± 2.74, 67.75 ± 1.31 and 29.34 ± 2.39 mg/kg, respectively. *A. wilkesiana* cannot act as arsenic hyper accumulator because hyper accumulator plant should accumulate As with concentration more than 1000 mg/kg in their above root biomass [48]. A lower As accumulation was observed by Caille et al.in *Pteris vittata* planted in contaminated soil, and this suggested that the decreased in As uptake may be due to phytotoxicity of Cu, Zn and other metals that existed in the soil [49].

CONCLUSION

Due to its abundance in most of the world, including Africa and Asia, *A. wilkesiana* is a species that is worth exploring for its possible application in metal phytoextraction. Present findings revealed that high accumulation of Co, Cr, Cu, Fe, Mn and Zn at the root of *A. wilkesiana* was observed. Co, Ni, and Pb were translocated to the stem of the plant, while Co, Cr and Fe were successfully translocated to the leaves. The highest accumulation at the root and leaves of *A. wilkesiana* was that of Fe with concentration 5002.00 and 1251.00 mg/kg, respectively. Ni accumulation (99.09 mg/kg) was recorded the highest at the leaves of the plant. The experiment also indicated that transportation of heavy metals in a different stage (soil to root, root to stem and stem to leaves) does not follow a specific pattern, it depends on the metal. The results obtained from this study are encouraging and indicated that *A. wilkesiana* is a promising species for phytoremediation of heavy metal contaminated soil owing to its very high biomass productivity. Moreover, the plant did not suffer from any stress or showed any sign of disease during the experiment. It could be suggested that this plant is more suited for metal removal, as it effectively reduced the number of heavy metals at the same time.

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