

## REMEDIATION OF ZINC CONTAMINATED SOIL USING *Acalypha wilkesiana*

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### AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between all authors. Authors AOP and OSB designed the study. Author EPO anchored the field study, gathered the initial data and performed preliminary data analysis. Author ASA wrote the protocol and interpreted the data managed the literature searches and produced the initial draft. All authors read and approved the final manuscript.

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### ABSTRACT

A study was premeditated to evaluate the phytoextraction potential of *Acalypha wilkesiana* for zinc (Zn) remediation. Sets of pot experiment were conducted. Stems of *Acalypha wilkesiana* were planted in five kilograms of soil placed in each plastic pot having 0 ppm, 5 ppm, 10 ppm, 15 ppm, 20 ppm and 25 ppm of Zn respectively. The study was carried out for a period of 12 weeks under natural conditions. Physicochemical properties of the soil were determined using standard methods. The results revealed that pH, phosphorous and moisture contents increased while nitrogen and organic carbon contents decreased in polluted soil remediated with *Acalypha wilkesiana* when compared to the zinc free soil. Leaves, stems and roots of the plant were analyzed for Zn uptake after 12 weeks. The plant mopped up substantial concentrations of Zn in the stem (6.21 ppm) and leaves (8.23 ppm) compared to concentrations in the roots (6.08 ppm). The phytoextraction ability of the plants was assessed in terms of its metal bioconcentration factor (BCF) and translocation factor (TF). It was observed that the levels of the Zn in the roots and shoots after 12 weeks showed that more bio-available pool of Zn was translocated from the root to the leaves and stem in that order. The results obtained suggests that the plant have phytoextraction capability and could be used in re-establishing soil polluted with Zn.

**Keywords:** Phytoremediation; heavy metal; translocation; zinc.

### 1. INTRODUCTION

Soils may become contaminated by the accumulation of heavy metals and metalloids through emissions from the rapidly expanding industrial areas, mine tailings, disposal of high metal wastes, leaded gasoline and paints, land application of fertilizers, animal manures, sewage sludge, pesticides,

wastewater irrigation, coal combustion residues, spillage of petrochemicals, and atmospheric deposition [1]. Heavy metals constitute an ill-defined group of inorganic chemical hazards, and those most commonly found at contaminated sites are lead (Pb), chromium (Cr), arsenic (As), zinc (Zn), cadmium (Cd), copper (Cu), mercury (Hg), and nickel (Ni) [2]. Soils are the major sink for heavy metals released into

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the environment by aforementioned anthropogenic activities and unlike organic contaminants which are oxidized to carbon (IV) oxide by microbial action, most metals do not undergo microbial or chemical degradation [3] and their total concentration in soils persists for a long time after their introduction [4]. Changes in their chemical forms (speciation) and bioavailability are, however, possible. The presence of toxic metals in soil can severely inhibit the biodegradation of organic contaminants [5]. Heavy metal contamination of soil may pose risks and hazards to humans and the ecosystem through: Direct ingestion or contact with contaminated soil, the food chain (soil-plant-human or soil-plant-animal-human), drinking of contaminated ground water, reduction in food quality (safety and marketability) via phytotoxicity, reduction in land usability for agricultural production causing food insecurity, and land tenure problems [6].

Most Zn is added during industrial activities, such as mining, coal, and waste combustion and steel processing. Many foodstuffs contain certain concentrations of Zn. Drinking water also contains certain amounts of Zn, which may be higher when it is stored in metal tanks. Industrial sources or toxic waste sites may cause the concentrations of Zn in drinking water to reach levels that can cause health problems. Zinc is a trace element that is essential for human health. Zinc shortages can cause birth defects. The world's Zn production is still on the rise which means that more and more Zn ends up in the environment. Water is polluted with Zn, due to the presence of large quantities present in the wastewater of industrial plants. A consequence is that Zn-polluted sludge is continually being deposited by rivers on their banks. Zinc may also increase the acidity of waters. Plants often have a Zn uptake that their systems cannot handle, due to the accumulation of Zn in soils. Finally, Zn can interrupt the activity in soils, as it negatively influences the activity of microorganisms and earthworms', thus retarding the breakdown of organic matter [7]. The industrial activity accelerates pollution of the biosphere, especially the soil. Nowadays soil pollution is getting considerable public attention since the magnitude of this problem is growing rapidly. Heavy metals are the most dangerous substances in the environment due to their high level of durability and toxicity to the biota [8].

Humans and ecosystem may be exposed to chemical hazards such as heavy metals like Zinc through the direct ingestion of contaminated soils, consumption of crops and vegetables grown on the contaminated lands or drinking water that has percolated through such soils [6]. Numerous studies have been conducted in this area aimed at developing an efficient and

economical way to remediate the soil contaminated with heavy metals. Conventional remediation methods such as physical, thermal and chemical treatments are very expensive, have high maintenance costs and may cause secondary pollution [9] or adverse effect on biological activities, soil structure and fertility [10]. However, the high cost of these approaches necessitated the need for a less expensive cleanup technique. A promising approach is the phytoremediation technology, where living plants are used to remove trace metals from impacted sites [9]. Phytoremediation is a developing technology which uses plants and their associated microbes for the remediation of soil contamination. This process is cost effective without creating disturbance to the landscape [11]. In the phytoremediating process, several sequential crops of selected plant species can be cultivated to reduce the concentration of heavy metals in contaminated soils to environmentally acceptable levels. However, successful phytoextraction require plants that are capable of producing high biomass while accumulating large amount of contaminants in the biomass from the soil [12].

*Acalypha wilkesiana* is a shrub. It grows 3 m high and spreads 2 m across. The stem is erect with many branches. The branches have fine hairs. It has a closely arranged crown. The leaves are coppery green with red splashes of colour. While, *Acalypha inferno* is also an evergreen shrub native to the Pacific islands, copperleaf bears brightly colored foliage in shades of red, purple, bronze and green. It is a popular landscape plant in subtropical and tropical zones and planted as a houseplant or annual in cooler zones. The leaf edges are distinctly toothed and sometimes differ in color from the rest of the leaf [13]. There has been a continuing interest in searching for native plants that are tolerant to heavy metals; however, studies have evaluated the phytoremediation potential of native plants under field conditions [14-16]. Heavy metals can cause severe phytotoxicity and may act as powerful force for the evolution of tolerant plant populations. Therefore, it is possible to identify metal tolerant plant species from natural vegetation in the field sites that are contaminated with various heavy metals. The aim of the study is to remediate Zinc contaminated soil using tropical plant; *Acalypha wilkesiana*.

## 2. MATERIALS AND METHODS

### 2.1 Collection and Processing of Samples

The soil sample used for this study was collected from a depth of 0–20 cm within the Federal University of Technology, Minna, Nigeria and transported in plastic pots to the Center for Preliminary and Extra-mural

Study (CPES) garden. The soil sample was air-dried and presieved with 2 mm diameter mesh. The physicochemical properties of soil used for the study is shown in Table 1. The taxonomic classification of the experimental soil was loamy sand with pH of 6.57. Mature stems of *Acalypha wilkesiana* were collected from botanical garden, of Biological Sciences Department, Federal University of Technology, Minna, Niger State, Nigeria.

## 2.2 Preparation of Heavy Metal Contaminants

The contaminants were added as zinc sulphate ( $Zn(SO_4)$ ), and 2.4771g of  $Zn(SO_4)$  was dissolved in 1,000 mL of distilled water to make stock solutions of 5, 10, 15, 20 and 25 milliliters. These different concentrations were then measured from the stock solutions into a 100-mL capacity measuring cylinder and made up to the mark to give 5 ppm, 10 ppm, 15 ppm 20 ppm 25 ppm and 0 ppm (control) metal concentrations. The soil was spiked with different concentration of zinc and it was thoroughly mixed [17].

## 2.3 Experimental Design and Treatment

Pot experiment study was conducted at Centre for Preliminary and Extra-mural Studies (CPES) Garden of the Federal University of Technology, Minna, Nigeria. The set up was a complete randomized design and the treatment was replicated three times. The experimental pots were filled with 5 kg soil presieved with 2 mm sieve size. Then the stems (one stem per pot) were planted in each pot. Sampling of the plants to monitor metal uptake and soil for residual metal contents was done 12 weeks after planting. The plants were irrigated with 200 mL (per pot) of tap water daily and sampling of the plants to monitor metal uptake and soil for residual metal contents was done 12 weeks after planting.

## 2.4 Zinc Analysis

After 12 weeks of planting, all the plants were harvested separately according to soil treatment, separated into three compartments, viz. roots, stem, and leaves. The 3 replicates of each treatment were pooled together to give composite sample of each treatment. The plants were washed in water to eliminate dust, dirt, possible parasites or their eggs and finally with deionized water [18]. Each sub-sample was oven-dried at 70°C for 24 hours. Acid digestion method of Yusuf et al. [18] was used for the digestion of grounded plant samples. 1g each of this was weighed into 50 mL capacity beaker, followed by addition of 10 mL mixture of analytical grade acids:  $HNO_3$ ;  $H_2SO_4$ ;  $HClO_4$  in the ratio 1:1:1. The beakers containing the samples were covered with watch

glasses and left overnight. The digestion was carried out at a temperature of 70°C until about 4 mL was left in the beaker. Then, a further 10 mL of the mixture of acids was added. This mixture was allowed to evaporate to a volume of about 4 mL. After cooling, the solution was filtered to remove small quantities of waxy solids and made up to a final volume of 50 mL with distilled water. Zinc concentrations were determined using Atomic Absorption Spectrophotometer (AAS), Accusys 211, Buck scientific, USA.

**Table 1. Physicochemical properties of soil sample used for phytoremediation study**

Parameters	Values
pH	6.57±0.15
Nitrogen (%)	0.21±0.01
Phosphorus (ppm)	25.67±1.20
Organic Carbon (%)	8.83±0.02
Moisture	8.00±1.15
Sand (%)	83.33±0.17
Silt (%)	4.83±0.09
Clay (%)	11.83±0.09
Na <sup>+</sup> (cmol/kg)	0.18±0.01
K <sup>+</sup> (cmol/kg)	0.05±0.12
Mg <sup>2+</sup> (cmol/kg)	1.37±0.01
Ca <sup>2+</sup> (cmol/kg)	2.24±0.02

## 2.5 Determination of Bioconcentration and Translocation Factor

Bioconcentration factor (BCF) and Translocation factor (TF) were calculated using the formula of Santosh et al. [19].

Bioconcentration factor (BCF)

$$= \frac{\text{Average metal concentration in the whole plant (mg/kg)}}{\text{Metal concentration in soil (mg/kg)}}$$

Translocation Factor (TF) =  $C_{\text{aerial}} \times 1/C_{\text{root}}$ ,

$C_{\text{aerial}}$  = Metal concentration in the aerial part of plant (stem and leaf)

$C_{\text{root}}$  = Metal concentration in root of plant

## 2.6 Statistical Analysis of Data

Statistical analysis was performed using the SPSS (version 16). Differences in heavy metal concentrations were detected using One-way Analysis of Variance (ANOVA), followed by multiple comparisons using Duncan tests.

## 3. RESULTS AND DISCUSSION

Table 2 shows the physicochemical properties of soil after 12 weeks of phytoremediation studies. The high

pH level (6.90) of the soil is generally within the range for soil which was established by FEPA [20]. Soils are complex environments, and the fraction of Zn that is in the soil solution is a function of soil type, soil properties (pH, redox potential, content of organic matter). Soil pH plays an important role in the sorption of heavy metals; it controls the solubility and hydrolysis of metal hydroxides, carbonates and phosphates and also influences ion-pair formation, solubility of organic matter, as well as surface charge of Fe, Mn and Al-oxides, organic matter and clay edges [21]. This indicates that metal uptake is influenced by soil factors including pH, organic matter and cation exchange capacity as well as plant species, cultivation and age. The mobility and availability of heavy metals in soil are generally low, especially when soil is high in pH, clay and organic matter [22,23]. The zinc free soil (Table 1) had higher organic carbon (8.83%) than the polluted soil (Table 2) which had 1.25%. There was a slight decrease in nitrogen content while phosphorous was higher in zinc polluted soil than the zinc free soil. The zinc free soil had 0.18, 0.05, 1.37 and 2.24 cmol/kg of  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Mg}^{2+}$  and  $\text{Ca}^{2+}$  respectively while 0.14, 0.05, 1.76 and 2.40 cmol/kg were observed for  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Mg}^{2+}$  and  $\text{Ca}^{2+}$  respectively in zinc polluted soil after harvesting the plants. When compared with other cations,  $\text{Ca}^{2+}$  had the highest value of 2.24 and 2.40 cmol/kg for zinc free soil and zinc polluted soil respectively. The bioavailability of metals in soil is a dynamic process that depends on specific combinations of chemical, biological and environmental factors.

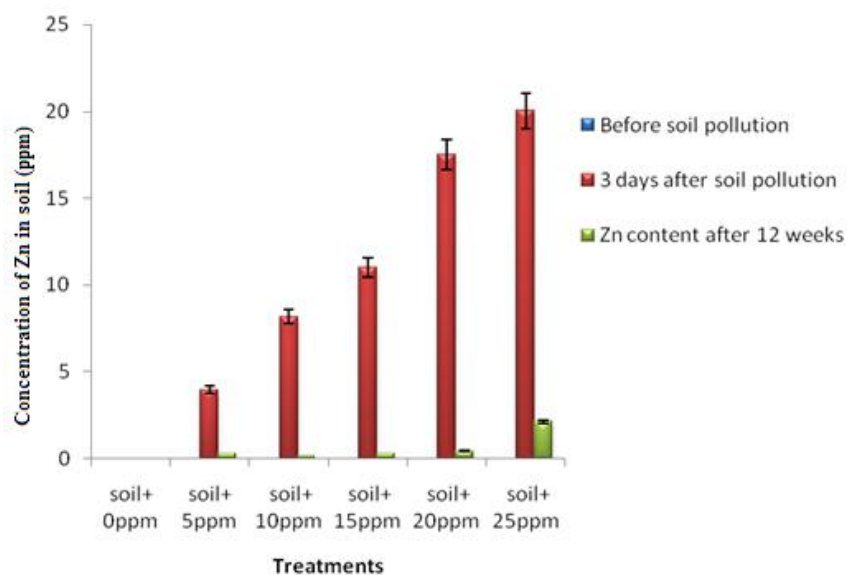
**Table 2. Physicochemical properties of Zn polluted soil after 12 weeks of phytoremediation**

Parameters	Values
pH	6.90±0.21
Nitrogen (%)	0.14±0.01
Phosphorus (ppm)	41.00±1.15
Organic Carbon (%)	1.25±0.01
Moisture	11.00±1.15
Sand (%)	78.93±0.07
Silt (%)	11.00±0.12
Clay (%)	10.07±0.18
$\text{Na}^+$ (cmol/kg)	0.14±0.01
$\text{K}^+$ (cmol/kg)	0.05±0.01
$\text{Mg}^{2+}$ (cmol/kg)	1.76±0.01
$\text{Ca}^{2+}$ (cmol/kg)	2.40±0.01

*C:N ratio of carbon to nitrogen*

### 3.1 Zinc Content in Soil Remediated with *Acalypha wilkesiana*

Fig. 1 shows the concentration of Zinc in the Zinc free soil, and Zn contaminated soil at the end of the experiment with *Acalypha wilkesiana* Fig. 1. show that zinc was not detected in the original soil used for the experiment but after 3 days of zinc pollution (artificially); this contaminant was determined and recorded before planting. Each corresponding concentration of Zn pollution in the soil after 3 days was lesser than their original values and the value was 3.97 ppm, 8.17 ppm, 11.03 ppm, and 17.53 ppm, 20.03 ppm for 5, 10, 15, 20 and 25 ppm pollution concentrations, respectively. This may be due to eveny distribution of Zinc in the experimental pots.



**Fig. 1. Zinc content in the experimental soil of *Acalypha wilkesiana***

After the harvest of the plants, residual Zinc contaminant was determined in the soil and it was found that 1.17, 1.42, 0.72, 1.03 and 1.16ppm remained in 5, 10, 15, 20 and 25 ppm, respectively. This indicated that large proportion of zinc was removed from the soil which could be traced to phytoextraction potential of the plants. It could also be possible that some of the zinc might have escaped into the atmosphere. USEPA [24] discovered the phytovolatilization of some plants and proved that heavy metals (when mopped up by plants) have the ability to escape into the atmosphere which could be in line with this finding.

### 3.2 Zinc in harvested parts of *Acalypha wilkesiana*

Fig. 2 revealed the phytoremediation potential of different compartments of *A. wilkesiana*. There was a high content of zinc in the leaves of *Acalypha wilkesiana* harvested from zinc contaminated soil after 12 weeks and the concentrations corresponded to the contents in the treated soils except in soil polluted with 10 ppm which had 1.3 mg/kg.

In contaminated soils planted with *A. wilkesiana* (Fig. 2), the concentrations of Zn after 12 weeks for leaves compartment were 2.60, 1.27, 2.17, 1.16 and 1.03 ppm, roots; 0.59, 1.37, 1.35, 1.37 and 1.40 ppm while 0.80, 1.14, 1.47, 1.60 and 1.20 ppm were observed in the stems at 5, 10, 15, 20 and 25 ppm respectively. The results indicated that *A. wilkesiana*

mopped up substantial concentrations of Zn in the above-ground biomass compared to concentrations in the roots. The results also showed that, at the end of sampling period, the leaves had the highest concentration of Zn followed by the stems and roots after 12 weeks in that order. The high Zn content in the leaves may be attributed to the high level of zinc in the soils because plants absorb metals based on their availabilities in the soil. This relationship is referred to as linear by Benzarti et al. [25].

The plant (*A. wilkesiana*) had potential to accumulate heavy metal and may be selectively used for phytoextraction of metal contaminated soil. According to Emerging technology for the phytoremediation of metals in soils, [26] phytoextraction is the ability of plants to absorb, concentrate, and precipitate toxic metals from contaminated soils into the above ground biomass (shoots, leaves and stem).

Based on the results reported in Fig. 2, it is clear that *Acalypha wilkesiana* can take up considerable amount of metal to the plant tissue as reported in the literature [27]. Besides, the results shows that zinc is present more in leaves than in the stem and roots. This result is in agreement with an early study conducted by Spirochova [27] who reported that zinc accumulated more in shoots of *Acalypha wilkesiana* than the leaves and stem.

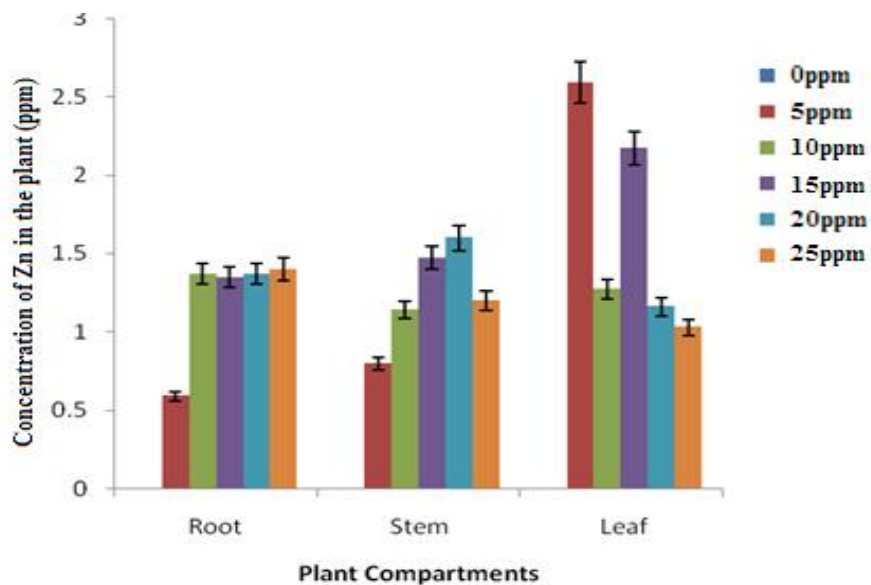


Fig. 2. Zinc concentration in roots, stems and leaves of *A. wilkesiana* harvested from Zn contaminated soil after 12 weeks

### 3.3 Bio-concentration Factor (BCF) and Translocation (TF) of Zinc in *Acalypha wilkesiana* Remediated Soil

Table 3 shows the Bio-concentration Factor (BCF) and Translocation Factor (TF) of Zn in *Acalypha wilkesiana* plant. Translocation factor is a measure of the ability of plants to transfer accumulated metals from the roots to the shoots. It is given by the ratio of concentration of metal in the shoot to that in the roots [28,29].

**Table 3. Bio-concentration Factor (BCF) and Translocation (TF) of Zinc in *Acalypha wilkesiana* remediated soil**

Treatment	BCF	TF (in leaf)	TF (in stem)
Soil + 0 ppm Zn	ND	ND	ND
Soil + 5 ppm Zn	1.14	1.02	1.36
Soil + 10 ppm Zn	0.89	0.93	0.83
Soil + 15 ppm Zn	2.31	1.61	1.09
Soil + 20 ppm Zn	1.34	0.85	1.17
Soil + 25 ppm Zn	1.04	0.74	0.86

ND=Not Detected

The highest BCF was recorded in soil polluted with 15 ppm Zn and the lowest BCF was recorded in soil polluted with 10 ppm. The highest TF in stems was also recorded in soil polluted with 15 ppm with 1.61 while the highest TF in leaves was recorded in soil polluted with 5 ppm with 1.36. There was no significant difference between the TF of Zn in the stems and leaves of *Acalypha wilkesiana* at  $p < 0.05$  significant level.

Bio-concentration Factor (BCF) is the capacity of metal accumulation in relation with plant biomass [19]. Results of this study agree with the study conducted by Baker [30] who showed that plant species may effectively and selectively act as accumulators and indicators. These findings partially agree with other studies indicating that the total metal concentration is a weak predictor of metal availability for plants [31]. Indeed, metal uptake by plants can be influenced by many factors including soil pH, cation exchange capacity, clay content, organic matter content and the presence of other ions [32].

Ability of a plant to accumulate metals from contaminated soils was evaluated by the BCF, according to studies of Santosh et al. [19]. This study assumed that plants with BCF values  $> 1$  are accumulators, while plants with BCF values  $< 1$  are excluders. Additionally, plants were classified as potential hyperaccumulators if the BCF values were  $> 1$  [33]. The results in this study showed that

*Acalypha wilkesiana* at 5 ppm, 15 ppm, 20 ppm and 25 ppm concentrations had BCF values  $> 1$ , indicating that the plant had the potential to be used as accumulators of Zn, while at 10 ppm the plant had a BCF value  $< 1$  for Zn (Table 3). The success of the phytoextraction process depends on heavy metal removal by the shoots [34]. Therefore, it is suggested that the plant species having the higher metal concentration in their shoots than in their roots can be considered as accumulators for phytoremediation. For the fact that this plant also showed BCF value  $< 1$ , it could also be an excluder in phytoremediation processes.

The ability of phytoremediation has commonly been characterized by a TF [34-36,], which is defined as the ratio of the metal concentration in the shoots to that in the roots. Plants with TF values  $> 1$  are classified as high-efficiency plants for metal translocation from the roots to shoots [33]. Only at 5 ppm, 15 ppm and 20 ppm in stem of *Acalypha wilkesiana* had TF value  $> 1$  while at 10 ppm and 25 ppm TF value was  $< 1$ . The concentration of Zn at 5ppm and 15ppm in leaves of the plant showed TF value  $> 1$  while at 10 ppm, 20 ppm and 25 ppm TF value was  $< 1$  indicating that the plant could be classified as high efficient plant for metal translocation from the roots to the above shoots. Wei and Chen [37] suggested that plant species with TF values  $> 1$  actively take up metals from the soil and accumulate them in their aboveground parts, therefore, they could be good phytoremediators. Generally, the higher metal accumulation in the aboveground components with BCF and TF values  $> 1$  have been shown to explain the higher potential for metal extraction from contaminated sites [37].

Generally, natural metal hyperaccumulators can accumulate large amounts of heavy metals in their aboveground tissues and should be tolerant of metal contaminants and other site conditions that may limit plant growth [38,29]. Ma et al. [33] and Srivastava et al. [35] identified the fern *Pteris vittata* as a novel hyperaccumulator for As, and it has received the most attention in the phytoremediation field to date. They explained that the efficacy of phytoextraction in metal contaminated soil is mainly determined by root uptake, translocation from the roots to shoots, accumulation in the aboveground components, and plant tolerance of metals. It is an important note that plant species with a higher BCF value combined with a lower TF value can be suitable for phytostabilization of soils contaminated with heavy metals [35,38,29]. Taken together, these findings indicate that phytoremediation may provide a sustainable option to remediate Zn contaminated soils, but the proper selection of plant species for specific target metals

must be achieved before implementation of phytoremediation technique.

#### 4. CONCLUSION

This study demonstrated the strong accumulative potential for Zn by *Acalypha wilkesiana*. The experiment also showed that this plant generally had the highest concentrations of Zn in its shoots (leaf and stem) at 12 weeks. Given these attributes, *Acalypha wilkesiana* is capable of continuous phytoextraction of metals from contaminated soils by translocating them from roots to shoots.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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