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Accumulation and Translocation of Potentially Toxic Elements (PTEs) from Industrial Soil by a Woody Drought Tolerant Tree, *Eucalyptus citridora*

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ABSTRACT

Field samples of *Eucalyptus citridora,* a drought tolerant tree, growing in an industrial area with high anthropogenic activities (Challawa) were collected and were separated into leaves, stems and roots. The aim was to assess the levels of accumulation and translocation of six Potentially Toxic Elements (PTEs) (Zn, Cu, Cd, Cr, Pb and Ni) from the soil media. Atomic Absorption Spectroscopy (AAS) was used to assess the concentrations. The bioaccumulation/ transfer of metals from roots to shoots and from soil to roots were evaluated in terms of translocation (TF) and bioconcentration factors (BCF). TF values of 1.58, 1.98 , 1.07, and 1.26 for Zn **,** Cu , Cr and Ni respectively indicated that *Eucalyptus citridora* was efficient in translocation of PTEs from roots to shoots and follows the trend Cu>Zn > Ni > Cr. This is an indication that the plant is therefore suitable for phytoextraction of Zn**,** Cu, Cr and Ni. BCF values of Zn (0.94), Cu (0.85), Cd (1.37), Cr (1.25), Pb (2.3) and Ni(1.66)were recorded for the elements under investigation. This show that *Eucalyptus citridora* may be a suitable candidate for phytostabilization of Cd and Pb in the contaminated soils as it retains high concentration of these metals in its roots as seen in the study area.

Keywords: Bioaccumulation, *Eucalyptus citridora*, Phytoextraction, Phytostabilization, PTEs, Translocation

INTRODUCTION

The widespread accumulation of potentially toxic metals (heavy metals) in the environment is on an alarming increase becoming a major source of concern worldwide (Si *et al*., 2021; Li *et al*., 2021). Agricultural soils are being contaminated by these trace elements at very high levels as a result of anthropogenic activities worldwide (Tang *et al*., 2020). Phytoremediation technology has recently been applied to develop an inexpensive and eco-friendly method to remove heavy metals from soil without damaging the environment (Wang *et al*., 2020). Phytoremediation of contaminated soils involves the use of plants to remove contaminants through harvestable tissue portions like the stem and the leaves which is known as phytoextraction or by stabilizing the contaminants in the root portion which is known as phytostabilization.(Yang *et al*., 2020). This is a cost-effective and eco- friendly alternative to known traditional remediation strategies that are frequently used like excavation, isolation and containment, mechanical separation, chemical treatment, electrokinetics, and bio- remediation especially for large areas(Antoniadis *et al*., 2021). Recently, increasing attention has been paid to plants, as an alternative for ecologically safe and cost-effective phytoremediation (Dobrikova *et al*., 2021). However, plant selectivity and soil quality are still potential green areas for research (Madanan *et al*., 2021).The uptake and translocation of metals in plants are controlled by various molecules, like complexing agents and metal ion transporters. This specific transporter, like H^+, coupled trans membrane protein; channel protein is in the root cell plasma membrane and is essential for the absorption of heavy metal ions from the ground(Sharma *et al*., 2021). During the process of absorption by plant, different metallic ions pass through the membranes and mediate metal translocation from roots to shoots (DalCorso *et al*., 2019).

Eucalyptus citridora (Family: *Myrtaceae*) was selected for this study. To the best of our knowledge, this is the first study investigating the accumulation capacity of *Eucalyptus citridora*in proportion to background metal concentration in this study area (Challawa Industrial Area).*Eucalyptus* has a huge shoot system which should be able to bioaccumulate large concentrations of heavy metals from the soil (Bilal *et al*., 2014).The use of *Eucalyptus* as bioaccumulator for toxic metals has a low risk in terms of entering the food chain because the plant is not consumed by humans and hardly ever used by mammals (Bilal, *et al*, 2014). Eucalyptus species have been proposed for phytostabilization of contaminated soils because of their moderate to low rates of heavy metal accumulation into aboveground biomass, thus reducing the risk of transmission of contaminants into the food web (Navarro-ferna *et al*., 2017) .The aim of this study is to assess the accumulation and translocation of six heavy metals in tissues of the plant growing naturally at Challawa Industrial Estate. The specific objective is to look at the suitability of using the plant for phytoextraction, phytostabilization and as potential bio-indicators for metal contaminated soils. A picture of an old and young *Eucalyptus citridora* tree is shown in plates 1a and 1b respectively.

Plate 1a *:(*A picture of an old *eucalyptus citridora tree)*

Plate 1b *:(*A picture of a young *eucalyptus citridora tree)*

MATERIALS AND METHODS

Preparation of Reagents

In the preparation of reagents, chemicals of analytical grade purity and deionized water were used throughout the analysis. All the laboratory apparatus (glass wares and the plastic containers) were first soaked in nitric-acid and thoroughly washed with detergent solution, followed by several rinses with tap water, deionized water and finally with the analyte samples.

Study Area

The field study was carried out in the vicinity of Challawa Industrial area. The area is located in Kumbotso local government of Kano state. Sampling was done at Yandanko village in Challawa Industrial area, located between latitudes 11°52´48.81" and along longitudes 8°28´17.25". The Global Positioning System (GPS) was used in recording the coordinates and Geographical Information System (GIS) was used to locate the map of the study area as shown in Figure 1.

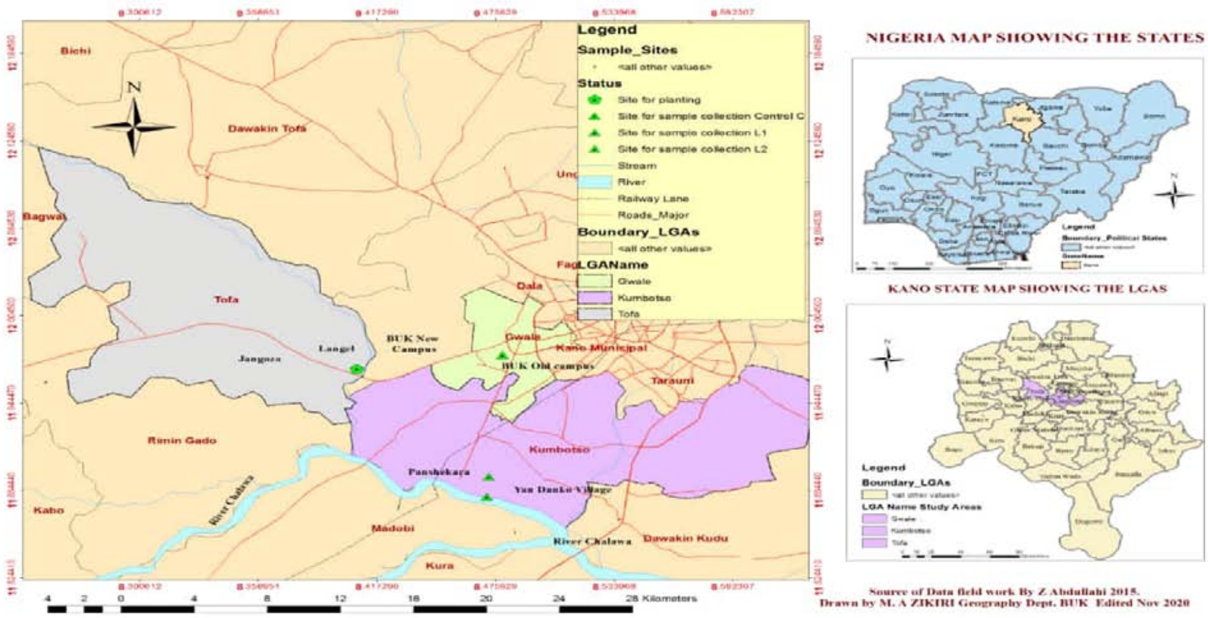

Figure 1: Map of Kumbotso and Tofa Local Government Areas Showing Sampling location

Field Sampling of Soil and Plant Species (Yandanko, Challawa and Langel)

Nine sampling points from three locations were systematically established after every 100m. *Eucalyptus citridora* was collected for analysis with at least three species per sampling point including the control site (Langel village), which was far away from Challawa Industrial area. The plant specie was collected from these sites at almost similar stage of growth as that from the Challawa sample and were used as the control.

Identification of the collected plant specie was done at the Plant Biology Department of Bayero University Kano and a herbarium number *Eucalyptus citridora* (BUKHAN 0028) was assigned to the plant. The sample was labeled, placed in polythene bags and transported to the University and air-dried. Three soil samples were also collected at each sampling point for the plant and composites obtained. The composite soil samples was air dried and ground into fine powder using pestle and mortar and sieved through 2 mm plastic mesh and stored in labeled polythene bags.

Digestion of Soil Samples

1g of the soil sample from Yandanko, at Challawa was mixed with 20cm^3 of nitric acid (HNO_3) (70% w/v, S.G 1.42g/cm^3) and allowed to stand for 1 hour. 15cm^3 of perchloric acid ($HClO_4$) (70% w/v, S.G 1.67g/cm^3) was then added and the mixture was placed in a sand bath and heated at 55°C until dense white fumes was observed. It was allowed to cool and filtered into the 100cm^{3}volumetric flask and made to the mark. The resulting solution was analyzed for metal concentrations using Atomic Absorption Spectrophotometer Buck scientific, Model-210VGP (Tanee aand Amadi, 2016).

Plant Tissue Analysis

Before the analyses root and shoot samples were thoroughly washed using distilled water to remove all adhering soil particles. Samples were then oven dried to constant weights at 105°C. Each dried sample was ground to powder and 0.5 gram of each sample was used for analysis. These samples were placed in a crucible and transferred to the muffle furnace and ashed at 550°C.The ash was then dissolved in 10ml 0.1M nitric acid, filtered and made up to the 100cm^3 mark and analyzed for metal content using Atomic Absorption spectrophotometer (Inuwa and Mohammed, 2018).

Statistical analysis

All data gathered were analyzed statistically using analysis of variance (ANOVA). When significant differences were detected between treatments, Tukey test (at $P < 0.05$) was calculated for each parameter and all graphs were plotted by employing Microsoft Excel.

RESULTS AND DISCUSSION

Soil properties

The soil physicochemical characteristics from the study area have been reported in our earlier works. Results revealed that the area is characterized by sandy texture (66.8%). As indicated from earlier report, the pH of soil was slightly acidic with a value of 6.0 while that of the control is 6.8 (Zakari and Audu, 2021).

Potentially Toxic Elements (PTEs) in *Eucalyptus citridora*

The data obtained from the field studies show that the PTEs contents in the tissues of *Eucalyptus citridora* varied from each other in the area under study. Overall, all studied PTEs occurred at elevated levels in plant biomass collected from the investigated site. Normal and phytotoxic concentrations of Pb, Cu, Zn and Cd were reported, which were 0.6–28 mg kg^{-1} for Pb, 20–30 mg kg^{-1} for Cu, and 100–300 mg kg^{-1} for Zn and 0.1– 3 mg kg^{-1} for Cd (Midhat *et al.*, 2017).

Fig 2-7 represents the charts showing the distribution of levels of Zn, Cu, Cd, Cr, Pb and Ni in the plant *Eucalyptus citridora*. In Fig 2, the Zn concentration in the *Eucalyptus citridora* tissues follows the decreasing order pattern as root > leaf > stem.

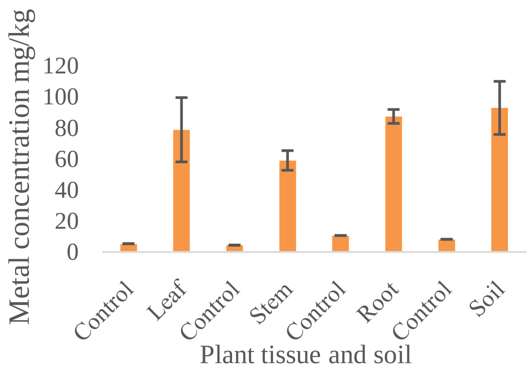

Fig 2:Distribution Levels of Zinc in Tissues and Soil Samples of *Eucalyptus citridora*

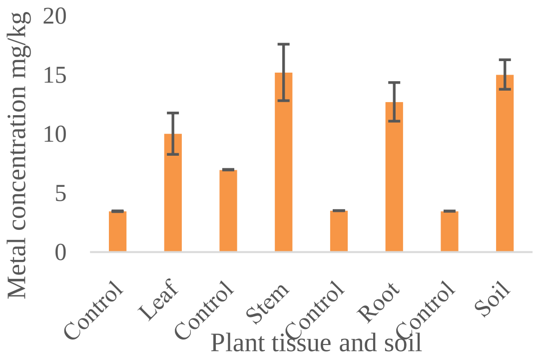

Fig 3:Distribution Levels of Copper in Tissues and Soil Samples of *Eucalyptus citridora*

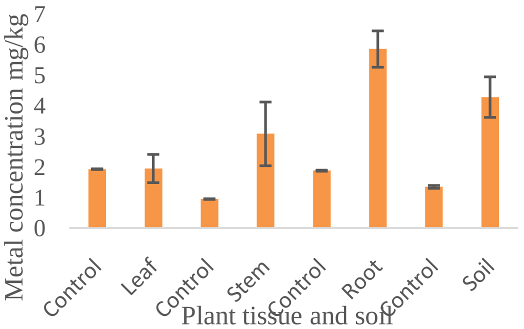

Fig 4:Distribution Levels of Cadmium in Tissues and Soil Samples of *Eucalyptus citridora*

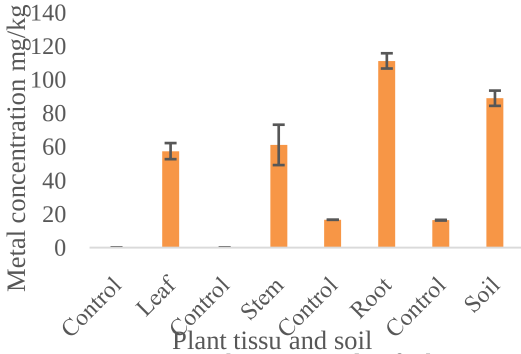

Fig 5:Distribution Levels of Chromium in Tissues and Soil Samples of *Eucalyptus citridora*

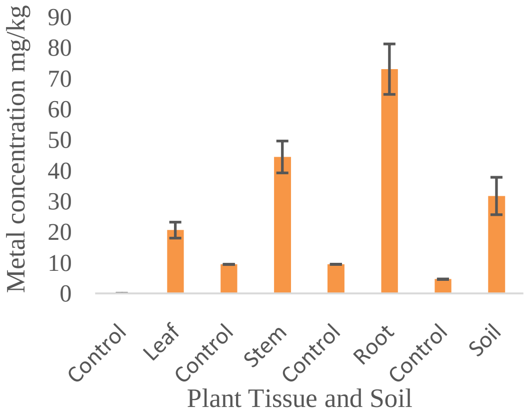

Fig 6:Distribution Levels of Lead in Tissues and Soil Samples of *Eucalyptus citridora*

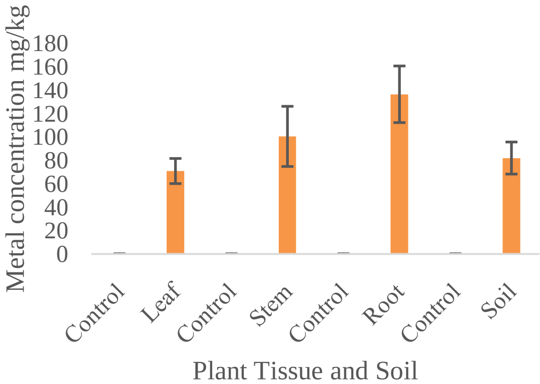

Fig 7:Distribution Levels of Nickel in Tissues and Soil Samples of *Eucalyptus citridora*

One way Anova shows that there is significant difference between the Zn levels in the leaf, stem, root and soil at $P < 0.05$. The Tukey test however, revealed that the Zn levels in the soil is significantly higher than those obtained in the tissues. However, there is no significant difference at $P < 0.05$ between the levels of zinc in leaf, stem and root. Also, the Zn levels in the leaf, root and soil are not significantly different at $P < 0.05$ from each other. Results showed that both the leaf and roots of *Eucalyptus citridora* were found to accumulate high amounts of zinc. This result is similar to the findings of Daniel *et al.*, 2012 who reported high Zn accumulation in the leaves of *Eucalyptus camaldulensis*. (Ibrahim & El, 2020) also modelled the accumulation of zinc in the tissues of *Nerium oleander* and predicted its accumulation in the aerial parts of this plant. Similarly, Bilal *et al.* (2014), reported *Eucalyptus* species to be effective in the bioaccumulation of Zn in its tissues. Zinc is an essential trace element required for plant growth and plays both structural and catalytic roles in numerous enzymes that participate in various important metabolic processes (Ji *et al.*, 2021).

Copper is an essential plant nutrient, therefore, higher concentrations of Cu in soils and its accumulation in roots and shoots are expected(Godwin & Oluwagbemiga, 2020).The Cu concentration in the *Eucalyptus citridora* tissues follows the decreasing order pattern as stem>root> leaf. One way Anova shows that there is significant difference between the Cu levels in the leaf, stem, root and soil at $P < 0.05$.The Tukey test however, revealed that there is no significant difference at $P < 0.05$ between the levels of Cu in the leaf and root. However, the Cu levels in the leaf of the plant is significantly lower than those obtained in the stem at $P < 0.05$.However, results showed that stem and roots of *Eucalyptus citridora* are capable of accumulating Cu than leaf as depicted by fig 3. This result is similar to the findings of Daniel *et al.*, (2012) who reported similar Cu accumulation in the roots of *Eucalyptus camaldulensis*. However, higher copper levels were reported in the shoots of *Hyptis Suaveolens Poit* by (Godwin & Oluwagbemiga, 2020).

The Cd concentration in the tissues follows the decreasing order pattern as root > stem > leaf. One way Anova shows that there is significant difference between the Cd levels in the leaf, stem, root and soil at $P < 0.05$. The Tukey test however, revealed that the Cd levels in the root of the plant is significantly higher than those obtained in the leaf and stem. However, results showed that roots of *Eucalyptus citridora* were found to accumulate fairly good amounts of Cd than leaf and stem as depicted by Fig 4. This results agreed with the findings of Zeng *et al.* (2018) in a similar work for Cd accumulation in roots of *Osmanthus fragrans* and *Ligustrum vicaryi*. Also, (Xiong Li & Yang, 2020)in another work reported high cd accumulation in the roots of *Sansevieria trifasciata*.

This is also consistent with our findings. Cd is has no nutritional value for plants and is toxic having no biological function in plants (Daryabeigi *et al*., 2020), while its accumulation can cause damage even at low concentrations (Dobrikova *et al*., 2021).

Chromium and its compounds are widely used in industrial production and are mostly used as raw materials in metal processing, electroplating, and tanning industries (Wang *et al*., 2021) .The Cr concentration in the tissues follows the decreasing order pattern as root > stem > leaf. One way Anova shows that there is significant difference between the Cr levels in the leaf, stem, root and soil at $P < 0.05$. The Tukey test however, revealed that the Cr levels in the root of the plant is significantly higher than those obtained in the stem and leaf. However, there is no significant difference between the levels of Cr in the leaf and the stem. Our results showed that roots of *Eucalyptus citridora* were found to accumulate high amounts of Cr than leaf and stem as depicted by fig 5. This result is consistent with the findings of Tauqeer *et al*. (2019) who reported high levels of Cr in the roots of *Conocarpus erectus*. However, our result differs with the findings of Daniel *et al*., (2012) who reported a slightly higher Cr accumulation in the leaves than the roots of *Eucalyptus camaldulensis.*

The Pb concentration in the tissues follows the decreasing order pattern as root > stem > leaf. One way Anova shows that there is significant difference between the Pb levels in the leaf, stem, root and soil at $P < 0.05$. The Tukey test however, revealed that the Pb levels in the root of the plant is significantly higher than those obtained in the stem, soil and leaf at $P < 0.05$. Results showed that roots of *Eucalyptus citridora* were found to accumulate substantial amounts of Pb than leaf and stem as depicted by fig 6. This result is consistent with the findings of Steliga & Kluk, (2020) who reported high concentrations of Pb in the roots of *Festuca arundinacea*. However, our result differs with the findings of Daniel *et al*., (2012) who reported a slightly higher Pb accumulation in the leaves than the roots of *Eucalyptus camaldulensis.* In plants, Pb is absorbed by roots and its effects are dependent on environmental conditions, concentration, exposure time, species, and plant development stage (Pires-Lira *et al*., 2020).

The Ni concentration in the *Eucalyptus citridora* tissues follows the decreasing order pattern as root > stem > leaf. One way Anova shows that there is significant difference between the Ni levels in the leaf, stem, root and soil at $P < 0.05$. The Tukey test however, revealed that there is no significant difference at $P < 0.05$ between the levels of Ni in the leaf, soil and stem. A similar observation was noted for the stem and the root at $P < 0.05$.However, results showed that roots of *Eucalyptus citridora* were found to accumulate considerable amounts of Ni than leaf and stem of as depicted by Fig 7. This result is consistent with the findings of Manikandan *et al*., (2016)who reported a high concentration of Cr in the roots of *Dalbergia sisso.* This result however, differs with the findings of Daniel *et al*., (2012) who reported a slightly higher Ni accumulation in the leaves than the roots of *Eucalyptus camaldulensis.* Nickel is an essential micronutrient for plants, but a high level of Ni acts as heavy metal, and it has adverse effects on photosynthesis, plant growth, and the quality and yield of plants (Heidari *et al*., 2020) .

Bioaccumulation and Translocation in Field Plants

The Bioconcentration factor (BCF) was determined as previously described for in-situ phytoextraction potential in native hyper accumulator plants(Sharma *et al*., 2020).While the translocation factor (TF) was evaluated by calculating the ratio of metal concentration in plant shoot and metal concentration in plant root (Sharma *et al*., 2020; Amir *et al*., 2020).

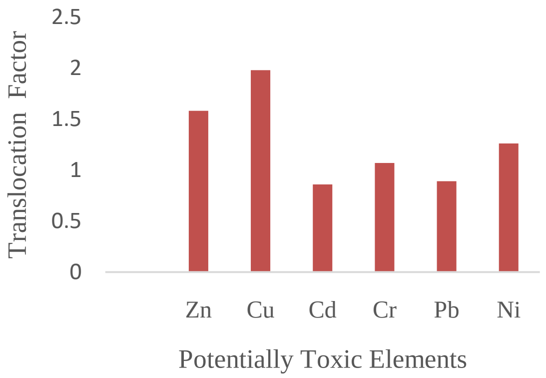

Fig 8a: Translocation factor of the six potentially toxic elements in *Eucalyptus citridora*

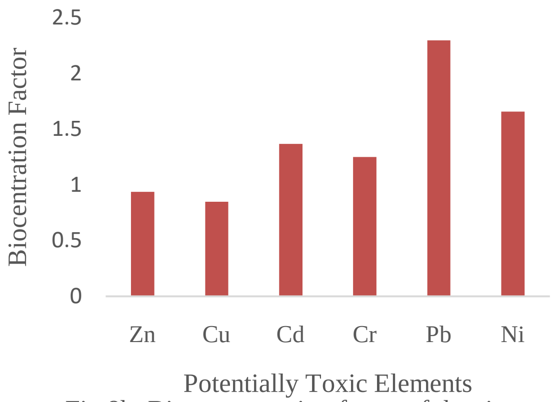

Fig 8b: Bioconcentration factor of the six potentially toxic elements in *Eucalyptus citridora*

The Translocation and Bioaccumulation in *Eucalyptus citridora* is as shown in Figs 8a and 8b respectively. The translocation factors (TF) expresses the ability of a plant to translocate heavy metals from the root to shoot in the soil-plant system (Álvarez-mateos & García-martín, 2019). TF determines plant efficiency in heavy metals translocation from the roots to the shoots. It shows whether the native plant can be classified as an accumulator, excluder or indicator. A plant is considered efficient in metal translocation from root to shoot when TF > 1; the reason being an efficient metal transport system.TF < 1, suggest an ineffective metal transfer indicating that such plant species accumulate metals mostly or substantially in the roots and rhizomes than in the shoot portions or the leaves of plants (Usman *et al*., 2019). Bioconcentration factor (BCF) on the other hand, can be used to evaluate a plant's phytoremediation potential. A BCF value > 1 indicate that a plant is a hyperaccumulator whereas, a value less than one is indicative of an excluder (Usman *et al*., 2019).Both BCF and TF have to be considered for evaluating whether a plant is a metal hyperaccumulator. A hyperaccumulator plant should have BCF>1 or TF > 1(Sharma *et al*., 2020). *Eucalyptus citridora* was screened for Zn, Cu, Cd, Cr, Pb, and Ni. Results showed that it has the ability to take up and translocate more than one heavy metal from roots to shoots as shown in figs 8a and 8bwith noticeable variations between TF and BCF.

Fig 8a depicts that *Eucalyptus citridora* was able to translocate PTEs from roots to shoots with TF values of 1.58, 1.98, 1.07 and 1.26 for Zn**,** Cu, Cr and Ni respectively. This is indicates that the plant is a likely candidate for phytoextraction of Zn**,** Cu, Cr and Ni. The exception being Cd and Pb with a TF value of 0.86 and 0.89 respectively. In Fig 8b, which illustrates the BCF values for *Eucalyptus citridora*, BCF > 1 were observed for the elements Cd (1.37),Cr (1.25), Pb (2.3), and Ni (1.66) with the exception of Zn (0.94) and Cu (0.85) which had a BCF < 1.Therefore, Cd and Pb with a TF value of 0.86 and 0.89 respectively on the one hand and BCF value of 1.37 and 2.3 on the other hand shows that Eucalyptus citridora accumulates these two metals (Cd and Pb) more in the roots than in the shoots indicating ineffective transfer.

In general, BCF values from this study show that *Eucalyptus citridora* may be suitable a candidate for phytostabilization of Cd and Pb in soil as it still retains high concentration of these metals in its roots.

CONCLUSION

The potential for phytoremediation through bioaccumulation of *Eucalyptus citridora* against six PTEs (Zn, Cr, Cd, Cu, Ni and Pb) was studied. In the course of this study, we can reasonably conclude that the plant is a resistant species containing in its tissues amounts of PTEs that were much higher than those considered toxic for normal plants. Based on the translocation factor (TF) and the bio concentration factor (BCF) values, the study showed the suitability of this plant for both phytoextraction of Zn**,** Cu, Cr and Ni and phytostabilization of Cd and Pb in the study area and where desired.

Disclosure statement: *Conflict of Interest:* The authors declare that there are no conflicts of interest.

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