Cadmium Tolerance and Phytoremediation Strategies of Selected Tropical plants Cultivated on Industrial Dump Site under the Influences of Two Mycobionts

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Abstract

This research was carried out on a waste disposal site of a paint industry in Ijebu- Ijesha, Osun State, Nigeria in an attempt to assess the cadmium toxicity tolerance and bioremediation strategies of selected tropical plants cultivated under the influence of two mycobionts. On the waste disposal site, two plots (Plot A and B) having size of about 9 m by 12 m each were prepared with a control plot (Plot C) which is a non-polluted site. The experimental design on the first plots (Plot A) was 4x2x3 in which viable seeds of the four selected weeds were grown and inoculated with two mycobionts (*Glomus intraradices* and *Glomus mosseae*) respectively in a randomized complete block design with three replicates. However, on the second (Plot B) and control plots, only the seeds of the weeds were grown without mycorrhiza treatment using the same experimental design of 4x3 respectively. After Twelve weeks of planting, each plant was harvested, separated into root and shoot tissues and analysed for Cd concentrations using Atomic Absorption Spectrophotometry (AAS). Data collected were subjected to descriptive and inferential statistics. The highest (18.51 mg/kg) concentrations of Cd were reported in *Amaranthus spinosus* with root and shoot bioconcentration factors; and transfer factors greater than 1.00. Out of the four plants, 75% act as cadmium phytostabilizers in the absence of inocula and were good candidates for the biomanagement of hazardous sites while all the plant displayed the characteristics of a cadmium phytoextractor under mycorrhizal inoculation with *Amaranthus spinosus* having the highest mobility indices of cadmium under the influence of *Glomus intraradices.* The study concluded that the four weeds are good Cd phytoextractors in the remediation and biomanagement of marginal lands under bioaugmentation.

Key words: *Glomus intraradices, Amaranthus spinosus*, Cadmium, Mobility indices

Introduction

Soil contamination by heavy metal (HM) species has become a significant environmental concern over the past few decades. Most conventional methods used to restore polluted soil-environment such as soil flushing, soil washing, electro-osmotic process, precipitation and solvent extraction among others are not eco-friendly (Lin et al.,2005; Yadav et al., 2017). Besides, the methods also need high operational and maintenance costs. This has drawn interest to develop a scientifically cost-effective remedial measure to remove heavy metals (HMs) from contaminated sites. Presently in most developing countries, there are lots of illegal brownfields and dumpsites awaiting restoration. In the year 2016, approximately one-fifth of the global cancer incidence was associated with exposures to environmental pollutants such as metals (Pure and Green, 2016). The adverse effect was disproportionately higher in developing countries than the developed. Moreover, dumpsites from industries such as industrial estates, smelting, artisanal small-scale gold mining (ASGM), product manufacturing, chemical manufacturing, and the dye are major sources of pollutant across the globe (Adei and Osibanjo, 2009; Dada and Awotoye, 2013; Dada et al., 2015; Awomeso et al., 2017). Using the Disability-Adjusted Life Years (DALYs) rating, deaths and disabilities of biotics were previously linked to other risk factors that are more accurately attributed to pollution by metals from these industries; with over 32 million people at risk accounting for 7 million to 17 million Disability-Adjusted Life Years (DALYs) in low- and middle-income countries (Pure and Green, 2016).

In Nigeria, the scourge of environmental pollution has reached a frightening scale especially in industrialized areas such as Ogun state, Lagos State, Delta state, and Bayelsa State. Moreso, artisanal small-scale gold mining (ASGM) activities are carried out in Zamfara and Osun state among others (Yusuff et al., 2002). As at 2002, Nigeria had about 5,000 registered industrial facilities and 10,000 illegal small scale industries without proper waste management facilities and Environmental Impact Assessment (E.I.A.) prior their establishment (Yussuf et al, 2002). This is evident as so many chemical companies lack adequate and proper facilities for the treatment of their waste prior to disposal. Thus, mixed pollution with heavy metals is one of the characteristics of spill areas, mine sites and industrial effluents dump sites.

According to Ethiopian Gazette (2019), Lagos state, Nigeria is the third most polluted cities in Africa. Presently, over 14,100,000 people were found residing in sub-urban of Lagos State while approximately 9, 000,000 people live in the city area (World Statistical Data, 2019) with pollution index of 82.49. Unfortunately, most developing countries of the world could not afford the cost of conventional remediation because of lack of adequate fund, misplacement of priorities, poor environmental management strategy and low level of technological advancement to handle problems of pollution.

To address these challenges, the application of a method that offers a cost effective and an environmentally friendly approach that utilizes bio-agents with approprioate techniques such as phytoextraction could be employed. Although, over 500 plants species had been identified as metalliferous or hyperaccumulators of metals from polluted sites, majority of them are exotic species with adaptation to a specific natural habitat (Krämer, 2010). Thus, the need to access the phytoremedial potentials of indigenous plant species whose potentials are yet to be known, as hidden potentials of some plants species are yet to be unveiled for human benefit particularly as it relates to improving the quality of the environment.

Among heavy metals needed to be removed from environments are Cadmium (Cd), Lead (Pb), Mercury (Hg), Chromium (Cr), Copper (Cu), Manganese (Mn) because of their toxicity. However, out of the non-essential ones, Cadmium, represented with symbol Cd is a heavy metal considered as one of the most harmful metal contaminants since the occurrence of Itai- Itai disease in Japan (Yukimasa, 1975). It is a HM having half-life of ten to thirty years. Cadmium is toxic at very low exposure levels and has acute and chronic effects on health of the environment. It is non-degradable in nature and hence; persist in the environment after release.

One of the established approaches to remove Cd from the environment is phytoremediation. Phytoremediation is an approach that proffers an environmentally-friendly alternative solution to the problem of pollution by HM and other contaminants. Specifically, phytoremediation process which involves the use of plant biodiversity to remove recalcitrant contaminants had been suggested to be a biotechnological approach that is efficient and economical in the remediation of heavy metal contaminated soils (Bacchetta, et al., 2013). The method is also cost-effective, non-intrusive and aesthetically pleasing.

Phytoremediation method exploits the inter-linked between physiological and molecular mechanisms in floras that tolerate heavy metal toxicity. This shows that metal hyperaccumulation and tolerance characteristics are genetically inherited traits in plant species that could be explored in bioremediation of metal contaminated environments. In plants, high tolerance to HM toxicity is based on reduced metal uptake or increased internal sequestration (Sarma, 2011). However, improvement of plants for phytoremediation process can be achieved by modifying characteristics like metal uptake, transportation, accumulation and plant's tolerance to metals.

Plants species have been known to possess a range of potential cellular mechanisms that may be involved in the detoxification of heavy metals and thus tolerance to metal stress (Sarma, 2011). These plant species can tolerate metal toxicity by reducing the concentrations of toxic metals from contaminated media; and taking up metals through the root system to the shoot system without showing toxicity syndrome (Xiao-min et al., 2013). However, some plant species are known to be adversely affected by showing various types of irreversible changes. For instance, direct consequences of such effects are inhibition of cytoplasmic enzymes and damage to cell structures due to oxidative stress (Jabeen et al., 2009). Oxidative stress is related to formation of reactive oxygen species (ROS) and cytotoxic compounds like methylglyoxal (MG). These compounds are known to disrupt the equilibrium of ionic homeostasis within plant cells (Hosman et al., 2017). Cd also induces changes in plants by affecting H^+-ATPase enzymatic activities associated with cell membranes in plant species (Krämer, 2010). Moreover, when exposed to Cd, photosynthetic apparatus in plants species were reported damaged with decrease in chlorophyll contents of plants while stomatal regulations are usually inhibited (Baker and Walker, 1989).

The adaptive responses of plants to HM contaminated environments are efficient processes that include many physiological, molecular, genetical and ecological traits. These traits give certain species the ability to survive, tolerate, and hyperaccumulate toxic metals (Singh et al., 1999). Most plant species responded to Cd induced oxidative stress by modulating non-enzymatic antioxidants such as Glutathione (GSH) and Non-Protein Thiols (NPSH) and enzymatic antioxidants; Superoxide dismutase (SOD), Ascorbate peroxidase (APX) and Glutathione reductase (GR). Some plants were able to tolerate Cd induced stress using effective antioxidative defense mechanisms (Ji et al., 2015) which may involve production of some antioxidants and chelating agents. It has been reported by Lee, et al. (2013) that phytochelatins produced in plant species play a significant role in plant metal tolerance. Phytochelatins are oligomers of glutathione. They are produced by the enzyme phytochelatin synthase. Phytochelatins act as chelators and are important for heavy metal detoxification.

Apart from the production of antioxidants to alleviate toxicity from HM pollution, plants rely on the efficiency of bioagents such as rhizobium species, mycorrhizals among others. These microbes may serve as bioaugument in phytoremediation processes. Bioaugument may not only shield the plants from potential effect of the stress of pollution but also enhance the efficiency of plants to phytoremediate. However, for the purpose of this study, two ecotype arbuscular mycorrhizal fungi (AMF) were used; and these are *Glomus intraradices* and *Glomus mosseae*. AMF are known to alleviate heavy metal stress in plants (Turnau et al., 2006, Cui et al., 2011) species. They are beneficial symbiont that assists plants in uptake; and synthesis of numerous nutrients and heavy metal binding. In addition, the oxalate crystals produced by mycorrhizal fungi are also known to immobilize and detoxify HM (Khan, 2005). Their filamentous hyphal structure deeply penetrates into the deeper soil aggregates and chelates or adsorbs HM.

In assessing the toxicity tolerance and phytoremediation strategies of plants some indices such as concentration of metals in plant tissues, Bioaccumulations factors (BCFs) and Transfer factors (TFs) may be considered as well as other plant growth factors such as plant biomass and survival rate of plants after exposure to contaminants. The BCFs is the ratio of the contaminant in plant tissues to that of the environmental loadings. This may be evaluated for the root and for the shoot of plant. Thus, the root BCF is the ratio of the metal concentration to that of environmental loading while the shoot BCF is the ratio of metal concentration in shoot to that of environmental loadings. The TF is the ratio of metal concentrations in the shoot to that of metal concentrations in the root of plant species. In plants, the strength of metal mobility indices determine the plant's degree of metal tolerance. A plant is referred to as cadmium hyperaccumulator, when the TF and the shoot BCF in plant is greater than 1.0 with ability to accumulate more than 100 μg/g of cadmium in the shoot. Furthermore, plants which have high levels of heavy metals in the roots but with shoot/root quotients that is less than 1 are classified as heavy metal excluders (Jabeen et al., 2009).

However, some of these species are edible with potentials to introduce pollutants into the food chain. Thus, the need to explore intrinsic potentials of indigenous tropical weeds for cleaning contaminated soils. Besides, many studies on phytoremediation in the tropics such as phytoextraction, phytostabilization among others are known to be conducted in a greenhouse under controlled environment (Fatoki and Ayodele, 1991; Adewole et al., 2010; Dada and Awotoye, 2013; Oseni et al., 2015; Oseni et al., 2016) in which the experiment may fail to replicate the precise cellular conditions of the model plant when grown on field *in situ*. Therefore, in this study four weeds namely *Amaranthus spinosus*, *Euphorbia heterophylla*, *Sida acuta* and *Synedrella nodiflora* were selected and grown on an industrial dump site at Ijebu-jesa. The plants were selected in order to reduce the risk of contaminating the ecological food chains (Wuana and Okieimen, 2011; Dada, 2013); as the plants are regarded as weeds in Nigeria (Akobundo and Agrawal, 1998). Moreover, this plants were selected because they were observed to thrive in most of the wastelands than other plant species. From field studies, some plant species that thrive naturally on contaminated soils are known to accumulate heavy metals more than the normal levels encountered generally in plants (Smith and Bradshaw, 1992; Escarre et al., 2000; Boularbah et al., 2006; Dada, and Awotoye, 2013). Thus, Baker et al (2000), reported that these plants could be used as model plants to remove heavy metal from metalliferous soils. According to Adei and Osibanjo (2009), In Nigeria there is dearth of information about the phytoremediation potentials of indigenous plants with metal tolerant traits. Hence, in this study, the cadmium toxicity tolerance and phytoremediation strategies of the weeds under the influence of *Glomus intraradices* and *Glomus mosseae* were assessed *in situ*. This is with a view to suggesting a model phytoremediation species with ability to cope with effects of cross pollution in multi-metals polluted systems.

Materials and methods

Study area

The research was carried out on an industrial dump site in Ijebu- ijesha, Osun State, Nigeria (Figure 1) and a non-polluted site located about 40 km from the polluted site. The dump sites

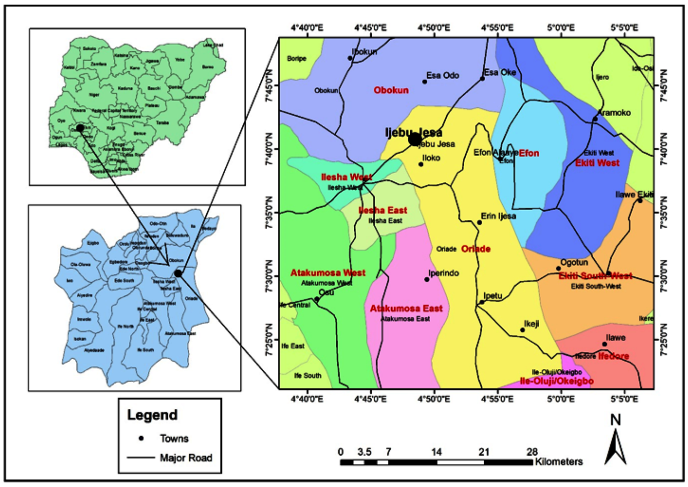

Fig. 1: Map of Nigeria showing the Osun State and the sampling area in Ijebu-Jesa

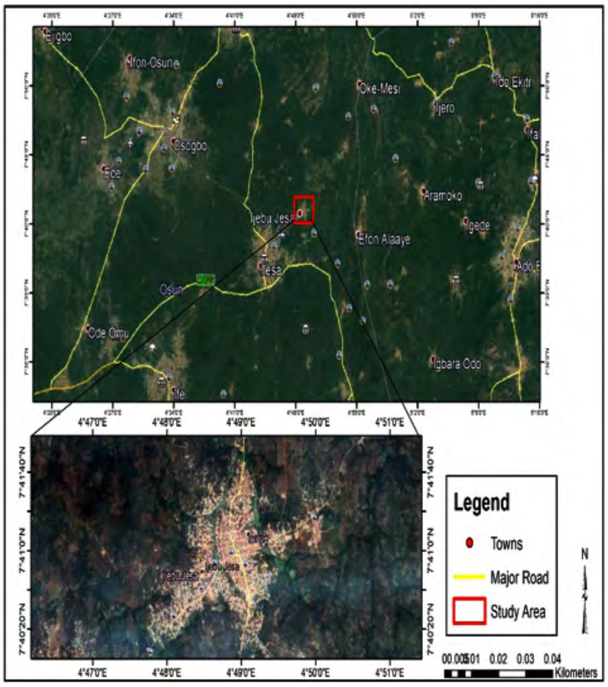

Fig. 2: Map of the Sampling area at Ijebu-Jesa

had been subjected to indiscriminate dumping of both solid and effluent discharges from the paint industry located around the study area. However, the level of human intrusion at the control plot is minimal as the plot is a forested area located far away from the dump site.

Ijebu- Ijesha town shares boundary with the city of Ilesha with lots of wetlands around the boundary. The industrial dump site is geographically located very close to the wetland area of the city of Ijebu-Ijesha. The town is between Latitude 04^0.00 and Longitude 04^0.30 and is geographically located in moderately hot, humid tropical climatic zone of Southwestern Nigeria (Figure 2). Rainfall lasts between March and October with two peaks in July and September while the dry season occurs between November and April. The temperature is relatively high during the dry season with the mean value around 30°C. However, the harmattan period at Ijebu- Ijesha is from December to January during the dry season. Low temperatures are experienced between July and August during rainy season when the minimum temperature could be as low as 17°C. The experiment was carried out from the month of December 2017 to March, 2018 during the dry season in order to reduce cross contamination of pollutants.

Being close to the wetlands, the water table of the polluted site was high, thus increasing the risk of cross pollution by the effluents and solid wastes discharged from most of the paint industries located around the wetland axis. The industrial dump site was divided into two plots (Plot A and B) with each of the plot having size of about 9 m by 12 m each. The two plots were cleared and tilled for the experiment. Plot A was the inoculated plot with mycorrhizars while Plot B was non-inoculated. The undisturbed land located almost 40 km away from the contaminated plots was the Control Plot. The contaminated plots were separated by creating a 100 cm space between the two plots. The average daily minimum and maximum temperature of about 21°C and 32°C respectively are recorded at the polluted site while the average daily minimum and maximum temperature of about 19°C and 31°C respectively were recorded at the control plot. There is a graduation from weakly acidic to moderately basic top soil. The vegetation on the three plots were rich in diversity. Members of the family Asteraceae, Poaceae, Euphorbiaceae, Amaranthaceae, Boraginaceae and Malvaceae were found growing on the three plots. However, the family Amaranthaceae and Malvaceae dominated the polluted site while the family Poaceae dominated the control plot.

Experimental Design

Viable seeds of the four selected weeds were planted separately on the three plots which were Plot A (Contaminated site inoculated with mycorrhizal), Plot B (Contaminated site only); and Plot C (Control site). On the Plot A the experimental design was 4x3x2 in which viable seeds of the four plants were cultivated in a randomized complete block design; replicated thrice with two ecotypes mycorrhizal species (*Glomus intaradices* and *Glomus mosseae*). Thereafter, on Plot B and Plot C seeds of each weed were planted using a randomized complete block design with three replicates on each plot in this design 4x3.

Pre-planting operations

The plots were cleared and stumps removed. Plants cleared from the plots were allowed to decay for almost six months on the soil before planting viable seeds of the weeds.

Collection of Soil

Composite soil samples of the study area were randomly collected from depths of 0–20 cm using soil agar. The soil samples were air-dried for a week and then sieved using 2-mm mesh gauze to remove debris and stones.

Soil analyses

The pH value was determined with a calibrated Jenway glass electrode pH meter on 1:1 soil: deionized water extracts (IITA, 1992). Particle size analysis was also determined using the hydrometer method (Bouyoucous, 1951). Phosphorus was determined by Vanado-molybdate yellow complex formation using colorimeter. Walkey-Black (1934) method was used to determine percentage organic carbon while cation exchange capacity (CEC) was determined following IITA (1992) method. To determine the concentrations of metals in soil, soil samples were air-dried for two weeks. The dried soil samples were crushed using mortar and pestle. The crushed soil samples were sieved using 2 mm sieve. Thereafter, less than 2 mm fraction of soil samples were digested. This was done using 2 M HNO_3 in centrifuge tubes which were placed in boiling water in a 1 L beaker on a hot plate for 2 h and shaken at 20 minutes intervals. The digested samples were filtered into standard flasks. Concentrations of Zn, Pb, Cd and Cu were determined using a Perkin Elmer Atomic Absorption Spectrophotometer while the concentrations of Ca, Mg, K and Na were determined using Flame photometer.

Post-planting Operations

Twelve weeks after planting, plants were harvested, separated into roots and shoots and washed with tap water to remove soils and rinsed with tap water. Parts of the roots were collected and stored in 50 % ethanol in McCartney bottles for mycorrhizal infection determination.

Plant analyses

Plant samples were carefully collected from the field and washed under running tap water to remove adhered soil. The plant samples were separated into root and shoot parts. The root and shoot samples were oven-dried at 70^0C for 3 hours using Gallen Kemp oven. Ground root and shoot samples were digested separately with concentrated H_2SO_4 and 50 % (v/v) H_2O_2 at 90^o C by macro-Kjeldahl method. The concentrations of Cd were determined using a Perkin Elmer Atomic Absorption Spectrophotometric method.

Calculation of Cd mobility indices in Plant tissues

Root bioaccumulation factor (BCFr)

The root bioaccumulation factor (BCFr) is the ratio of metal concentration in root of plant to the soil environmental loadings by adopting the method of Cui et al. (2007).

Shoot bioaccumulation factor (BCFsh)

The Shoot bioaccumulation factor (BCFsh) is the ratio of metal concentrations in shoot of plant to the soil environmental loadings by adopting the method of Yoon et al. (2006).

Transfer factor (TF)

The Transfer factors were also determined for each weed species as the ratio of metal concentrations in plant shoot (mgkg^{-1} dry mass) to that of the metal concentration in the root Yoon et al. (2006).

Data analyses

The data obtained were subjected to analysis of variance (ANOVA) to test for treatment effects using SAS mixed Model Systems. Test of significance for differences in means was obtained using Duncan's Multiple Range Test (DMRT).

Result

The Physico-chemical characteristics of the soils in the contaminated and control site

The Physico-chemical characteristics of the soils in the contaminated and control site were presented in Table 1. The concentrations of metals analysed from soil samples collected from the industrial dump site and the control site was in the order of Fe> Pb > Cu > Zn >Cd (Table 1). Although, the concentrations of some of these metals are acceptable and do

TABLE 1
Physico-chemical characteristics of the soils in the contaminated and control site

Properties	Contaminated sites (Plot A &B)	The control site (Plot C)
pH (1:1) Soil-water	8.40	8.01
Organic Matter	3.60	1.18
N (g/kg)	6.21	2.13
Available P(mg/kg)	6.83	4.46
Exchangeable Acidity (c mol kg^{-1})	0.20	0.35
Effective cation exchange capacity	33.05	10.25
Exchangeable cations (c mol kg^{-1})		
Ca	31.72	29.92
Mg	1.09	5.05
Na	0.10	0.30
K	0.48	0.51
Extractable micronutrient (mg kg^{-1})		
Fe	1,173.80	101.01
Zn	45.43	9.72
Heavy metals (mg kg^{-1})		
Pb	210.01	7.03
Cd	16.10	0.14
Cu	122.51	12.41
Clay (g kg^{-1})	108.00	103.00
Silt (g kg^{-1})	726.00	789.00
Sand (g kg)	858.00	108.00
Particle size	Loamy sand	Loamy sand

TABLE 2
Survival rate (%) of the four selected weeds grown on contaminated and non – contaminated sites under two bioaugments

Family of selected species	Selected weed species	Plot identify	Types of bioaugment	Weeks After Planting (WAP)					
				2	4	6	8	10	12
Amaranthaceae	*Amaranthus spinosus*	Plot C	NI	100.00^a	100.00^a	100.00^a	100.00^a	100.00^a	100.00^a
Euphorbiaceae	*Euphobia heterophylla*		NI	100.00^a	100.00^a	100.00^a	100.00^a	100.00^a	100.00^a
Malvaceae	*Sida acuta*		NI	100.00^a	100.00^a	100.00^a	100.00^a	100.00^a	100.00^a
Asteraceae	*Synedrella nodiflora*		NI	100.00^a	100.00^a	100.00^a	100.00^a	100.00^a	100.00^a

TABLE 2 *cont.*
Survival rate (%) of the four selected weeds grown on contaminated and non – contaminated sites under two bioaugments

Amaranthaceae	*Amaranthus spinosus*	Plot B (Contaminated Site)	NI	100.00[a]	100.00[a]	100.00[a]	100.00[a]	100.00[a]	100.00[a]
Euphobiaceae	*Euphorbia heterophylla*		NI	100.00[a]	100.00[a]	100.00[a]	100.00[a]	83. 33[b]	66.67[c]
Malvaceae	*Sida Acuta*		NI	100.00[a]	100.00[a]	100.00[a]	100.00[a]	100.00[a]	100.00[a]
Asteraceae	*Synedrella nodiflora*		NI	100.00[a]	100.00[a]	100.00[a]	100.00[a]	83.33[b]	83.33[b]
Amaranthaceae	*Amaranthus spinosus*	Plot A (Contaminated site + mycorrhizas)	GI	100.00[a]	100.00[a]	100.00[a]	100.00[a]	100.00[a]	100.00[a]
			GM	100.00[a]	100.00[a]	100.00[a]	100.00[a]	100.00[a]	100.00[a]
Euphobiaceae	*Euphorbia heterophylla*		GI	100.00[a]	100.00[a]	100.00[a]	100.00[a]	100.00[a]	100.00[a]
			GM	100.00[a]	100.00[a]	100.00[a]	100.00[a]	100.00[a]	100.00[a]
Malvaceae	*Sida Acuta*		GI	100.00[a]	100.00[a]	100.00[a]	100.00[a]	100.00[a]	100.00[a]
			GM	100.00[a]	100.00[a]	100.00[a]	100.00[a]	100.00[a]	100.00[a]
Asteraceae	*Synedrella Nodiflora*		GI	100.00[a]	100.00[a]	100.00[a]	100.00[a]	100.00[a]	100.00[a]
			GM	100.00[a]	100.00[a]	100.00[a]	100.00[a]	100.00[a]	100.00[a]

Legend: NI=Non – inoculation, GI = *Glomus intraradices*, GM =*Glomus mosseae*, Plot A= Contaminated site inoculated with mycorrhiza, Plot B = Contaminated site without mycorrhiza inoculation, Plot C = Control site

not require urgent clean-up according to US EPA (2002) permissible limit for hazardous substance, the amounts (16.10 mgkg-1) reported for Cd might still contribute to human health risk. The Fe, Zn Cu, Pb and Cd contents of the contaminated soil were almost 12, 5, 10, 30-fold higher respectively than when compared with the control.

Survival Rate

Table 2 revealed the survival rate of the weeds within 12 weeks of planting on the contaminated and the control sites. At 10 and 12 weeks after planting (WAP), the survival rate of some of the weeds were affected on contaminated sites with and without the influence of Glomus species. For example *Euphorbia heterophylla* had survival rate reduced by 16.27% at 10 WAP while at 12 WAP the survival rate was 66.67% (Plate 1). Similarly, *Synedrella nodiflora* had the survival rate of 83.33% both at the 10 WAP and 12 WAP. However, *Amaranthus spinosus* (Plate 2) and *Sida acuta* had 100% survival rate throughout the period of biomonitoring of growth parameters.

Thus, 50% of the weeds survived the stress of pollution. On control site and Plot A which was

Plate 1: *Euphorbia heterophylla,* weed with lowest survival rate on contaminated site under bioaugmentation

Plate 2: *Amaranthus spinosus,* weed with highest survival rate on contaminated with highest survival rate on contaminated site under bioaugmentation with no chlorotic expresssions

contaminated sites inoculated with *Glomus intraradices* and *Glomus mosseae*, the four selected weeds survived the pollution stress.

Cadmium accumulations, Bioaccumulation factors (BCFs) and Transfer Factors (TFs) in the tissues of the selected plants grown on industrial dump site.

Table 3 showed cadmium accumulations and bioaccumulation factors (BCFs) in the tissues of the four selected weeds grown on the industrial dump site while Figures 3 revealed the TFs of the test weeds. On contaminated site, cadmium accumulations reported in the roots of the four selected plants ranged from 1.20 mg/kg to 5.24 mg/kg Cd while that of the shoots ranged from 0.64 mg/kg to 3.03 mg/kg. The root of *Amaranthus spinosus* accumulated highest (5.24 mg/kg) concentration of cadmium with BCF (0.45) and TF (0.58) less than 1. However, for the shoots of *A. spinosus*, cadmium concentrations of 3.03 were obtained with shoot BCF (BCFsh) less than the root BCF (BCFrt). In tissues of *Sida acuta*, not less than 2.43 mg/kg Cd were estimated in the root while 1.51 mg/kg Cd were reported in the shoot with BCFr and BCFSh of 0.12 and 0.07 respectively. In the same weed, the translocation of Cd was limited to the root with respect to the soil pollutant loadings; however with improved TF value (0.62) more than the TFs of other

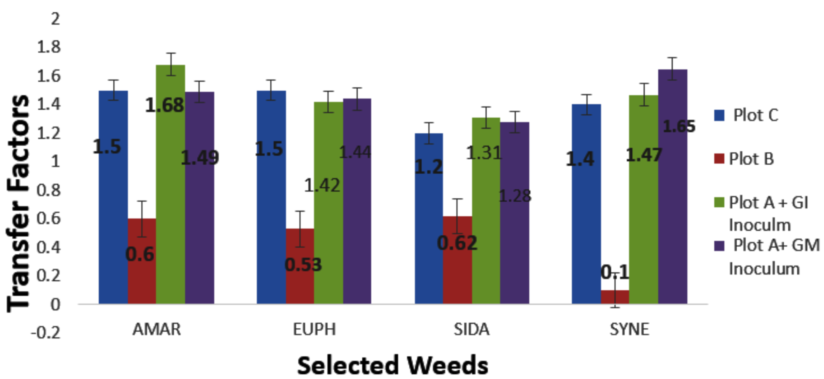

Fig. 3: Transfer factors of the four selected Weeds

Legend: GI = *Glomus intraradices,* GM =*Glomus mosseae,* Plot A= Contaminated site inoculated with mycorrhiza, Plot B = Contaminated site without mycorrhiza inoculation, Plot C = Control site, AMAR = *Amaranthus spinosus,* EUPH = *Euphorbia heterophylla* SIDA = *Sida acuta, SYNE* = *Synedrella nodiflora* plants. When compared with *Synedrella nodiflora* grown on the contaminated sites, Cd accumulations (1.90 mg/kg) in roots was 83% more than concentration accumulated in the roots of *Synedrella nodiflora* cultivated on the Control sites. However, up to 1.64 mg/kg Cd were analyzed from the shoot of *Synedrella nodiflora* with root BCF and shoot BCF values of 0.05 and 0.10 respectively and TF of less than 1.0. Similarly, from the report, least cadmium accumulations of 1.20 and 0.64 were both in root and shoot of *Euphorbia heterophylla* respectively with BCF of 0.06 and 0.09 and Transfer factor (0.53) of less than 1 (Figure 3). Across the four selected plants grown on the contaminated sites, cadmium accumulations in the roots were higher than cadmium accumulations in the shoots with BCF (Table 3) and TF (Figure 3) below 1.0. Hence, the trend of the cadmium accumulations in the root was *Amaranthus spinosus* > *Sida acuta* > *Synedrella nodiflora* > *Euphorbia heterophylla* while that of the shoot was *Amaranthus spinosus* > *Synedrella nodiflora* > *Sida acuta* > *Euphorbia heterophylla*.

However, under the influence of two ecotypes mycobionts, all plants grown on the contaminated sites accumulated higher concentration of Cd in their tissues than those grown without mycorrhiza inoculation. Correspondingly, BCF and TF (Figure 3) values reported for all the plants also increased

TABLE 3
The mobility indices of Cadmium in the root and shoot of the four selected plants grown in the contaminated and control sites

Selected weed species	**Plot Identity**	**Types of bioaugument**	**Root Cd**	**Shoot Cd**	**Soil Cd**	**BCFRt**	**BCF Sh**
AMAR	Plot C (Control)	NI	0.23 ^{a}	0. 15^{a}	0.16^{b}	1.44 ^{a}	0.94^{a}
EUPH		NI	0.03^{c}	0.02^{c}	0.25^{b}	0.12^{d}	0.08^{d}
SIDA		NI	0.11 ^{b}	0.09^{b}	0.41 ^{a}	0.27^{c}	0.22^{c}
SYNE		NI	0.19 ^{a}	0.14^{a}	0.22 ^{b}	0.86 ^{b}	0.64^{b}
AMAR	Plot B	NI	5.24^{a}	3.03^{a}	11.66^{c}	0.45^{a}	0.36^{a}
EUPH	Contaminated soils	NI	1.20d	0.64d	16.79a	0.06c	0.09b
SIDA		NI	2.43^{b}	1.51^{c}	20.32^{a}	0.12^{b}	0.07^{b}
SYNE		NI	1.90^{c}	1.64^{b}	15.89^{b}	0.11^{b}	0.05^{b}
AMAR	Plot A	GI	6.12^{a}	10.29^{a}	4.58^{b}	1.34^{a}	2.25^{a}
		GM	5.23^{b}	7.81^{b}	6.11^{a}	0.86^{b}	1.28^{b}
EUPH	(contaminated soils+mycorrhiza inocula)	GI	2.51^{b}	3.57^{b}	6.17^{a}	0.41^{b}	0.58^{b}
		GM	3.62^{a}	5.18 ^{a}	5.67^{b}	0.64^{a}	0.91^{a}
SIDA		GI	3.09^{a}	4.05 ^{a}	7.42^{b}	0.42 ^{a}	1.08 ^{a}
		GM	2.83^{b}	3.62 ^{b}	10.6^{a}	0.27 ^{b}	0.34^{b}
SYNE		GI	6.02^{a}	9.07 ^{a}	5.45^{b}	1.13 ^{a}	1.66^{a}
		GM	4.29^{b}	7.06 ^{b}	11.22^{a}	0.37 ^{b}	0.65 ^{b}

Legend: NI=Non – inoculation, GI = *Glomus intraradices*, GM =*Glomus mosseae*, Plot A= Contaminated site inoculated with mycorrhizas, Plot B = Contaminated site without mycorrhiza inoculation, Plot C = Control site

TABLE 4
The Cadmium uptake values in the root and shoot of the four selected weeds grown in the contaminated and control sites

Selected weed species	Plot Identity	Types of bioaugument	Biomass of Root (g)	Biomass of shoot (g)	Root Cd (mg/kg)	Shoot Cd (mg/kg)	Root Cd uptake (10-3mg/kg)	shoot Cd Uptake (10-3mg/kg)
AMAR	Plot C (Control)	NI	2.80 [a]	5.77 [a]	0.23 [a]	0.15[a]	0.64 [a]	0.42 [a]
EUPH		NI	0.31[d]	2.43 [c]	0.03 [d]	0.02 [c]	0.01 [c]	0.01 [d]
SIDA		NI	2.64[b]	5.21[b]	0.11 [c]	0.09[d]	0.29 [b]	0.24 [b]
SYNE		NI	1.28[c]	5.17 [b]	0.19 [b]	0.14[b]	0.24 [b]	0.18 [c]
AMAR	Plot B	NI	2.2 [a]	3.13 [a]	5.24 [a]	3.03[a]	11.53[a]	9.48 [a]
EUPH	Contaminated soils	NI	0.22 [b]	2.06 [c]	1.20 [d]	0.64[d]	0.26 [d]	1.32 [d]
SIDA		NI	2.45 [a]	3.60 [b]	2.43 [b]	1.51 [c]	5.96 [b]	5.44 [c]
SYNE		NI	0.71[b]	3.40 [a]	1.9 [c]	1.64 [b]	1.35 [c]	5.58 [b]
AMAR	Plot A	GI	3.5 [a]	4.17[a]	6.12 [a]	10.29 [a]	28.42 [a]	42.91 [a]
		GM	2.39[b]	3.92 [b]	5.23 [b]	7.81 [b]	7.72 [b]	30.62 [b]
EUPH	(contaminated soils+mycorrhiza)	GI	0.52[a]	3.44 [a]	2.51 [b]	3.57 [b]	1.31 [b]	12.28 [b]
`		GM	0.48[a]	2.97 [b]	3.62 [a]	5.18 [a]	1.74 [a]	15.39 [a]
SIDA		GI	2.00 [a]	5.70 [a]	3.09 [a]	4.05 [a]	6.18 [a]	23.09 [a]
		GM	1.83 [b]	5.13 [b]	2.83 [b]	3.62 [b]	5.18	18.57 [b]
SYNE		GI	1.2 [a]	4.21 [a]	6.17 [a]	9.07	7.28 [a]	38.18 [a]
		GM	1.0 [b]	4.01 [b]	4.19 [b]	7.26 [b]	4.19[b]	29.11 [b]

Legend: NI=Non – inoculation, GI = *Glomus intraradices*, GM =*Glomus mosseae*, Plot A= Contaminated site inoculated with mycorrhizas, Plot B = Contaminated site without mycorrhiza inoculation, Plot C = Control site

significantly at p> 0.05. In addition, across all the plant cultivated under mycorrhiza inoculation, concentration of cadmium accumulated in the shoot tissues were higher than concentrations reported in the root tissues (Table 3). For example, in the root of *Amaranthus spinosus*, cadmium concentration of 6.12 mg/kg obtained increased by 14% when compared with *A. spinosus* grown on polluted sites without mycorrhiza augmentation while the shoot cadmium concentration (10.29 mg/kg) also increased by 71%.

Likewise, Cd concentrations in the roots of *Sida acuta* (3.09 mg/kg), *Synedrella nodiflora* (6.02 mg/kg) and *Euphorbia heterophylla* (3.62 mg/kg) increased by 6%, 68% and 52% after mycorrhiza bioaugmentation, when compared with the same plants species cultivated on contaminated sites without mycorrhiza augmentation. However, increments of 63%, 83% and 82% were reported in the shoots of the same plants respectively following this trend *Sida acuta* (9.07) > *Synedrella nodiflora* (4.05) > *Euphorbia heterophylla* (5.18).

From Table 3, highest BCF values were reported for the shoot of the selected plants when exposed to mycorrhiza (*Glomus intraradices* and *Glomus mossaea*) treatment. *A. spinosus* and *S. nodiflora* had BCFr above 1.0 while *Euphorbia heterophylla* and *Sida acuta* had BCFr below 1.0 although with improved values (Table 3). Similarly, for all the plants, their TFs are above 1.0. After inoculation with *Glomus intraradices* (G.I.), all the plants had shoot BCF above 1.0 except for *Euphorbia heterophylla* in which the highest BCFsh was reported after treatment with *Glomus mosssae*.

The Biomass and cadmium uptake values of the four selected plants grown on contaminated soils

The biomass and Cd uptake mean values of the four selected weeds grown on contaminated plots and the control were presented in Table 4. For, *Amaranthus spinosus*, the root (3.50 g) and shoot (4.17 g) biomass increased by 37% and 27% respectively when grown on polluted sites under G.I. inoculation. Although, under *G. mossaea* the biomass of the root (3.50 g) and shoot (4.17 g) increased significantly at $p > 0.05$ by 8% and 20% respectively when compared with the biomass of the *Amaranthus spinosus* grown on contaminated plot without augmentations. Highest uptake value of 42.91×10^{-3} mg/kg were reported in the root of *A. spinosus* under *Glomus* intraradices inoculation while up to 28.42×10^{-3} mg/kg were recorded in the shoot of *Amaranthus spinosus* (Table 4). The cadmium concentration of 7.72×10^{-3} mg/kg and 3.0 x10-3 mg/kg were reported for both root and shoot uptake of *Amaranthus spinosus* when inoculated with *Glomus mossae*. This result shows that *Glomus intraradices* enhanced root uptake of *A. spinosus* by almost 4-fold while the shoot uptake were enhanced by almost 1.5-fold when compared with *Glomus mossae* inoculated ones. However, when compared with the uninoculated ones grown on contaminated site (Plot B), the root (2.20 g) and the shoot (3.13 g) biomass of *A. spinosus* were lower than the biomass of bioaugmented ones with 37% and 25% reduction both in root and shoot mean uptake values. Similarly, the same trend was observed in all other plant species except for *Euphorbia heterophylla* in which the inoculation of *Glomus moseae* enhanced the uptake of cadmium than that of *Glomus intraradices* induced weeds. Thus, comparing the enhancement strengths of the two mycobionts, the order is *A. spinosus (G.I.)>A. spinosus (G.m.).>Synedrella nodiflora (G.I.) > Synedrella nodiflora (G.m.)> Sida acuta (G.I.)> Sida acuta (G.m.)> Euphorbia heterophylla (G.m.)> Euphorbia heterophylla (G.I.)*.

However, for *Sida acuta* with root and shoot cadmium uptake values of 5.96×10^{-3}mg/kg and 5.44^{-3} mg/kg, their root and shoot biomass were 2.45 g and 3.60 g respectively with 7% and 31% reduction in dry weight when compared with the control result.

compared with values obtained for plants grown on control plot. However, the root of *Euphorbia heterophylla* uptake up to 0.26 x10-3 mg/kg with root biomass reduction of 29% (0.22 g) while shoots biomass (2.43 g) was significantly reduced by 15%. Thus, under mycotic influence of *G.I.*, all the plants species grown on polluted sites showed significant increase in their weight and mean uptake values when compared with plants grown on polluted soils without mycorrhizal influence.

Discussion

This study is a field experiment in which the toxicity and phytoremediation strategies of four weeds were evaluated using some phytoremediation indices such as pollutant concentrations in plant tissues, root and shoot Bioaccumulation factors (BCFr and BCFsh), Transfer factor (TF), Survival and Growth rate. According to Ji, et al., (2011), the success of phytoremediation stems from the tolerance and accumulation capacity of the model plants not only in the greenhouse but also on the field. A fruitful phytoremediation strategy, depend largely on the ability of model plant species to accumulate and tolerate heavy metal species in its systems without showing toxicity syndrome; although in some cases this is not so because HM toxicity inhibits plant growth and development after accumulation (Jan et al., 1999; Hosman, et al., 2017)

In this study, after harvesting, the roots of the weeds cultivated on contaminated site without bioaugmentation had the highest Cd concentrations compared with their shoots with BCF and TF less than 1.0. This may be attributed to the fact that Cd had first contact with the roots in soils which are the first to experience Cd accumulation and toxicity (Sharma and Sachdeva, 2015). Mostly, cadmium penetrates the root through the cortex and is translocated to above ground tissues. Moreover, some concentrations of Cd may also bind to cell walls while chelation of Cd in the xylem with various ligands, such as phytochelatins might have occurred as reiterated by Girdhar, et al., (2014). In addition, the production of metallothioneins and other metal-binding proteins may also occur in the root tissues as a strategy to phytoaccumulate. In this wise, only small amount of Cd is transported to shoots as roots retain these ions. As reported by Sarma et al. (2017) many plant species achieved this by using metallothioneins, phytochelatins and Cys-rich mem-brane proteins for metal compartmentalization and therefore use metal exclusion method as phytoremediation strategies. Hence, the weeds displayed the characteristics of phytostabilizers in the absence of bioaugments. This result is in line with the result obtained by Waziri et al. (2016) in which the roots of *Azadira chtaindica* accumulated higher concentration of Cd, and other metals such as Cu, Fe, Mn, Ni, Pb, and Zn more than the shoot. Generally, the gradient of Cd concentration in the four weeds is in the order *Amaranthus. spinosus* > *Synedrella nodiflora*> *Sida acuta*> *Euphorbia heterophylla.* The capability of plants to grow well in soils with concentrations of heavy metals similar to the ones studied in this research were reported. Contrarily, when the weeds were inoculated with mycorrhiza species (*Glomus intraradices* and *Glomus mossaea*), the concentrations of Cd in shoot obtained were improved than that of Cd concentration recorded in roots of the four weeds. Similarly, there were increase in concentration of cadmium in the roots of the weeds after mycorrhiza bioaugmentation, with *Amaranthus spinosus* having the highest Cd in its root. Likewise, bioaugmentation also increases the concentration of Cd in shoots of the weeds respectively following this trend *Amaranthus spinosus* >*Sida acuta* >*Synedrella nodiflora* >*Euphorbia heterophylla*. As noted in this research, high load of HM in contaminated soils is not a challenge for most mycorrhizal species (Sheoran and Sheoran, 2010). This is because various intracellular functions of AM fungi and other rhizosphere microbes are driven by binding metal ions present in the external environment on the cell surface or to transport them into the cell. According to Kaewdoung et al. (2016), mycorrhizal in soil, can change metal speciation, mobility, dissolution, and toxicity strength of pollutants while Phieler et al. (2014) revealed that excretion of chelators and acidification of soil environment by mycorrhizars species at the rhizosphere of plants are known to be useful for phytoremediation processes. Hence, the increment in the concentration of Cd observed in the aboveground and belowground of the weeds. Here, metal sequestration and compartmentalization by mycorrhizal cells might further increase both metal tolerance and metal accumulation of plants.

Without bioaugmentation, the four selected weeds accumulated significant amount of Cd at P>0.05 but none of them were hyperaccumulator due to BCFs and TFs that were less than 1 (Seneviratne, et al., 2017) on the field. The root BCFs were in this trend *Amaranthus spinosus>Sida acuta > Synedrella nodiflora >Euphorbia heterophylla* while that of the shoot BCFs was *Amaranthus spinosus>Synedrella nodiflora > Sida acuta >Euphorbia heterophylla*. Meaning that the weeds were suitable as phytostabilisation agents with BCF and TF mean values of less than 1. Similarly, when the same weeds were exposed to various Cd pollution strength without augmentation in a greenhouse study, some of the plants acted also as phytostabiliser (Dada, 2013; Oseni, et al. 2015). The reduction in the BCFs and TFs values of the weeds may be due to presence of multiple metal species present on the industrial waste dump site as well as other environmental conditions (Jabeen and Ahmad 2009). However, under mycorrhizal treatment, the BCFs and TFs values of all the weeds improved significantly at P>0.05 with TFs above 1.0. All the plants had highest shoot BCF after inoculation with *Glomus intraradices (G.I.)*, except for *Euphorbia heterophylla* in which the highest BCFsh was reported after treatment with *Glomus mosssae*. The outcome of the study revealed that mycorrhizal treatment, enhanced the BCFs and TFs values of the weeds. Here, *Glomus intraradices* enhanced the phytoextraction capability of all the selected weeds than when inoculated with *Glomus mosssae* except for *Euphorbia species* that responded to *Glomus mosssae* enhancement better than *Glomus intraradices*. Therefore, under bioaugmentation by mycorrhizal treatment the plants acted as phytoextractors of Cd. This output corroborated with the findings of Dada, 2013; Dada and Awotoye, 2013 in which plants inoculated with Glomus species performed better than plants without inoculation. Likewise Oseni, et al. (2016) reported improved BCFs and TFs values of *Sida acuta* and *Chromolaena odorata* grown under augmentation. In addition, the TFs of the plants improved with highest transfer factor (1.68) recorded in *A. spinosus* inoculated with *Glomus intraradices*. Across the four plants, the TFs of plants treated with *Glomus species* were over 60% higher than TFs of plants grown on polluted sites without bioaugmentations in this trend *Amaranthus spinosus > Synedrella nodiflora > Euphorbia heterophylla. > Sida acuta.* Not only were the BCF and TF values of the weeds were affected, the biomass and uptake values of the weeds were shown to improve after treatment with *Glomus species* significantly at p>0.05. According to Sheoran, et al. (2010) arbuscular mycorrhizal fungi are allied colonizers and biofertilizers that form mutualistic associations between plants and fungi. By affecting the success of individual plants, the association may play a role in the success of reclamation efforts by their presence (improving the growth and fitness of desirable species) or in failure by their absence. They could provide plants with benefits crucial for ecosystem restoration. However, considering the influence of the two mycobionts on the TFs of the plant species, *Glomus intraradices* inoculated plant species performed better than those inoculated with *Glomus moseae*, except for *Euphorbia heterophylla* and *Synedrella nodiflora*. From the report, *Euphorbia heterophylla* and *Synedrella nodiflora* treated with *Glomus moseae* on the contaminated site had their TFs improved than TFs of *Glomus intraradices* treated *Euphorbia heterophylla* and *Synedrella nodiflora*. This may be attributed to the fact that some plants are specific in the symbiotic relationship they have with some soil microflora. Arbuscular mycorrhizal fungi are known to confer protection and tolerance traits on plants species exposed to heavy metal pollution in soils. (Sinha et al., 2007).

The result revealed that on contaminated site under inoculation, *Glomus intraradices* enhanced the survival rate of the four selected weeds by 100%, whereas *Glomus mosseae*, enhanced the survival rate of the four selected weeds by 83% except for *Euphorbia heterophylla* recording 66.67 % survival rate in the 10 WAP and at the end of 12 WAP. This was evident in the chlorotic expression on leaves of *Euphorbia heterophylla* grown on the contaminated site after augmentation (see plate 1). Mycorrhizal fungi inoculation assisted the tested plants with improved productivity. According to a study by Kaewdoung et al. (2016) oxalate crystals produced by white-rot fungi *Fomitopsis cf. meliae* and *Ganoderma aff. steyaertanum* assisted in metal tolerance by transforming the toxic metal to less toxic derivates of such metals. Specifically, zinc sulfate was transformed into zinc oxalate dehydrate while toxic cadmium sulfate into cadmium oxalate trihydrate, and lead nitrate into lead oxalate. According to Seneviratne et al. (2017), mycorrhizars in rhizosphere resolve problems of metal tolerance in plants by modulating plant growth as well as by altering physico-chemical properties of soil to enhance metal bioavailability which trigger rapid detoxification or removal of HM from soil.

Conclusion

Based upon the outcome of the research carried out in situ on the industrial dump site, it can be concluded that all the weeds undertaken in the study are capable of accumulating significant amount of Cd as phytostabilizers in the absence of bioaugment. The weeds were also capable of maintaining their survival rates, growth rate, BCFs; and TFs when compared to controls. Specifically, indigenous species of *Amaranthus spinosus* and *Synedrella nodiflora* can tolerate the toxicity of Cd in the phase of multi-metal pollution. These weeds are thus suitable species for phytostabilization and revegetation projects. Moreover, the use of arbuscular mycorrhizar fungi as bioaugments was also able to improve and assist all the weeds to phytoextract Cd with improved values of BCF and TF; uptake and growth rates. All the plants phytoextract Cd when inoculated with *Glomus intaradices* except *Euphorbial heterophylla* that phytoextracted Cd better when treated with *Glomus mosseae* than when treated with *Glomus intaradices*. *In situ* technique of phytoremediation process is a promising technique for the biomanagement of hazardous substances in soil environment. Hence, the study concluded that the four weeds could be used to phytoremediate cadmium polluted soils in tropical environment. The study also recommended that further researches be conducted on native plants of the tropics and the synergy they established with indigenous beneficial rhizosphere microbes in restoring metal polluted soils tropical environment.

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