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Comparative Studies on the Efficacy of *Azadirachta indica* and *Moringa oleifera* in Phytoremediation of Some Selected Heavy Metals from Contaminated Soil

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

This research was carried out to investigate the efficacy of *Azadirachta indica* (Neem) and *Moringa oleifera* (Drumstick) in phytoremediation of heavy metals (Ni, Pb and Zn) from the potted contaminated soil. The three selected heavy metals were used to pollute three blocks; each block was subdivided into two subsets of three replicates each per the heavy metal. The two studied plants were transplanted from the nursery into each bag containing contaminated soil. The result of the metal accumulation at the roots of the studied plants indicates that *A. indica* had the higher mean value of Ni, Pb and Zn absorption (170.0 mg/kg, 119.1 mg/kg and 1.5 mg/kg respectively), whereas the *M. oleifera* had a lower mean value (0.4 mg/kg, 118.2 mg/kg and 0.9 mg/kg), with no significant difference. The levels of heavy metals in the soil containing *A. indica* was lower with

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variation in the level of Pb (p=0.043) but the levels of Zn and Ni (p=0.380 and p=0.144 respectively) was not significant. The bioaccumulation factor shows that *A. indica* accumulates a higher concentration of heavy metals. This research reveals that both studied plants accumulate high concentration of Ni, Pb and Zn at its root, though *A. indica* is shown to possess greater ability in accumulating heavy metals.

Keywords: Phytoremediation; pollution; heavy metals; Moringa oleifera; Azadirachta indica.

1. INTRODUCTION

Heavy metals pose several adverse health effects have been known for a long time, exposure to heavy metals continues and is even increasing in some parts of the world, particularly in less developed countries [1]. Heavy metals can occur in any ecosystem; terrestrial or aquatic, and when they occur, it affects all the components of the ecosystem (biotic and abiotic). The effect ranges from physical and chemical contamination of soil, air and water to deleterious impact on flora and fauna [2]. Increasing awareness of the hazard caused by environmental pollution has led to the search in many countries for method, not only re-cultivating land, but also preventing the contamination of environment and food. The environmentally friendly solutions to these problems are phytoremediation, which is defined as the use of plants to remove pollutants from the environment or to render them harmless [3].

Land and water are precious natural resources on which rely the sustainability of agriculture and the civilization of mankind. Unfortunately, they have been subjected to maximum exploitation and severely degraded or polluted due to anthropogenic activities [4]. The pollution includes point sources such as emission, effluents and solid discharge from industries, vehicle exhaustion and metals from smelting and mining, and nonpoint sources such as soluble salts (natural and artificial), use of insecticides/pesticides, disposal of industrial and municipal wastes in agriculture, and excessive use of fertilizers [5,6]. Each source of contamination has its own damaging effects to plants, animals and ultimately to human health, but those that add heavy metals to soils and waters are of serious concern due to their persistence in the environment and carcinogenicity to human beings. They cannot be destroyed biologically but are only transformed from one oxidation state or organic complex to another [7,8]. Therefore, heavy metal pollution poses a great potential threat to the environment and human health.

In order to maintain a good quality of soils and waters and keep them free from contamination, continuous efforts have been made to develop technologies that are easy to use, sustainable and economically feasible. Most heavy metals occur naturally and are usually conserved, so their indefinite persistence in the soil environment is in great danger [9]. Metals such as Cu, Pb and Zn are important since high quantities of them can decrease crop production due to the risk of biomagnifications and bioaccumulation in the food chain. There is also the risk of underground and surface water contamination [10,11]. Among various types of soil pollutants, heavy metals pollution appears to be a great concern especially in developing countries where it has caused soil quality deterioration either by aerial deposition or wastewater discharge. Heavy metals accumulation in crop plants is a great concern due to its potential for food contamination through the soil-root interface [12,13]. Contaminated soil can be amended by conventional ways such as physical, chemical, bioremediation and phytoremediation. In recent years, efforts have focused on the remediation strategies that are less expensive and less destructive than current approaches [14]. Phytoextraction emerged as an intense research effort for more efficient and less hazardous techniques to remediate contaminated soils. It comprises the removal of metals by plants through uptake and accumulation into body parts. However, progress in making phytoextraction a practical commercial technology is hindered by a lack of strategies to optimize plant uptake of metals [15]. This technology can be applied to both organic and inorganic pollutants present in soil, water and air. In this respect, plants can be compared to solar driven pumps which can extract and concentrate certain elements from the environment [3].

M. oleifera and *A. indica* are valuable plant species that are widely used traditionally due to their multi-purpose in medicine, domestic and industrial values. *M. oleifera* is widely cultivated and has become naturalized in many locations in the tropic [16]. *M. oleifera* tolerates most soil types and grow well in full sun, water regularly and protect from wind and frost, in a cooler climate. They can be grown in warmed greenhouse, propagate from seed or cutting [17]. Seed from *M. oleifera* has been used for water purification and to remove microorganisms in water for many years in rural areas of Africa and Asia. Previous studies have shown that *M. oleifera* is effective in removal of heavy metals including lead from water [18]. Neem (*A. indica* juss*)* is one the most suitable and valuable tree species for arid and semi-arid region especially for reclamation of degraded land. *A. indica* is a plant that is used traditionally as food and medicine. Extract of the leaves and bark are used in the treatment of a wide range of diseases and infections. Neem tree is a popular tree amongst the populace and has served as a medicinal plant [19].

Heavy metals are the major group of inorganic contaminants and a considerate large area of land is contaminated with them due to the use of sludge or municipal compost, pesticides, fertilizers and emission from wastes, incinerates, exudates residues from metalliferous mines and smelting industries [20]. The decomposition of litter is one of the most important processes in the nutrient cycle in the forest ecosystem. It has suggested that the accumulation of heavy metals in the litter layer can be toxic to micro-organism and that this phenomenon is responsible for the inhibition of decomposition of the organic matter in the heavily polluted forest [21]. Irrespective of the origin of the metals in the soil, an excessive level of many metals can result in soil quality degradation, crop yield reduction and poor agricultural product, posing significant hazard to human, animals and ecosystem health [22]. The environmentally friendly solutions to these problems are phytoremediation, which is defined as the use of plants to remove pollutants from the environment or to render them harmless [3].

Therefore this research aims to assess the efficacy of two plant species (*M. oleifera* and *A. indica*) in the phytoremediation of some heavy metals from contaminated soil.

2. MATERIALS AND METHODS

The experiment was carried out in Federal College of Forestry Jos, Plateau State Nigeria. Jos is located in Northern Guinea Savanna and it is situated between latitude 8° and 30’ and 10° 10’N and longitude 8° 20’ and 9° 30’E. It has an average elevation of about 1, 250m above sea level and stands at a height of about 600m about the surrounding plains. The temperature in Jos ranges between 21℃ to 25℃. The climate of the state is cool due to its high altitude. The mean annual rainfall is 1,260 mm. The relative humidity increases from May to October and decreases gradually from November to April [23]. The soil analysis was carried out at Ahmadu Bello University soil laboratory.

2.1 Preparation of Materials

Polyethylene pots were filled with 5kg of soil each, weighed with a weighing balance (Setra 480s USA) calibrated in kilogram.

The polyethylene bags were arranged in three blocks (designated as A, B, C) of three (3) replicates each, a complete randomized design (CRD) was used for the experiment. Each block was polluted with 100 mg of the studied metal solution, the soils were thoroughly mixed in the bags to enhance the harmonization of the metal solution with the soil, then the bags were allowed to stand for two weeks, after the two weeks of post pollution treatment, each block (treatment) was subdivided into two subsets of three replicates each. Then two months seedlings of *M. oleifera* and *A. indica* were transplanted from the nursery into each bag of one subset of each block, while the other subset act as the control for each block with planting. This experiment was conducted during the raining season, plants were watered with natural rains.

2.2 Soil Analysis

The soil pH before and after raising the seedlings was determined. Soil samples were collected at the end of the experiment. The heavy metals (Zn, Ni and Pb) were analyzed for the soil samples.

At the end of the 4 weeks, the plants were carefully harvested from each bag, and then the shoots were separated from the root by cutting, then samples collected from each block (treatment) were taken to the laboratory immediately for analysis. The root parts and the soils were analyzed for the heavy metals content. Plant samples were analyzed by first rinsing with distilled water and dried. The plants were ground to fine powder and 1.0 g of the powder was digested and analysed. 1.0 g of the dried soil sample was placed in 100 ml beaker and 3 ml of perchloric acid and 5 ml of nitric was then added, the mixture was allowed to stand for 15 mins before digestion by gently heating at low temperature on a hot plate and as well allowed to cool for 5 mins, then the digest was been filtered into 50 ml standard flask, the filtrate was analysed for heavy metals using atomic absorption spectrophotometer (AAS).

Plant samples were dried for 24 hours at 70℃ temperature and digested using the Milestone Ethos One closed vessel microwave system. About 200 mg of the sample was placed in a Teflon vessel and digested with 8 ml of HNO_3 (65%) and 2 ml of water (30%) in a microwave digestion system for 25 minutes. For the quantitative determination of metals in plant material, a Shimadzu AA-6800 atomic absorption spectrometer equipped with deuterium background correction, and single-element hollow cathode lamps as radiation sources were used. All the plastic and glassware were cleaned by soaking in 5% HNO_3 for 24 hours and rinsed with distilled water prior to use. The element standard solutions were prepared by diluting a stock solution of 1000 mg /L (Pb, Ni and Zn). Measurements of pH values of soil were carried out in deionized water according to the NF ISO 10390/2005 procedure. PH determination was performed with Consort P501 pH-meter.

Plate 1. Seedlings of *M. oleifera*

Plate 2. *A. indica* seedlings

Plate 3. Seedlings of *A. indica* and *M. oleifera* subjected to contaminated soil

Plate 4. Solutes of zinc, lead and nickel oxides used for the experiment

2.3 Statistical Analysis

The data obtained from the chemical analysis of heavy metals in the soil and root parts of the studied plant were subjected to T-test comparative analysis. Statistical package for social science (SPSS) version 16 statistical tools was used for the determination of the significant differences between the level of heavy metals in the soil and the roots of the two studied plants. The Bioaccumulation factors were analysed. The bioaccumulation factor (BF) from soil to root parts of two studied plant, expressed as the ratio of metal concentration in part divided by the concentration of metal in soil, may be an indicator of the *A. indica* and *M. oleifera* accumulation behavior.

$$BF = C_{root} / C_{soil}$$

Where *Croot* and *Csoil* represent the heavy metal Concentration in the root and soils respectively [14]. The mean and standard error mean was calculated from the data generated and was subjected to analysis of variance (ANOVA).

3. RESULTS

Fig. 1 revealed that *A. indica* accumulated the highest nickel in its root of 17.2 mg/ kg while *M. oleifera* had the lowest 0.4 mg/kg at its root. Whereas the level of nickel left in the soil used to grow *M. oleifera* had highest nickel concentration of 38.00 mg/kg to compare to the soil used for *A. indica* with the lowest nickel concentration of 0.4 mg/kg.

Fig. 2 shows the Lead concentration of *A. indica* at its root is 119.1 mg/kg whereas *M. oleifera* accumulated 118.2 mg/kg at its root but the soil used to grow *M. oleifera* had highest lead residue of 43. 1 mg/kg. *A. indica*, on the other hand, had a lower lead residue of 35.6 mg/kg.

Table 2 revealed that lead concentration left in the soil between the two studied plant species was significant (p = 0.043).

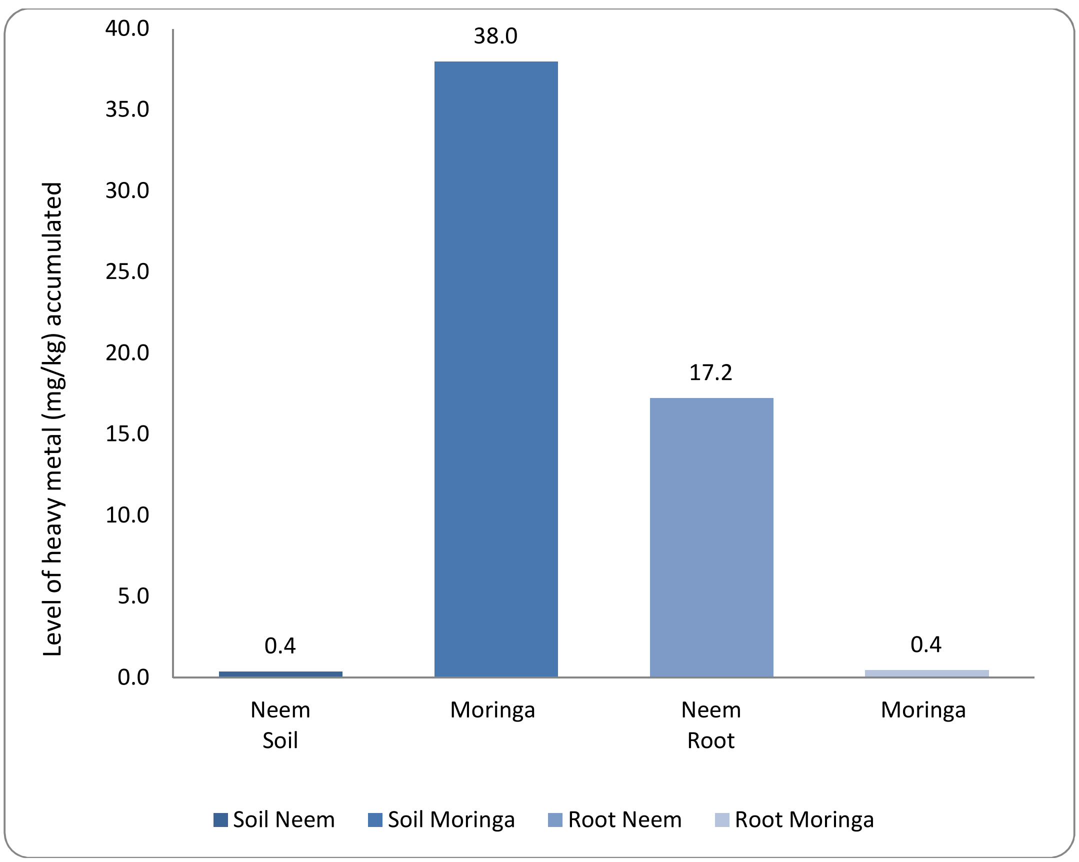

Fig. 1. Nickel concentration in soil and roots of Neem and Moringa

Table 1. T-test of heavy metals in root between Neem and Moringa

Levene's test for equality of variances			T-test for equality of means						
Heavy metals	**F**	**Sig.**	**T**	**df**	**Sig. (2-tailed)**	**Mean Diff**	**SE Diff**	**95% CI Diff**	
								Lower	**Upper**
Ni	6.456	0.044	1.444	6	0.199^{ns}	16.778	11.621	-11.657	45.212
Pb	1.130	0.329	0.274	6	0.793^{ns}	0.938	3.415	-7.420	9.295
Zn	5.835	0.052	0.589	6	0.577^{ns}	0.637	1.081	-2.008	3.282

** Significant; ns: Not significant*

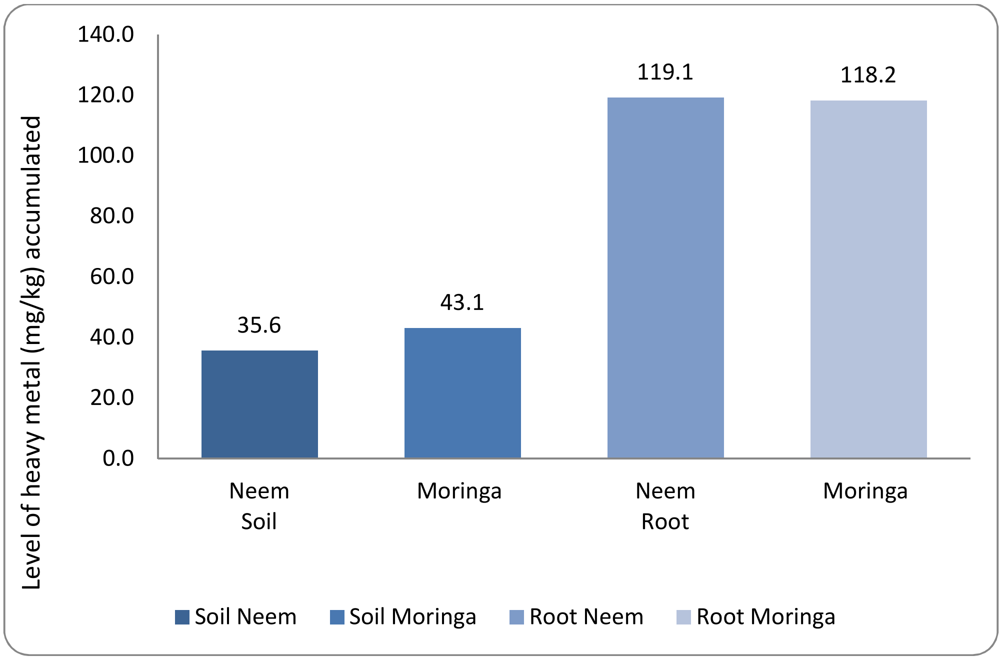

Fig. 2. Lead concentration in the soil and roots of Neem and Moringa

Table 2. Independent T-test of heavy metals in soil between Neem and Moringa

Levene's test for equality of variances			T-test for equality of means						
Heavy metals	**F**	**Sig.**	**t**	**df**	**Sig. (2-tailed)**	**Mean Diff**	**SE Diff**	**95% CI Diff**	
								Lower	**Upper**
Ni	46.018	0.001	-1.680	6	0.144^{ns}	-37.607	22.381	-92.371	17.158
Pb	1.752	0.234	-2.560	6	0.043*	-7.489	2.925	-14.647	-0.331
Zn	13.399	0.011	-0.947	6	0.380^{ns}	-0.085	0.090	-0.305	0.135

** Significant; ns: Not significant*

Fig. 3 revealed that *A. indica* accumulated the highest zinc of 1.5 mg/kg at its root whereas the *M. oleifera* accumulate lower Zinc of 0.9 mg/kg at its root. The concentration of zinc left in the soil used to raise *A. indica* plant was 0.1 mg/kg lower than the concentration of zinc left in the soil used to grown *M. oleifera* plant 0.2 mg/kg.

The T-test statistical analysis of the concentration of lead in the soil and roots of *A. indica* and *M. oleifera* as shown in the Table 3 revealed that Lead concentration level was highly significant (p = 0.000) compared to the soil and root.

The average values of the pH of the soil were 6.17 and 6.07 for pre and post-cultivation respectively as shown in Fig. 4. The pH of the soils is observed to moderately acidic and temperature of soil was 22.4℃ and 22.5℃ for pre and post cultivated soils.

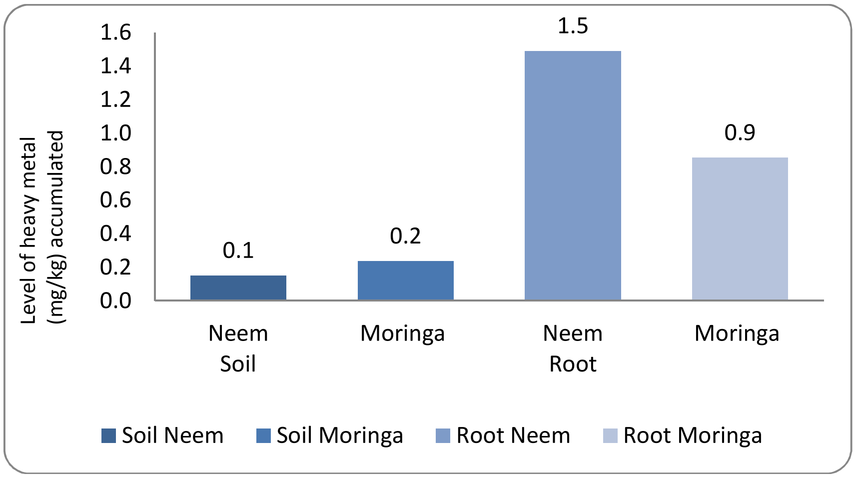

Fig. 3. Zinc concentration in the soil and root of Neem and Moringa

Table 3. Independent T-test of heavy metals in plants between soil and root

Levene's test for equality of variances			**T-test for equality of means**						
Heavy metals	**F**	**Sig.**	**t**	**df**	**Sig. (2-tailed)**	**Mean Diff**	**SE Diff**	**95% CI Diff**	
								Lower	**Upper**
Ni	4.008	0.065	0.737	14	0.473^{ns}	10.341	14.030	-19.750	40.431
Pb	0.068	0.798	-31.426	14	0.000*	-79.301	2.523	-84.713	-73.889
Zn	4.860	0.045	-1.894	14	0.079^{ns}	-0.978	0.517	-2.086	0.130

** Significant; ns: Not significant*

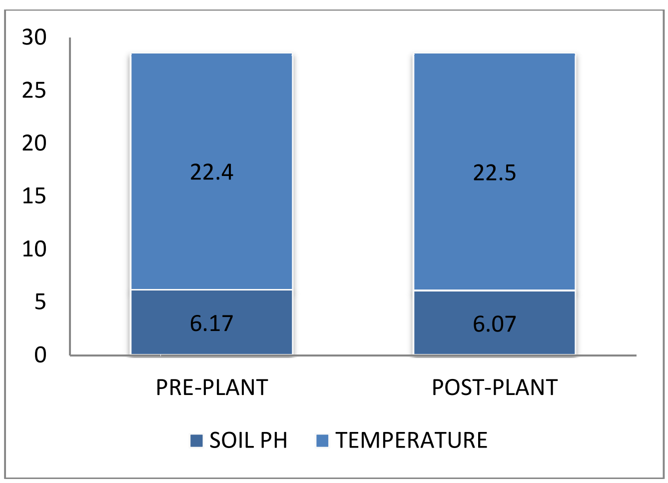

Fig. 4. Soil pH and temperature of the pre and post cultivated soils

Bioaccumulation factors of metals in both studied plants root organs in ratio to soil as listed in Table 4 below indicated that root of *A. indica* accumulated more metals with mean of 42.5 mg/kg of Nickel; 3.35 mg/kg of lead and 14 mg/kg of zinc respectively while *M. oleifera* accumulated lower heavy metals at its roots with a mean of 0.011 mg/kg of Nickel; 2.74 mg/kg of lead and 4 mg/kg of zinc.

4. DISCUSSION

Soil pH directly influences the phyto-availability of metals as soil acidity determines the metal solubility and its ability to move in the soil. Metals cations are most mobile under acidic condition while anions tend to absorb oxide minerals at low pH range. The bioaccumulation factor of the heavy metals from the soil to the roots of the two studied plant species, revealed that for metals of Pb, Ni and Zn accumulation in *A. indica* were mostly higher than that of *M. oleifera a*s shown in Table 4. The ratio of the concentration of Pb in the root parts of the studied plant is more than the ratio concentration to the soil. According to *Baker et al*., *A. indica* should be considered as the best and the right heavy metal accumulator which agrees with these results [24]. This study indicates that *A. indica* accumulates the Pb ion in its root (119.10 mg) while, *M. oleifera* accumulated a lower concentration of Pb (118.16 mg). The levels of heavy metals in the soil containing *A. indica* was lower with variation in the level of Pb (P=0.043). The rhizosphere is where interactions take place between roots and soils constituents [25]. When a root absorbs water or nutrients from the soil, ions and molecules move toward this organ both by mass flow with soil water and by diffusion [26]. Pb may be present in different fractions in the soils. It was previously thought that Pb had low solubility and availability for plant uptake because it forms precipitates with phosphates, sulfates, and chemicals in the rhizosphere [12]. These geo-chemical forms of Pb in soils affect its solubility, which directly influences its mobility. However, roots produce and excrete protons, exudates and several metabolites, which can modify the soil pH and thus interfere with the dissolution processes and formation of soluble metal–organic complexes [27]. In most plants, 90% of the total Pb is accumulated in roots [28]. Most Pb in roots is localized in the insoluble fraction of cell walls and nuclei, which is linked with the detoxification mechanism [29]. After exposure to Pb, cell mechanisms that minimize the potential for toxicity are rapidly activated, uptake and tolerance to Pb depend on root system conditions.

Pb accumulation and cell response was shown to differ between seedlings with a primary root system (PRS) and seedlings with adventitious root systems (ARS) only (in which the primary roots were cut off). The ARS was found to be more tolerant to Pb than the PRS in some plants such as Sunflower, *Helianthus annuus* L. and *Allium cepa* [30,31]. This suggests that ARS have additional mechanisms that protect them against Pb penetration and Pb-induced oxidative stress. However, these mechanisms are still unknown. The uptake of Pb is based mainly on the plant species and the interaction between roots (structures and synthesized exudates) and the rhizosphere (biochemical properties). Indeed, several factors must be taken into account when developing strategies for phytoremediation of Pb, besides the organic and mineral composition of the soil and rhizopheric organisms and microorganisms, the ability of roots to modify the mobility and the bioavailability of Pb by changing rhizospheric conditions can significantly contribute to a successful phytoremediation program. Thus *A. indica* adventitious root system contributes as a hyperaccumulator of lead. It is reported that roots are the first organ in contact with the various components of rhizosphere [25]. Roots have evolved various mechanisms to reduce Pb uptake and transfer to the aboveground parts of the plant and limit its deleterious effect. Although Pb is not an essential element, some plant species proliferate in Pb-contaminated area and accumulate it in different parts. Lead uptake is greatly affected by rhizospheric processes. Lin et al., explained the ability of certain plants to absorb high levels of Pb from soil by a decrease in soil pH due to root exudates, solubilization of Pb by rhizosphere microorganisms and complexation of Pb with organic matter at the soil-root interface [32].

Table 4. Bioaccumulation factor of heavy metals

Treatment		Roots (g/L)
Ni	Neem	43.00
	Moringa	0.01
Pb	Neem	3.35
	Moringa	2.74
Zn	Neem	15.00
	Moringa	4.50

Table 5. Relationship of heavy metals in root between Neem and Moringa

Proximate composition	Location	N	Mean	Std. deviation	Std. error mean
Ni	Neem	4	17.22	23.22	11.61
	Moringa	4	0.44	0.89	0.44
Pb	Neem	4	119.10	3.45	1.73
	Moringa	4	118.16	5.89	2.95
Zn	Neem	4	1.49	2.13	1.07
	Moringa	4	0.85	0.36	0.18

Results showed that the best nickel accumulator at root was *A. indica* as shown in Fig. 1 which had 17.2 mg/kg and *M. oleifera* had the lowest nickel accumulator having 0.4 mg/kg. The proportions of heavy metals among the roots of the studied plants were different from each other which suggests that *A. indica* had a great ability to be used in phyto-extraction of nickel, lead and zinc in the soil contaminated with such heavy metals, so *A. indica* can be used for post Ni, Pb and Zn mining land remediation, which agrees with findings by [33,34]; metal concentrations in plants vary with plant species. According to Istvan et al., toxic concentrations of heavy metals for various plant species are 100, 200, 300 and 500 mg/kg for Zn, Cu, Pb and Fe, respectively [35]. Plants that can grow in the presence of toxic elements are categorized as “tolerant” and “hyperaccumulator”. A tolerant species is one that can grow on soil with concentrations of a particular element that is toxic to most other plants. *A. indica* had shown its ability as hyperaccumulator than *M. oleifera* at the root system.

As shown in Fig. 3, *A. indica* accumulated higher zinc ion at its root (1.4 mg/kg) than *M. oleifera* (0.8 mg/kg), although there was no significant difference. The results suggest that plant of *M. oleifera* with a lower ability to accumulate Zn from soil showed higher values of concentration ratio. On the other side, plant such as *A. indica* with a higher ability to accumulate Zn from soil showed lower values of concentration ratio. This fact can be related to the ability of these plants to bind and detoxicate metals such as Zn, in root cells and cellular compartments (e.g. vacuoles). Thus, in this case, the root system acts as a protective barrier to further metals movement into the above-ground parts of plants [33]. This character results in a high tolerance to metals and metals accumulation in plants or in given plant tissues. A study by Netty et al. reported that Zn was mostly complexed to histidine in roots, transported as Zn^{2+} in the xylem sap, and complexed to organic acids in leaves [34]. The similarity between studied plants individual plant species on the basis of the obtained variables and conditions of the experiments were subjected to Independent T-test analysis of zinc at the soil and roots of *A. indica* and *M. oleifera* as was illustrated in Fig. 3. The zinc ion accumulation at the root of *A. indica* had a mean value of 1.49 mg/kg greater than that of *M. oleifera* at 0.85 mg/kg. According to the analysis, it can be expected that *A. indica* had similar characteristics as *M. oleifera,* which are defined as Zn hyper accumulators, in terms of Zn^{2+} accumulation and other positive parameters for their utilization in phytoremediation processes and techniques.

5. CONCLUSION

The studied plants possess the ability to accumulate metals in their roots. This research shows that the bioaccumulation factor for the selected heavy metals was higher in *A. indica* which makes it a better accumulator than *M. oleifera,* although *M. oleifera* can also be used as an alternative since the values for zinc and nickel were not significant.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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