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Studies of the Uptake of some Heavy Metals by Okra *(Abelmoschus esculentus)*, Amaranth *(Amaranthus dubius)* And Rosselle*(Hibiscus sabdariffa L) Grown* in Artificially Contaminated Soils

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ABSTRACT

Phytoremediaion is an effective and affordable technology used to remove inactive metals and metal pollutants from contaminated soil and water. This research study was designed to assess the naturally enhanced phyto extraction potential of three different vegetable plant species from heavy-metal contaminated soil. The uptake ofZn, Ni, Cr, Cd and Pb by plant species in a heavy metal contaminated soil was studied in pot culture experiment. Five metals Zn, Ni, Cr, Cd andPb contents of root, stem and leaf in the three plant varieties okra (*Abelmoschusesculentus*), amaranthus (*Amaranthus dubius*) and rosselle *(Hibiscus sabdariffa L*) were determined using pot experiments. The highest average metal concentration of1.11 ± 0.01 (mg/kg) were found in okra and0.87 ± 0.03 (mg/kg) in rosselle plants. They both showed higher tendency to accumulate all the five metals studied, while amaranthus has the lowest affinity towards these metals. Transfer factor (T F) index also gave supporting values, the result indicate that okra and rosselle have the high tendency of accumulation of the heavy metals investigated than amaranthus, CF values > 1 and TF values > 1 indicating that the metals were stored in the stems and leaves of these vegetable plants. The plant species showed relatively good response to the higher level of heavy metal concentration in the roots> stem > leafs suggest that these plant species can be good metal excluders with the possibility of extracting these heavy metals from artificially contaminated soils.

Keywords:- phytoremediation, Bioaccumulation factor, Transfer factor and Vegetable.

INTRODUCTION

Phytoremediation basically refers to the use of plants and associated microorganisms to partially or completely remediate selected contaminants from soil, sludge, sediments, wastewater and ground water.It has emerged as an alternative to the engineering-based methods. Phytoremediation can be used for removal of heavy metals, radionuclides as well as organic pollutants [1, 2, 3, 4]. In this new approach, plants are used to absorb contaminants from the soil and translocate them to the shoots. The metal-rich plant material may be safely harvested and removed from the site without extensive excavation, disposal cost and loss of top soil associated with traditional remediation practices [5, 6, 7, 8]. Phytoermediation of soil has attracted much attention in recent years due to its multiple advantages such as maintaining the biological activity and physical structure of soils, being potentially inexpensive, visually unobtrusive and providing the possibility of biorecovery of metals [9, 10]. Identification of native species for Phytoremediation is a key to the success of the method [11, 12].

Heavy metals pollution considered to be a worldwide threat now-a-days and responsible for environmental contamination [13] due to their high toxicity and persistency in the environment [14]. There are also many factors which contribute to heavy metal contamination such as contaminated irrigation water, fertilizers and pesticides applications, emissions of different waste materials from industries. Rapid urbanization leads to higher demand of food crops hence in peri-urban areas of mega cities vegetables are grown in shorter periods with greater profit. Leafy vegetables are teeming for accumulation of heavy metals in food chain. The heavy metals accumulation causes two impacts. First they enter in our diet and second crop production decline due to inhibition of metabolic processes [15, 16, 17, 18]. Reports have shown that, vegetables grown in heavy metal rich soils are also contaminated [19,20]. A common example of contamination includes bioaccumulation of heavy metals in vegetables. Bioaccumulation refers to the increase in concentration of a particular chemical or element in biological organisms over time and can pose a threat to the well-being of plants, animals and human beings [21].

Heavy metals accumulation and distribution in plant strongly depend on the plant species and metals level in the soil and air, soil pH and chemical exchange capacity, vegetation period and some other parameters [22]. The total metal concentrations in soil do not necessarily correspond with metal bioavailability. The bioavailability of heavy metals depends on a number of physicochemical properties such as pH, organic matter contents, cation exchange capacity, redox potential, soil texture and clay contents [23, 24].

Since the beginning of the industrial revolution, heavy metal contamination of the biosphere has increased considerably and has become a serious environmental concern. This study is aimed at determining the uptake of some heavy metals by okra (*Abelmoschus esculentus*), amaranth (*Amaranthus dubius*) and rosselle (*Hibiscus sabdariffa L*) grown in artificially contaminated soils.

MATERIALS AND METHODS

Materials

Seeds of Amaranth*(Amaranthus dubius)*, Okra *(Abelmoschus esculentus)* and Rosselle*(Hibiscus sabdariffa L)* were obtained from Mubi market, Adamawa State.The seed species were selected for the present study because of the qualities, such as large biomass production, robust rhizome suitable for accumulation of heavy metals.

The seeds were washed thoroughly in deionised or distilled water; a clean and new suitable plastic pots,dimension:(30cm × 40cm) were also provided.

Chemicals, Reagents and Instruments

Referncce standards of sulphuric acid, concentrated perchloric acid and concentrated nitric acid were used for digestion of vegetable and soil samples for heavy metal determination. All chemicals and reagents used were of analytical grade: HNO_3 69–72%, HCl 70 %, A.C.S. reagent and 98% H_2S0_4 70 %. The glassware and polyethylene containers used for analysis were washed with tap water, then soaked in 4 M HNO3 solution and rinsed several times with deionized water. Deionized water was used throughout the experiment for preparation and dilution of the sample solutions.

Air circulating oven (Genlab Limited, UK), electronic blending device (K-M20, IKA-WERKE, Germany), ceramic mortar and pestle (Haldenwanger, Germany), a digital analytical balance (MettlerTolendo, Switzerland), stainless steel Auger, Kjeldahl block digester (Gallenkamp, England) were used. (Buck Scientific Model 210VGP AAS, East Norwalk, USA) with air-acetylene flame and deuterium background correction was used for the analysis of the target metals.

EXPERIMENTAL DESIGN

Pot experiment was carried out from February to May of 2018 in Mubi, AdamawaState, Nigeria. Vegetable seeds of okra *(Abelmoschus esculentus)*, Amaranth *(Amaranthus dubius)* and rosselle *(Hibiscus sabdariffa)* were planted in contaminated and normal soil in pots of measured size, and were watered once every day with 500 mL of borehole water. The vegetable plants were grown for three months (90days) after which the vegetables were harvested and separated into roots, stem and leaves. Top garden soil (0–20 cm) was collected and sieved with a<4 mm size sieve and then thoroughly mixed to produce a homogenous soil composite. All of the soil was air-dried for a week before being artificially spiked with (50 mg/kg) metal salts of Pb, Zn, Cr,Cd and Ni, using nitrate and chloride salts of the metals respectively. Each pot of 23 cm × 22 cm area size were filled up with five kilograms (5kg) of garden soil that has been treated with Pb (50 mg Pb/kg soil), Zn (50 mg Zn/kg soil), Cd (50 mg Cd/kg soil),Cr(50 mgCr/kg soil) and Ni(50 mgNi/kg soil) The concentrations of artificially spiked metal treatments were prepared based onthe Malaysian guidelines for soil contamination (DOE 2009) and the European Unionheavymetals threshold limits [25].

Sample Collection and Treatment

Whole plant samples of Okra *(Abelmoschus esculentus)***,** Amaranthus*(Amaranthus Dubius)* And Rosselle*(Hibiscus sabdariffa L)* were harvested from each of the spiked and the control pots at the end of (90 days); Soil samples (150 g) both spiked and the control were collected from the surface to a depth of 20cm around each plant root zone.. The collection was done by dividing the spiked and the and control samples each into four quadrants, plant samples and soil samples were collected from each quadrant in a diagonal basis by following the methods of [26]. The soil samples were taken to the laboratory, air-dried, ground and sieved through a 2mm mesh,then kept in polythene bags forAtomic Absorption Spectrophotometer (AAS) analysis. Plant samples were washed with running tap water and then rinsed with distilled water. The roots, stems and leaves of each plant were separated, air-dried and again dried in an oven at 60^{0}C for 16hrs. The dry plant materials were ground placed in polythene bags and kept dry in a desiccators for AAS analysis.

Digestion of the Soil and Plant Samples

This was carried out according to the method described by [27]. The plant samples were subsequently oven dried at 75 °C for 48 h until it achieved a constant weight and weighed once again and then homogenized using a mortar and pestle. Approximately, 0.5 g of homogenized powder (shoot or root) was transferred into a 100 ml conical flask and 5 ml of concentrated H_2SO_4 added, followed by 25 mL of concentrated HNO_3 and 5 mL of concentrated HCl in a conical flask. The contents of the conical flask was then heated at 200 °C for 1 h in a fume cupboard and cooled down to room temperature. After cooling, 20 ml of distilled water was added and the mixture was filtered using Whatmann filter paper number one (110 mm). Subsequently, the mixture was transferred into a 50 mL volumetric flask and distilled water added up till to the mark. Finally, the volumetric flask was left to settle down for 15 h. The supernatants obtained were then analyzed in triplicates for total Zn, Ni, Cr, Cd and Pb metal concentrations using Buck ScientificAAS.

Soil samples were air-dried for 48 hrs until it reached a constant weight before being subjected to similar analytical procedures.

Quality Assurance (Analytical Method Validation)

The efficiency of the optimized procedure used was tested by using spiked samples that were later used as reference samples. This was done by spiking the pre-digested samples with multielement standard solutions of (Zn, Ni, Cr, Cd and Pb) as reported by [28].

Determination of the Movement of Metals From Soil To Plant

The accumulation factor (AF) of metals was used to determine the quantity of heavy metals that is absorbed by the plant from the soil.

$$AF = \frac{\text{metal concentration in plant tissue (Root + Stem + Leaf)}}{\text{Concentration of metal in substrate (soil)}}$$

This is an index of the ability of the plant to accumulate a particular metal with respect to its concentration in the soil [29].The higher the AF value the more suitable is the plant for phytoextraction [29]. BCF Values > 2 were regarded as high values.

Determination of the Movement of Metals From Roots to Plants

To evaluate the potential of plants for phytoextraction the translocation factor (TF) was used.

$$TF = \frac{\text{metalconcentration (stems + leaves)}}{\text{Metal concentration In Root}}$$

This ratio was an indication of the ability of the plant to translocate metals from the roots to the aerial parts of the plant [30]. Metals that are accumulated by plants and largely stored in the roots of plants are indicated by TF values < 1 with values > 1 indicating that the metals are stored in the stems and leaves.

Soil Analysis

Soil particle fractionation, moisture content and soil pH were determined by the method of [31]. The determination of organic matter was carried out according to the method of [32], and cation exchange capacity was determine according to the method described by [33].

Data Interpretation and Statistical Analysis

All treatments in the experiment were done in triplicates. The meanand standard deviations (D) were calculated using the Microsoft Office Excel 2007.

RESULTS

Evaluation of the analytical method

In this study, the method validation was made by a spiking experiment in which known quantities of the metal standard solution were added to the samples to be studied. Percentage recovery values for individual analysis for soil, effluent, and vegetable samples were presented in

Table 1. The percentage recovery values of the metals for soil, effluent, and vegetable samples were found to be 85%, 90%, 80%, 85% and 90%,respectively. These were within the acceptable range as reported by U.S. Environmental Protection Agency (USEPA, 2011) which confirmed the validity of the method utilized in the current study.

Table 1: Percentage metal recovery for Zn, Ni, Cr, Cd and Pb in spiked and unspiked Soils

Heavy Metal	Concentration of Spiked Soil	Concentration of Unspiked Soil	% Recovery
Zn	**0.51±0.03**	**0.33±0.01**	**85**
Ni	**0.49±0.001**	**0.30±0.02**	**90**
Cr	**0.47±0.09**	**0.29±0.05**	**80**
Cd	**0.49±0.02**	**0.32±0.01**	**85**
Pb	**0.53±0.01**	**0.37±0.04**	**90**

Physicochemical Parameters of Soils

The physicochemical parameters of soil are shown in Table 2 below. The soil texture of the growth media composed of 70.4% sand, 35.1% silt and 12.0% clay, with an average pH recorded to be 7.33 (slightly alkaline). Organic matter content of soil was determined to be 23.72 g%. The soil CEC was calculated to be 7.03 C mol/Kg and initial Zn, Ni, Cr, Cd and Pb concentration of soil, were determined to be 0.37 ± 0.01, 0.30 ± 0.03, 0.25 ± 0.02, 0.30 ± 0.01 and 0.20 ± 0.02 mg/kg.

Table 1: Physicochemical parameters of soils

Characteristic unit	Mean ± SD
Soil texture	
Sand %	70.4
Silt %	35.1
Clay %	12.0
Soil Ph	7.35
Organic matter %	23.72
Cation exchange capacity (CEC)	7.03
Soil metal content (mg/kg)	
Zn	0.37±0.01
Ni	0.20±0.02
Cr	0.25±0.02
Cd	.30±0.01
Pb	0.20±0.01

Accumulation of Heavy Metals (Zn, Ni, Cr, Cd and Pb) in root, stem and leaf of Okra *(Abelmoschus esculentus),* Amaranth *(Amaranthus dubius) And* Rosselle *(Hibiscus sabdariffa L)*(Mg/Kg Dry Weight Mean ±SD).

Concentration of trace heavy metals in vegetables plants depends upon the soil of the cultivated area, the atmospheric condition and the irrigated water. In this study, determination of heavy metals such as Zn, Ni, Cr, Cd and Pbwere done by atomic absorption spectroscopy in (mg/kg dry weight mean ±SD). The concentrations of the metals (Zn, Ni, Cr, Cd and Pb) in the investigated vegetables are given in Figures 1-3 for the okra, Amaranth, and rosselle samples, respectively.

The metal average concentrations (mg /kg) in Okra sample were found to be 1.11 ± 0.01, 0.32 ± 0.02, 0.34 ± 0.02, 0.28 ± 0.05 and 0.09 ± 0.05 (mg/kg). The concentration of the same in the control soil were found to be 0.95 ± 0.01, 0.29 ± 0.02, 0.31 ± 0.04, 0.07 ± 0.01 and 0.07 ± 0.01 (mg/kg) respectivelyforZn, Ni, Cr, Cd and Pb(Figure 1).

The concentrations (mg/ kg) of heavy metals in Amaranth, also shown that the average concentration of Zn, Ni, Cr, Cd and Pbin root, stem and leave spiked soil were found to be 1.35 ± 0.04, 0.26 ± 0.01, 0.32 ± 0.01, 0.02 ± 0.01 and 0.15 ± 0.03 (mg/kg), the concentration of the same in the control soil were found to be 0.69 ± 0.01, 0.27 ± 0.02, 0.31 ± 0.04, 0.07 ± 0.01 and 0.09 ± 0.02 [mg/kg](Figure 2).

The average concentration ofZn, Ni, Cr, Cd and Pbin root, stem and leave ofrossellespiked soil were found to be 0.87 ± 0.03, 0.29 ± 0.05, 0.30 ± 0.02, 0.16 ± 0.01 and 0.27 ± 0.04 (mg/kg). The concentration of the same in the control soil were found to be 0.75 ± 0.03, 0.26 ± 0.01, 0.25 ± 0.04, 0.03 ± 0.02 and 0.19 ± 0.02 [mg/kg] (Figure 1).

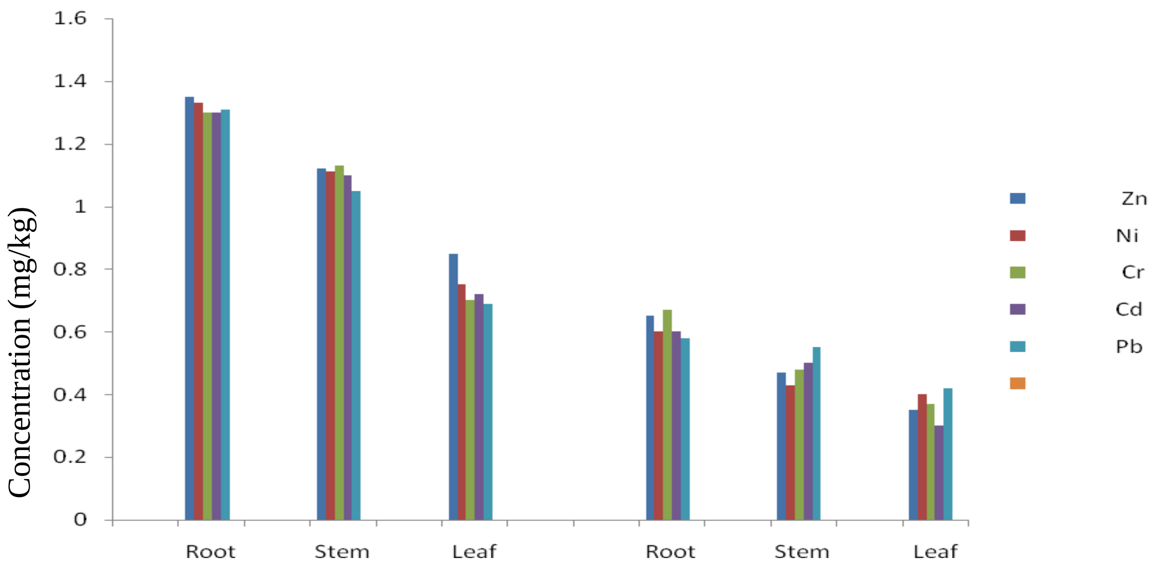

Figure1. Concertration of elements in parts of Okra (mg/kg) (Mean±SD)

Spiked Soil Control Soil

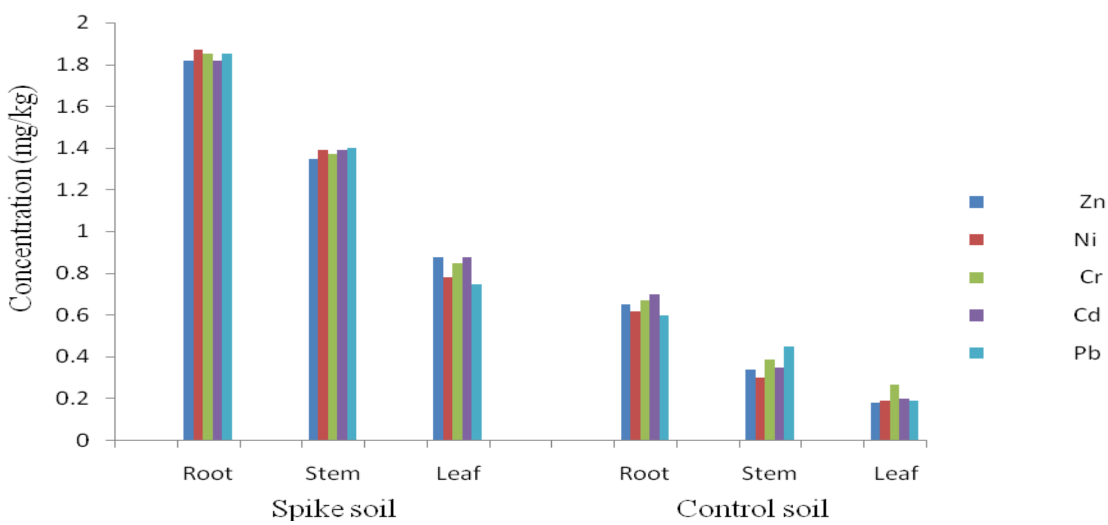

Figure 2.Concertration of elements in parts of Amaranthus [mg/kg] (Mean±SD)

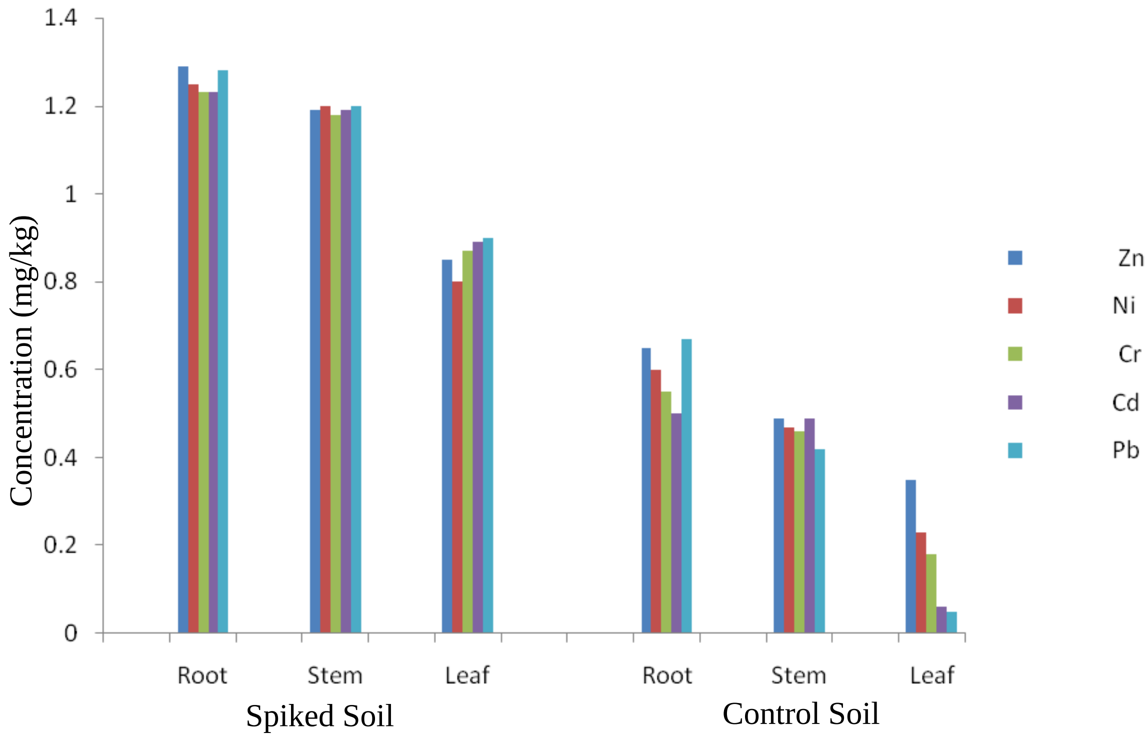

Figure 3.Concertration of elements in parts of rosselle [mg/kg] (Mean±SD)

Accumulation Factor (AF) And Transfer Factor (TF) Of Heavy Metals (Mg/Kg) In Okra, Amaranthusand Rossellein Spiked and Control Soil.

The result of accummulation factor (AF) and transfer factor (TF) of okra, Spinach and rosselle is shown in Figure 4 below. The result indicate Zn has the highest (AF) in okra, while Ni and Cr have the highest TFs in in Amaranthus and rosselle,respectively.

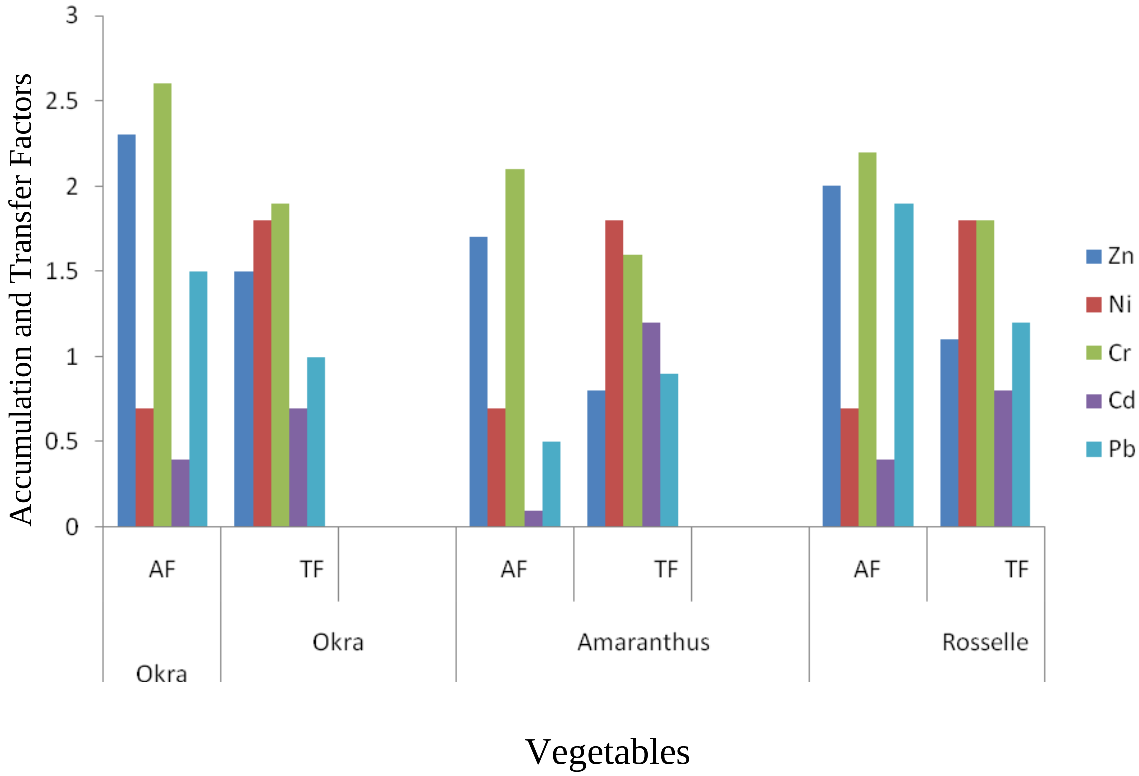

Figure 4. Accumulation factor (AF) and Transfer factor (TF) of heavy metal (mg/kg) in okra. Amaranthus and rosselle in spikesd soil

DISCUSSION

Physicochemical Properties of Soil

Soil texture was sandy loam with an average pH recorded to be 7.33 (slightly alkaline). The soil pH was within the permissible levels for appropriate growth and efficient uptake of metals from soil (Cempel, 2006). Organic matter content of soil was determined to be 23.72 %. The soil CEC was calculated to be 7.03 C mol/Kg. The initial Zn, Ni, Cr, Cd, Pb concentration of uncontaminated soil was determined to be 0.37 ± 0.01, 0.20 ± 0.02, 0.25 ± 0.01, 0.30 ± 0.02 and 0.20 ± 0.01 mg/kg. The presence of organic carbon increases the cation exchange capacity of the soil which retains nutrients assimilated by plants Van (1987).

Accumulation of Heavy Metals in Parts of Okra*(Abelmoschus esculentus),* Amaranth *(Amaranthusdubius)* and Rosselle*(Hibiscus sabdariffa)*

Figures 1 - 3 shows the average concentration of Zn, Ni, Cr, Cd and Pb in root, stem and leaf of Okra, Amaranthus and Rosselle.

The result of okra spiked soil were found to be 1.11 ± 0.01, 0.32 ± 0.02, 0.34 ± 0.02, 0.28 ± 0.05 and 0.09 ± 0.05 (mg/kg). The concentration of the same in the control soil were found to be 0.95 ± 0.01, 0.29 ± 0.02, 0.31 ± 0.04, 0.07 ± 0.01 and 0.07 ± 0.01 (mg/kg). The results in the spiked soil indicate that Zinc has an higher accumulation while lead has the lowest accumulation in the root, stem and leaf. The concentrations are in this order:Zn > Cr > Ni > Cd >Pb,

and are higher in the root and lower in the leaf. This result is in agreement with the report given byPivetz (2010). In Amaranth, the result also shown that the average concentration of Zn, Ni, Cr, Cd and Pb in root, stem and leave spiked soil were found to be 1.35 ± 0.04, 0.26 ± 0.01, 0.32 ± 0.01, 0.02 ± 0.01 and 0.15 ± 0.03 (mg/kg), the concentration of the same in the control soil were found to be 0.69 ± 0.01, 0.27 ± 0.02, 0.31 ± 0.04, 0.07 ± 0.01 and 0.09 ± 0.02 (mg/kg). The result indicates that Zinc in spiked soil has the highest accumulation, while Cd has the lowest concentration in amaranthusroot, stem and leaf. The concentrations are in this order:Zn > Cr >Ni >Pb> Cd.

The metal levels are higher in the root followed by the stem and the leaf has the lowest concentration of these heavy metals, their concentrations were higher than the control (Bigaliev*et al.,* 2013) also reported the same accumulation of these heavy metals in parts of amaranth*(Amaranthusdubius).*

The average concentration of Zn, Ni, Cr, Cd and Pbin root, stem and leave of rosselle spiked soil were found to be 0.87 ± 0.03, 0.29 ± 0.05, 0.30 ± 0.02, 0.16 ± 0.01 and 0.27 ± 0.04 (mg/kg). The concentration of the same in the control soil were found to be 0.75 ± 0.03, 0.26 ± 0.01, 0.25 ± 0.04, 0.03 ± 0.02 and 0.19 ± 0.02 (mg/kg). Zinc has the highest accumulation, while Cadmium has the lowest concentration in the spiked soil.The concentrations are in this order: Zn > Cr > Ni >Pb>Cd.

This current study indicated that rosselle plant showed a steady increase in the absorption of these metals in the root and lower in the leaf,which is in agreement with the report byMadaan and Mudgal (2009).

Accumulation Factor (AF) and Transfer Factor (TF) of Heavy Metals (Mg/Kg) in Okra, Amaranth and Rossellein Spiked and Control Soil.

Figure 4 shows the accumulation factors (AFs) and transfer factors TFs) for Zn, Ni, Cr, Cd and Pb, for Okra, Amaranth and Rosselle. The result shows that, the phytoextraction potential of each vegetable specie was determined by its AF and TF. The metals were extracted by each of the vegetable species and were translocated from the roots to the shoot. In okra, the AF and TF for all the studied metals were > 1 except for Cd. In Amaranth, the BCF were found to be > 1 except for Ni and Pb, while the TF were >1 except for Zn andPb. And for rosselle, the AF and TF values were all > 1except for Ni and Cd. A value > 1 indicates that the vegetable is a metal accumulator appropriate for phytoextractionGarbisu and Alkorta (2010). These indices were important values that estimate the potential of a plant for phytoextraction and phytostabilization (Saraswet and Rai, 2009**).** The results indicate that, okra, Amaranth and rosselle could be considered as a phytoremediant in heavy metal polluted soil with Okra (*Abelmoschus esculentus)* having the highest remediation potential while Amaranth having the lowest remediation potential. It has been reported that only a longer growth period produces higher biomass that can enhance metal hyperaccumulation by the plant [33].

CONCLUSION AND RECOMMENDATION

A pot experiment to investigate the phytoextraction of Zn, Ni, Cr, Cd and Pb by okra *(Abelmoschus esculentus)*, Amaranthus*(Amaranthus dubius)* and rosselle*(Hibiscus sabdariffa L,* planted on contaminated and control soils was carried out in the months of February to May, 2018. The soil texture was sandy loam with an average pH of 7.35 (slightly alkaline). The soil pH was within the permissible levels for appropriate growth and efficient uptake of metals from soil. Organic matter content of soil was determined to be 21.95 g%. The soil cation exchange capacity (CEC) was calculated to be 7.09 C mol/Kg. The mean concentrations of the metals in spiked soils were higher than the amount in controls. The results of the validation of analytical procedure of this work were within the acceptable range [20]. The accumultion and translocation factors determined for the metals by the tested vegetable species indicated that these vegetable species have the potentials for phytoremediation of soils.

CONCLUSION

Metal-contaminated soils are notoriously hard to remediate. Current technologies resort to soil excavation and either land filling or soil washing followed by physical or chemical separation of the contaminants phytoremediation is an effective and affordable technology used to remove inactive metals and metal pollutants from contaminated soil and water. Pot experiment for the three plant species, okra *(Abelmoschus esculentus)*, Amaranthus*(Amaranthus dubius)* and rosselle *(Hibiscus sabdariffa L)* used for this study indicate that they all have the potential to be used for phyto extraction. Each showed a significantly higher absorption of Zn, Ni, Cr, Cd and Pb (except Amaranthus which shows weak absorption of these metals) when compared to their respective controls, which led to the reduction in the level of the studied metals in the soils of the pot experiment. The accumulation and translocation factors (AF and TF >1) of the studied metals by the test plant species an indication that these species have the potentials for phytoremediation Zhang et al., (2009). The order of the phytoextraction potentials for these plants were found to be in the following order: *(Abelmoschus esculentus)* okra >*(Hibiscus sabdariffa L)*rosselle> *(Amaranthus dubius)* Amaranthus. A common conclusion can be drawn that Okra vegetable plant shows higher affinity towards Zn, Ni and Cr which was consistent with previous studies.

The results of this study were of considerable significance in demonstrating the practical application of these vegetable plants for phytoremediation of heavy metal in contaminated soil. It was observed that the heavy metals reduced considerably in the soil of the plants for both spiked and control soil this trend shows that in terms of phytoremadiation, *(Abelmoschus esculentus)* okra, *(Amaranthus dubius)* and *(Hibiscus sabdariffa)*rosselle can clean up contamination in a very short period of time without any cost implications and no time consuming.

RECOMMENDATIONS

The following recommendations are hereby made: There is a need to modify these plants (genetically) in other to increase the remediation potential of these plants so as to offer a viable remediation solution for polluted soils or farmlands. Investigation can be carried out on how to recover these metals extracted to avoid reintroducing the metal contaminants into the environment.

Further research is necessary in order to find the potential transformation mechanisms for heavy metal phytoextraction in vegetables.

Other local plants with the same morphology as the studied plants could be investigated for better phytoremediation of these metals and other metals not studied in this research.

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