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PHYTOEXTRACTION OF HEAVY METALS IN CONTAMINATED SOILS USING AMARANTHUSVIRIDIS

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Abstract: The phytoextraction potential of Amaranthus for heavy metals in soil was investigated in this study using *Amaranthusviridis*. The screen house experiment was carried out at the Department of Botany, Obafemi Awolowo University, Ile-Ife, to evaluate the phytoremediation potential of *Amaranthusviridis* on soil samples collected from gold mine site, Itagunmodi, Osun State, Nigeria. The heavy metals analyzed were: Lead (Pb), Cadmium (Cd), Copper (Cu), Zinc (Zn) and Iron (Fe), using Atomic Absorption Spectrophometry (AAS) Method. The heavy metals were analyzed in the soil samples before and after phytoremediation process and plant's parts (Root, Steam and Leaf) grown on the soil.The Bioconcentration Factor (BCF) and Translocation Factor (TF) were also evaluated. The average concentrations of Pb, Cd, Cu, Zn and Fe in the soil before and after phytoremediation process respectively are 0.012±0.001 ppm, 0.010±0.001; 0.029±0.003, 0.024±0.002; 0.064±0.007, 0.055±0.005; 0.111±0.012, 0.105±0.009; and 0.023±0.004, 0.022±0.003.The BCF > 2 was obtained for Zn, TF < 1 was obtained for Pb and Zn and TF > 1 was obtained for Cd, Cu and Fe. The results suggest that *viridis* is good for phytoextraction of Cd, Cu, Fe and Zn in contaminated soil.

Keywords: Phytoextraction, Bioconcentration factor, Translocation factor, Contaminated soil, Heavy metals.

1.0 INTRODUCTION

Soil and water are precious natural resources on which rely the sustainability of agriculture and the civilization ofmankind. Unfortunately, they have been subjected to maximum exploitation and severely degraded or polluted due to anthropogenic activities. 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 (McGrath *et al.,* 2001; Nriagu and Pacyna, 1988; Schalscha andAhumada, 1998). Each source of contamination has its own damaging effects on plants, animals and ultimately on human health, but those that add radionuclides and heavy metals to soils and waters are of serious concern due to their persistence in the environment, non-biodegradable nature and carcinogenicity to human beings. They cannot be destroyed biologically but are only transformed from one oxidation state or organic complex to another (Garbisu and Alkorta, 2001; Gisbert*et al.*2003). Therefore, radionuclides and heavy metals pollution pose a great threat to the environment and human health (Mohammad *et al*. 2008).

Apparently these toxic substances entered the food chain via grazers, such as cows and other livestock that feed on plants grown in contaminated soils. The toxins then accumulated and concentrated in the meat and milk products eventually consumed by humans. They can also find their ways into human system when the plants grown in contaminated soil are consumed directly by human beings (McGraw, 2000). Hence, efforts must be made to either remove these contaminants from the soil environment or render them harmless in the environment.

In recent years, phytoremediation has emerged as a promising eco-remediation technology, particularly for cleanup of large volumes of contaminated soil and water. The exploitation of plants to remediate soils contaminated with radionuclides and heavy metals could provide a cheap and sustainable technology for bioremediation. Phytoremediation is a technology in which theplants are employed to absorb and bio-magnify elements from a polluted environment and metabolize them into various biomolecules in their tissues (Pant *et al.*2011).

2.0 MATERIALS AND METHOD

2.1 The Study Area

The study area is Itagunmodi town, Atakumosa West Local Government Area, Osun State, Southwest Nigeria. The Itagunmodi mining site, where gold is predominantly mined, is located between latitudes 7°30′N and 7°36′N and longitudes 4°37′E and 4°42′E (Salami *et al.* 2003). It is about 347 m above sea level and has an approximate population of 12,655(NPC, 2006). It is a town very close to Ile-Ife, Ilesa and Modakeke in Osun State, Nigeria. The climate of the area is influenced by tropical maritime (mT) and tropical continental (cT) air masses. The mean annual rainfall of the area is about 1220 mm, and the meanannual temperature is about 27^{o}C (Balogun and Salami, 1995), while relative humidity over the area varies from 60 to 80%. The study area has a record of heavy rainfall ranging from 1200 mm to 1500 mm over 8 months of each annual season with a peak in June and September, (Adeoye, 2007). The area is underlain by the Precambrian basement complex of the southwestern, Nigeria and it is composed of biotite-and-biotite-hornblende-gneiss, migmatites, and schist associated with amphibolites (Adetoyinbo*et al.* 2010). The whole area is drained by tributaries of Osun, Sasa and Oora rivers which flow south ward and southwest ward directions.

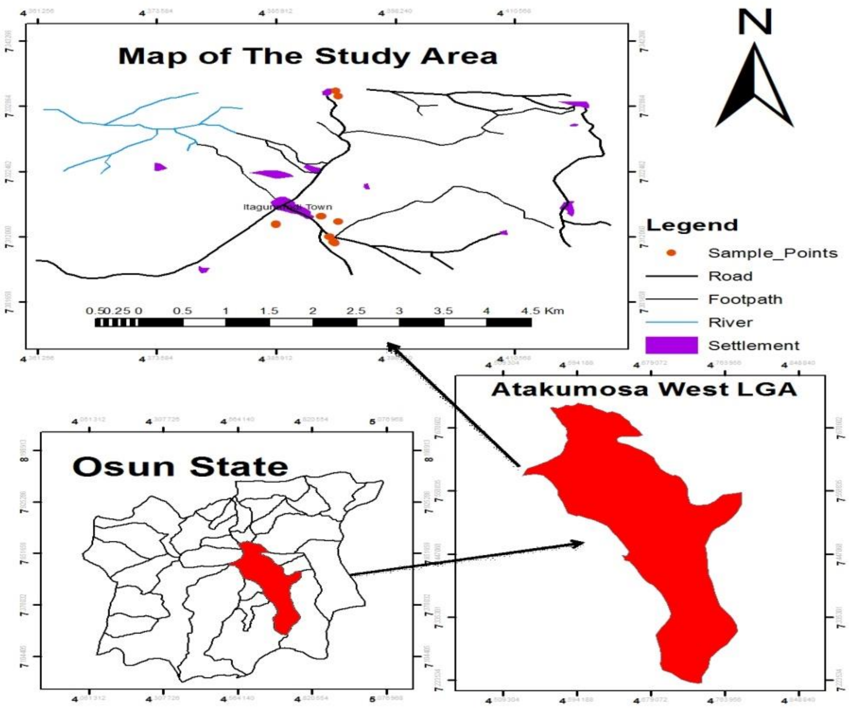

Figure 1: Map of the study area showing sample points

2.2 Soil sampling and preparation before plants cultivation

Soil samples werecollected from five major gold mine pits around Itagunmodi gold mine sites (Faweya, 2014). These five mine pits comprised of three pits where gold is currently mined and two pits where gold has been mined more than a year ago. Four soil samples were collected per pit to make a total of 20 soil samples. Plate 1 (A, B and C) show the degraded areas where gold is mined during soil samples collected in Itagunmodi. Four soil samples were collected from an agricultural field near Itagunmodi town as control soil samples. In this study, a total of 24 soil samples were investigated using Amaranthusviridis as phytoremediation plant. Samples were collected at 0 - 15 cm depth to obtain representative samples using soil auger. The soil samples collected were packed and labeled reflecting the location of each sampling point and then taken to the laboratory. The coordinates of each sampling point were recorded with the aid of a global positioning system (GPS). Small portion of each sample were taken for laboratory analysis to determine the initial conditions and concentrations of heavy metals before phytoremediation process takes place. The soil samples were air dried at room temperature (about 25^{o}C), subsequently grounded, and sieved with 2 mm mesh. The samples used to analyze for heavy metals concentrations were taken to the laboratory for the measurement of the five selected heavy metals viz: Pb, Cd, Cu, Zn, and Fe with the use of atomic absorption spectrometer (AAS).

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Plate 1: Soil sample collection from gold mine site, Itagunmode, Osun State.

Figure 2: Experimental set up showing *A. viridis*grown in pots under greenhouse 7 WAP

2.2.1 Experimental set up

The 24 soil samples used for the study were packed inside polypropylene pots to prevent leakage and seepage of the contaminants to the immediate environment with the pots properly labeled. The bottoms of the pots were perforated for proper drainage of the excess water in the soil during wetting. The seed of the A. viridis was obtained from Department of Plant Breeding and Seed Technology, Federal University of Agriculture Abeokuta, Ogun state, Nigeria. They were cultivated in a greenhouse environment at the Department of Botany, Obafemi Awolowo University, Ile-Ife, Osun State. Each pot was filled with 3 kg of soil sample from different sampling locations, and the seeds were broadcasted into each pot by hand (Watt, 2004). One week after germination, the seedlings were thinned to twenty (20) stands per pot to ensure uniform number of seedling per pot. The seedlings were grown for seven weeks before harvested.

2.2.2 Plants sampling and preparation

Plants preparation for laboratory analysis was carried out at the Department of Botany, ObafemiAwolowo University, Ile-Ife, Osun State. The sampling of *Amaranthus* seedlings was carried out 7 weeks after planting (Alonge*et al.* 2007). Ten seedlings were uprooted from each pot as replicates and washed thoroughly to remove dirt and soil particles from the plants' parts. The leaves, stems and roots were removed from the plant of each pot using stainless steel scissors (Pungtip*et al*. 2016). Each plant part was put in a labeled envelope and placed in an oven maintained at 70^{o}C for a period of about 24 hours, after which continuous weighing was observed using a digital scale till a constant weight was obtained (Alonge*et al.* 2007). Each sample was grounded to a fine powder and sieved through a 2-mm nylon mesh sieve. The samples were carefully packed, labeled and taken to the laboratory for the measurement of five selected heavy metals; Pb, Cd, Cu, Zn, and Fe with the use of AAS.

2.2.3 Soil sampling and preparation after cultivation

Soil samples were collected from each pot after cultivation for analysis. The samples were air dried at room temperature (about 25ºC), grounded and sieved, then analyzed for heavy metals concentrations.

2.3 Heavy metals and physico-chemical parameters concentrations measurement

2.3.1 Digestion of soil/plant samples

0.5g of the dried, grinded soil/plant sample was weighed accurately into a clean Teflon beaker, 20ml HF was added and heated to near dryness, 15 ml of HNO_3 was then added and heating resumed to mop up the residue, again on near dryness, it was allowed to cool, 20ml distilled water was added to boil off the acid. After boiling to one-third its volume, the sample was allowed to cool and filtered. The filterate was made up to 50ml mark in a standard flask with distilled water. Concentrations of the heavy metals and physicochemical parameterswere determined in the digest by AAS.

2.3.2 Analysis of the heavy metals and physico-chemical parameters

The sample was analysed with PG990 AAS of the Centre for Energy Research and Development (CARD), ObafemiAwolowo University, Ile-Ife, Nigeria for the metals in the samples by flame atomization, using air-acetylene flame and single element hollow cathode lamp and following the equipment procedures.The Atomic absorption spectrometric technique makes use of the flame as the atomizer. The sample solution is aspirated into the flame and the sample element is converted into atomic vapour, the flame thus contains atoms of the element. Some are thermally excited by the flame but most remain in the ground state where they can absorb radiations given off by a special 'source' made from that element. The wavelength of radiation given off by the source is the same as those absorbed by the atoms in the flame. This absorption follows Beer-Lambert's law which states the absorbance to be directly proportional to the path length in the flame and to the concentration of atomic vapour in the flame. Both of these variables are difficult to determine but the path length can be held constant and the concentration of atomic vapour in the flame is directly proportional to the concentration of the analyte in the solution being aspirated into the flame.

2.4.1 Determination of the movement of heavy metals from soil to plant's parts

2.4.1 Bioconcentration factor

The bioconcentration factor (BCF) is defined as the ratio of the total concentration of element in the harvested plant tissue (C_{plant}) to its concentration in the soil in which the plant was growing (C_{soil}). The bioconcentration factor (BCF) is used to determine soil to plant movement of the heavy metals. It is used to determine the quantity of heavy metals that is absorbed by the plant from the soil. This is an index of the ability of the plant to accumulate a particular metal with respect to its concentration in the soil (Ghosh and Singh, 2005) and is calculated using the formula:

$$\mathrm{BCF}=\frac{\text{Metal concentration in plant tissue (whole plant/portal)}}{\text{Initial concentration of metal in substrate (soil)}} \quad \ldots(1)$$

The higher the BCF value the more suitable is the plant for phytoextraction (Blaylock *et al*. 2000). BCF Values > 2 were regarded as high values.

2.4.2 Translocation factor (TF)

Translocation factor (TF), defined as the ratio of the total concentration of elements in the aerialparts of the plant (C_{shoot}) to the concentration in the root (C_{root}). To evaluate the potential of plants for phytoextraction the translocation factor (TF) is used. This ratio is an indication of the ability of the plant to translocate metals from the roots to the aerial parts of the plant (Marchiol*et al*. 2004). It is represented by the ratio:

$$\mathrm{TF}=\frac{\text{Metal concentration in plant tissue in (stem+leaves)}}{\text{Metal concentration in roots}} \quad \ldots \ (2)$$

Metals that are accumulated by plants and largely stored in the roots of plants are indicated by TF values < 1, whereas when TF > 1 indicates that the metals are stored in the stems and leaves.

3.0 Results and Discussion

Table 1 shows the coordinates in Northing/Latitude and Easting/Longitude of the sampling locations using GPS. The physico-chemical parameters of the samples soils were presented in Table 2. The pH of the soil samples used in the experiment ranged from 7.71 and 8.51, while the electrical conductivity ranged from 900.8 to 1214.8 µs/cm. The cations tested are Ca and Mg having their concentrations ranged from 0.188 to 2.60 ppm; and 0.059 to 0.095 ppm respectively.While the tested anions NO_3 and Cl_2 with range values of 2.096 to 7.089 ppm and 20.118 to 27.181 ppm respectively.

Table 1: Coordinates of sampling locations using GPS.

Location	Sample ID	UTM Northing/Latitude (N)	UTM Easting/Longitude (E)	Elevation (m)	Accuracy (m)
A	S1	0682236/07°33'52.70"	0836440/04°39'06.30"	335	3
	S2	0682226/07°33'52.90"	0836445/04°39'05.90"	330	6
	S3	0682231/07°33'52.90"	0836445/04°39'06.10"	330	3
	S4	0682222/07°33'52.80"	0836439/04°39'05.80"	334	3
B	S1	0682771/07°33'44.40"	0836184/04°39'23.70"	350	3
	S2	0682776/07°33'44.30"	0836184/04°39'23.80"	345	3
	S3	0682781/07°33'44.60"	0836190/04°39'24.00"	345	3
	S4	0682775/07°33'44.40"	0836187/04°39'23.80"	348	3
C	S1	0682502/07°31'20.10"	0831747/04°39'14.60"	327	3
	S2	0682504/07°31'20.20"	0831753/04°39'14.40"	323	3
	S3	0682500/07°31'20.20"	0831754/04°39'14.30"	322	3
	S4	0682502/07°31'20.20"	0831748/04°39'14.30"	324	3
D	S1	0682637/07°31'12.60"	0831521/04°39'18.20"	318	3
	S2	0682642/07°31'12.30"	0831512/04°39'18.40"	324	3
	S3	0682637/07°31'12.00"	0831502/04°39'18.30"	323	3
	S4	0682631/07°31'12.10"	0831507/04°39'18.50"	323	3
E	S1	0682646/07°31'11.70"	0831490/04°39'19.10"	326	3
	S2	0682658/07°31'11.90"	0831499/04°39'19.40"	326	3
	S3	0682676/07°31'10.30"	0831452/04°39'20.00"	320	3
	S4	0682695/07°33'52.90"	0831442/04°39'20.60"	318	3
Control	S1	0682031/07°31'40.70"	0832382/04°38'59.10"	352	3
	S2	0682039/07°31'40.50"	0832375/04°38'59.30"	351	3
	S3	0682021/07°31'39.90"	0832375/04°38'58.70"	350	3

	S4	0682021/07º31'39.50"	0832375/04º38'58.80"	347	3

Table 2: Physico-chemical parameters of the soil used in greenhouse experiment before phytoremediation process

Location	Ca (ppm)	Mg (ppm)	NO_3 (mg/l)	Cl_2 (mg/l)	EC (μs/cm)	pH
A	0.201	0.087	3.659	25.395	1001.5	8.11
B	0.223	0.091	3.239	26.961	1214.8	8.51
C	0.188	0.059	2.096	20.118	900.8	8.02
D	0.200	0.082	3.887	25.415	973.2	7.91
E	0.198	0.095	3.582	26.369	992.1	7.82
F (Living Area)	0.260	0.079	7.089	27.181	1200.5	7.71

Table 3: Average heavy metal concentrations in the soil samples from mining and living areas in Itagunmodi before and after phytoremediation exercise

Location	Heavy Metals Concentration in Soil (ppm)									
	Pb		Cd		Cu		Zn		Fe	
	Before	After	Before	After	Before	After	Before	After	Before	After
A	0.011± 0.001	0.008± 0.001	0.029± 0.002	0.024± 0.002	0.068± 0.004	0.055± 0.006	0.106± 0.013	0.105± 0.013	0.023± 0.005	0.022± 0.003
B	0.011± 0.001	0.008± 0.001	0.026± 0.005	0.024± 0.002	0.069± 0.009	0.057± 0.005	0.115± 0.020	0.098± 0.003	0.025± 0.004	0.023± 0.004
C	0.011± 0.002	0.011± 0.001	0.027± 0.003	0.023± 0.002	0.058± 0.006	0.052± 0.004	0.107± 0.008	0.105± 0.008	0.023± 0.004	0.021± 0.003
D	0.012± 0.001	0.011± 0.002	0.029± 0.003	0.024± 0.003	0.060± 0.007	0.056± 0.006	0.111± 0.008	0.110± 0.007	0.022± 0.005	0.021± 0.003
E	0.013± 0.002	0.010± 0.002	0.032± 0.003	0.023± 0.003	0.065± 0.007	0.057± 0.006	0.117± 0.012	0.109± 0.013	0.024± 0.004	0.022± 0.004
Mean (mining area)	0.012± 0.001	0.010± 0.001	0.029± 0.003	0.024± 0.002	0.064± 0.007	0.055± 0.005	0.111± 0.012	0.105± 0.009	0.023± 0.004	0.022± 0.003
F (Living area)	0.010±0. 001	0.004± 0.001	0.021± 0.002	0.013± 0.002	0.055± 0.004	0.034± 0.005	0.107± 0.008	0.060± 0.009	0.022± 0.004	0.015± 0.002

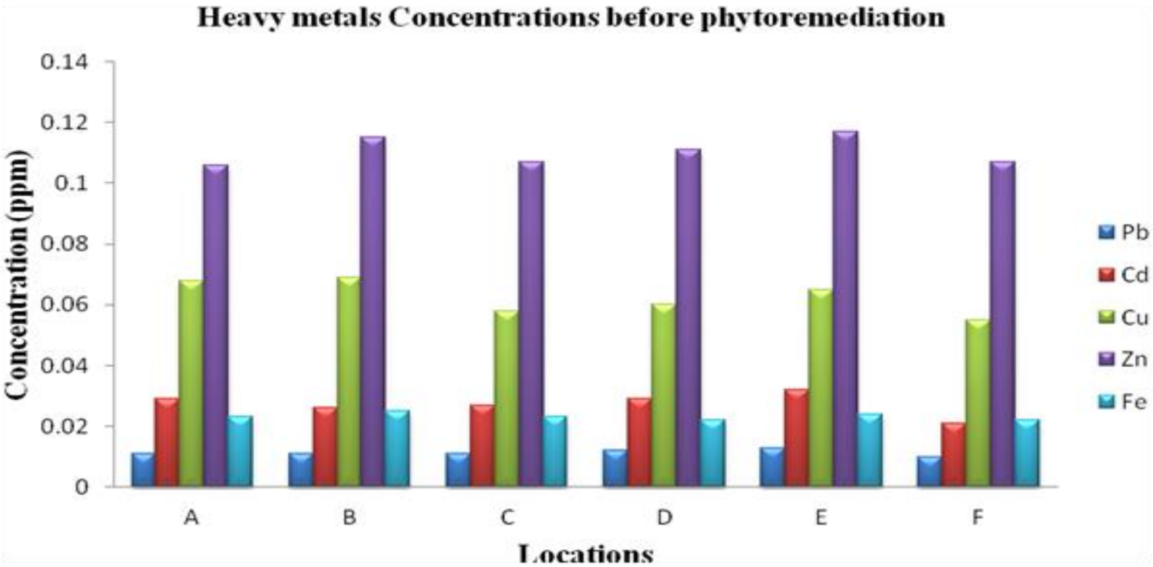

Figure 3: Comparison of concentrations of Pb, Cd, Cu, Zn and Fe before phytoremediation

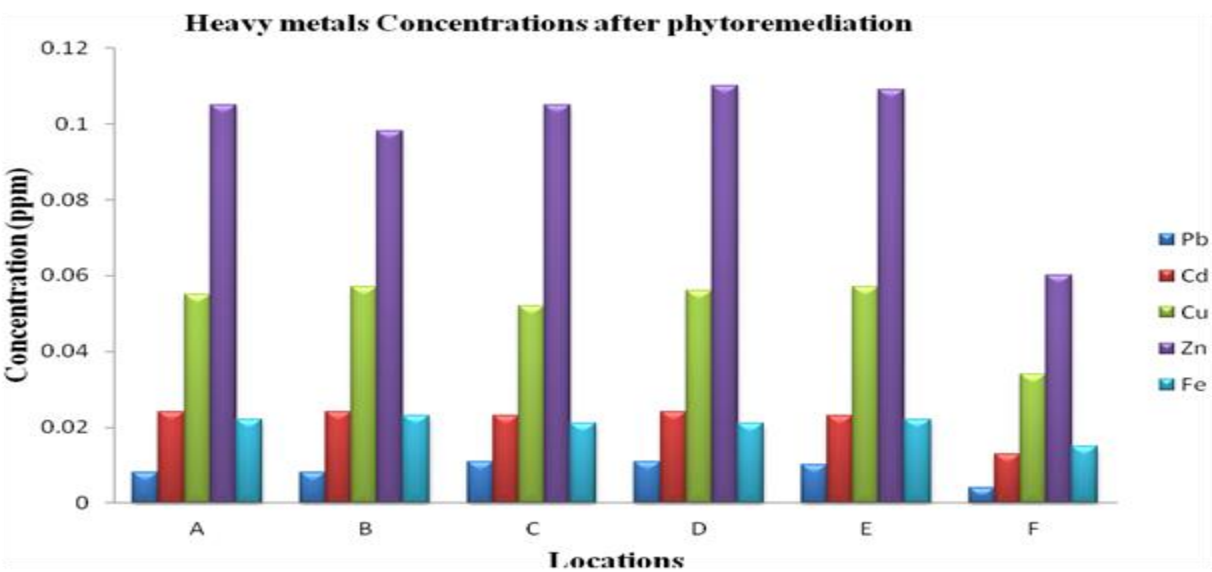

Figure 4: Comparison of concentrations of Pb, Cd, Cu, Zn and Fe after phytoremediation

The concentrations of the investigated heavy metals were generally greater in all the samples before the phytoremediation exercise than after the phytoremediation exercise. Compared with the living areas, the mean concentrations of the heavy metals in the mining areas were higher than their mean values obtained in the soil samples from living area. While the mean values of Pb, Cd, Cu, Zn and Fe before the phytoremediation exercise were 0.012±0.001 ppm, 0.029±0.003 ppm, 0.064±0.007 ppm, 0.111±0.012 ppmand 0.023±0.004 ppm, while after phytoremediaton exercise their respective mean values were 0.010±0.001 ppm, 0.024±0.002 ppm, 0.055±0.005 ppm, 0.105±0.009 ppmand 0.022±0.003 ppm. The mean concentrations of Pb, Cd, Cu, Zn and Fe in living areas before phytoremediation process were respectively 0.010±0.001 ppm, 0.021±0.001 ppm, 0.055±0.004 ppm, 0.107±0.008 ppmand 0.022±0.004 ppm; and after phytoremediation process, their mean concentrations were 0.004±0.001 ppm, 0.013±0.002 ppm, 0.034±0.005 ppm, 0.060±0.009 ppmand 0.015±0.002 ppm.

Figure 3 shows the comparison of concentrations of Pb, Cd, Cu, Zn and Fe before phytoremediation process, the mean concentrations of the heavy metals were in the order Zn>Cu>Cd>Fe>Pb in locations A, C, D and E. Whereas, in locations B and F, the mean concentrations of the heavy metals were in the order Zn>Cu>Pb, mean concentrations of Cd and Fe were almost the same. In general, before the phytoremediation process, Zn had the highest concentrations compared with other heavy metals investigated in this study. After the phytoremediation process, the concentrations of the heavy metals were in the order Zn>Cu>Cd>Fe>Pb in the mining sites. While, in the living areas, the concentrations of the heavy metals were in the order Zn>Cu>Fe>Cd>Fe, and generally after the phytoremediation process, Zn had the highest concentrations compared with other heavy metals investigated in this study as shown in Figure 4.

Table 4: Lead (Pb) levels (ppm) in soil, root, steam and leaf

Location	Concentration of Lead (Pb) (ppm)				BCF	TF
	Soil	Root	Steam	Leaf		
A	0.011	0.007	0.004	0.006	1.55	1.43
B	0.011	0.009	0.005	0.008	2.00	1.44
C	0.011	0.005	0.004	0.006	1.36	2.00
D	0.012	0.008	0.003	0.004	1.25	0.88
E	0.013	0.007	0.008	0.007	1.70	2.14
F (Living Area)	0.010	0.003	0.002	0.002	0.70	1.33

Table5: Cadmium (Cd) levels (ppm) in soil, root, steam and leaf

Location	Concentration of Cadmium (Cd) (ppm)				BCF	TF
	Soil	Root	Steam	Leaf		
A	0.029	0.011	0.009	0.015	1.21	2.18
B	0.026	0.012	0.01	0.011	1.27	1.75
C	0.027	0.013	0.011	0.014	1.41	1.92
D	0.029	0.015	0.012	0.015	1.45	1.8
E	0.032	0.016	0.011	0.016	1.34	1.69
F (Living Area)	0.021	0.012	0.008	0.013	1.57	1.75

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Table6: Copper (Cu) levels (ppm) in soil, root, steam and leaf

Location	**Concentration of Copper (Cu) (ppm)**				**BCF**	**TF**
	Soil	**Root**	**Steam**	**Leaf**		
A	0.068	0.023	0.033	0.028	1.24	2.65
B	0.069	0.032	0.024	0.035	1.32	1.84
C	0.058	0.031	0.027	0.041	1.71	2.20
D	0.06	0.021	0.034	0.038	1.55	3.43
E	0.065	0.035	0.027	0.032	1.45	1.69
F (Living Area)	0.055	0.022	0.032	0.021	1.36	2.41

Table7: Zinc (Zn) levels (ppm) in soil, root, steam and leaf

Location	**Concentration of Zinc(Zn) (ppm)**				**BCF**	**TF**
	Soil	**Root**	**Steam**	**Leaf**		
A	0.106	0.067	0.056	0.043	1.57	1.48
B	0.115	0.078	0.039	0.091	1.81	1.67
C	0.107	0.09	0.073	0.089	2.36	1.80
D	0.111	0.096	0.054	0.087	2.14	1.47
E	0.117	0.078	0.054	0.066	1.70	1.54
F (Living Area)	0.107	0.088	0.012	0.055	1.45	0.76

Table8: Iron (Fe) levels (ppm) in soil, root, steam and leaf

Location	**Concentration of Iron(Fe) (ppm)**				**BCF**	**TF**
	Soil	Root	Steam	Leaf		
A	0.023	0.006	0.005	0.008	0.83	2.17
B	0.025	0.009	0.007	0.01	1.04	1.89
C	0.023	0.007	0.009	0.01	1.13	2.71
D	0.022	0.01	0.007	0.005	1.00	1.20
E	0.024	0.007	0.009	0.009	1.04	2.57
F (Living Area)	0.022	0.008	0.009	0.004	0.96	1.63

Bioaccumulation factor (BCF) of the five heavy metals in A. viridis under investigation is presented in column 6 of Tables 4 to 8. The BCF of lead (Pb) is presented in Table 4 and ranged from 0.7 to 1.70, BCF of cadmium (Cd) shown in Table 5 which ranged between 1.20 and 1.57; BCF of copper (Cu) ranged from 1.24 to 1.71 as shown in Table 6; BCF of zinc (Zn) is presented in Table 7 with range value of 1.45 to 2.36; and Table 8 shows the BCF of iron (Fe) of range value between 0.83 and 1.13. Considering the investigated heavy metals, only Zn has the BCF > 2 for locations C and D, this value is considered high value of BCF with the greater concentration of the heavy metal accumulated in the plant's parts than in the roots.

Translocation factor (TF) of the heavy metals accumulated in Amaranth under consideration is shown in column 7 of Tables 4 to 8. The TF of the heavy metals ranged from 0.88 to 2.14; 1.75 to 2.18; 1.69 to 3.43; 0.76 to 1.47; and 1.20 to 2.71 for Pb, Cd, Cu, Zn and Fe respectively. The results revealed that forPb in location D and Zn in location F, TF >1, this indicates that the metals are accumulated by the plants and largely stored in the roots of the plants. Whereas for other heavy metal and locations the TF > 1, implying the greater concentration of the heavy metals is accumulated in aerial parts of the plant than the roots.

CONCLUSION

In this study, A. viridis was used as a phytoremediation plant of heavy metals in the soil samples from gold mine site in Itagunmodi, Osun State. The concentrations of the heavy metals accumulated in the plants were influenced by their initial concentrations in the soil. Based on the results obtained in this study, the study concludes that the A. viridis was a good phyto extractor with BCF > 2 and TF > 1 for some of the samples under investigation. The plants actively removed the heavy metals from the soil matrix and translocated it from the roots to the aerial parts of the plants. This study recommended further research using other plants for the phytoremediation of heavy metals in the soil.

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