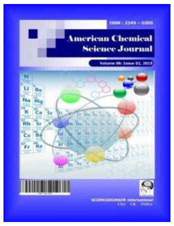

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Heavy Metal Accumulation in Tubers Grown in a Lead-zinc Derelict Mine and their Significance to Health and Phytoremediation

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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ABSTRACT

Four common tubers namely *Colocosia and Xanthosama* (Cocoa yam), *Manihol eaculenhis* (Cassava), *Dioscoreu rolundata* (White Yam) and *Ipomoea batatas* (Sweet potato) were grown within Enyigba lead-zinc derelict and samples of their leaves (aerial part) and roots (underground part) alongside the soil where they were grown were analyzed for heavy metal using Proton Induced X-ray Emission (PIXE) technique. The results revealed that heavy metals in soil (mean pH = 6.5±0.29) decreased in the order Pb > Zn > Cu > Mn > Cd > Ni > As > Cr. The mean concentration (mg/kg) of heavy metals in soil was found in the range of Pb (0.01-0.41); As (0.02-0.54): Cd (0.02-0.12); Cu (0.82-62.12); Cr (0.01-0.40); Zn (24.18-122.12); (Mn 18.46-624.26) and Ni (8.24-34.66). The results revealed that levels of Pb in white yam (0.41 mg/kg) and cassava (0.32 mg/kg); As in cassava (0.54 mg/kg), white yam (0.12 mg/kg) and sweet potato (0.14 mg/kg) and Cd in cocoyam (0.12 mg/kg) all exceeded the World health Organisation Maximum Limit (WHO ML) and thus they are unfit for human consumption. High value of bioaccumulation factor (BAF) observed for Mn in cassava (1.78) as well as high values of Translocation Factor (TF) observed in sweet potatoes for cadmium (2.0) and copper (1.47); in cocoyam for cadmium (1.2) and also in cassava for chromium (1.25) and zinc (1.13) suggest that the affected tubers were

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potential hyperaccumulators. The variation in the parameters determined were found to be statistically significant ($p < 0.05$) as determined by one way analysis of variance.

Keywords: Heavy metals; tubers; bioaccumulation factor; translocation factor; pixe; enyigba; lead-zinc derelict.

1. INTRODUCTION

Tubers are fleshy underground food-storing plant. It is a fleshy swollen part of a root such as a dahlia root or of an underground stem such as a potato that stores food over winter and produces new growth in spring. In Nigeria like most African countries, tubers form the major staple for most families. Young plants developing from tuber buds are nourished from starch stored in the tubers until mature enough to develop root systems. Heavy metals from the environment enter into plants via either the roots and/or foliar surfaces [1]. Metal uptake and accumulations are affected by some factors such as soil pH, metal solubility, conductivity, stages of plant growth, plant species, soil type and fertilizers [2]. Different plants have different capacity to absorb and accumulate heavy metals which often leads to contamination of the food chain and as a result causes varying degrees of illness based on acute and chronic exposures. There are three major bases for classifying a plant as a hyperaccumulator for heavy metal(s). First is when Translocation factor (TF) is greater than one ($TF > 1$). Translation Factor is shoot/root quotient (the ratio of concentration of heavy metal in the shoot to the root). Second is when Bioaccumulation factor (BAF) is greater than one ($BAF > 1$). Bioaccumulation factor is the extraction coefficient which is the level of heavy metal in the shoot divided by total level of heavy metal in the soil. Extraction coefficient gives the proportion of total heavy metal in the soil which is taken up by the plant shoot/aerial part of the plant [3]. Third is when there is higher level of heavy metal of 10 – 500 times the levels in normal plants (uncontaminated plants). The percentage of heavy metals accumulated by plant varies according to the heavy metals involved and previous studies have shown plants accumulating more than 1000 mg/kg of dry weight for nickel, copper, cobalt, chromium or lead and more than 10,000 mg/kg for zinc or manganese [4]. Capacity for accumulation is as a result of hypertolerance, (*phytotolerance*) which came from adaptative evolution of plants in hostile environments along multiple generations [5].

The present study was carried out within the vicinity of a lead-zinc derelict mine, Enyigba located 14 Km South of Abakaliki (latitudes 4°20' and 7°00'N and longitudes 5°25' and 9°35'). Enyigba is a rural area with sparse rural population of farmers who cultivate their crops especially tubers around the mine waste. More importantly, the community serve as food basket of Abakaliki city as farmers regularly supply their farm produce to the town. Mothers often fry or boil potatoes and white yam which is served as breakfast to young school children and adults. Cocoyam is usually boiled overnight and served as breakfast at the family table. Cassava is commonly prepared by fermenting it into *"fufu"* or further drying and frying to give what is known as *"garri"* and itthey are served as a daily staple all over Nigeria. The contamination status of many edible plants especially tubers from Enyigba is yet to be established. Previous works in Enyigba [6,7] focused on soils and few plants respectively. However, this present study focused on two aims. The first one was to determine the Pollution Index or the contamination status of the soil where the tubers are planted. The second one was to use bioaccumulation factor (BAF) and translocation factor (TF) to evaluate the metal uptake abilities of the investigated tubers which gives a clue to their phytoremediation potentials [8].

2. METHODOLOGY

2.1 Collection of Soil Samples

Composite top-soil and sub-soil samples were collected, 20 m apart at 0-30 cm (n=6) and at 60-90 cm (n=6) depths respectively. The former represents the top soil while the later represents the sub soil [9]. The soil samples were air-dried, ground mechanically with stainless steel soil grinder and sieved to obtain < 2 mm fraction. 30 g sub-sample was drawn from the bulk (< 2 mm fraction) and reground with laboratory mortar and pestle to obtain < 200 µm fraction. The sample was further dried in an open inert vessel in a muffle furnace at 105°C for 2 hours so as to remove soil moisture, after which the samples were cooled in desiccators [10].

2.2 Collection of Tubers Samples

Samples of *Colocosia and Xanthosama* (Cocoa yam), *Manihol eaculenhis* (Cassava), *Dioscoreu rolundata* (White Yam) and *Ipomoea batatas* (Sweet potato) were collected, authenticated, labelled and stored in pre-treated storage containers. For this work the fleshy underground part is regarded as the root. The roots and leaves of the tubers were separated in each case and the components were cut into pieces. The plant tissues were cleaned to remove dust, soil and other particles by putting them through a three step washing sequence [10]. First they were washed with water, then with P-free detergent and followed by de-ionized water. The moisture and water droplets were removed with the help of blotting papers. The samples were air dried, and placed in a dehydrator at approximately 80°C for 48 hours. This was followed by mechanical grinding with the aid of an agate mortar which was done with utmost care to avoid contamination with the elements to be analyzed. The ground tissues were further dried at 65°C in an oven to obtain a constant weight upon which to base the analysis [11,12].

2.2 PIXE Analyses of Heavy Metal in Plant and Soil Samples

The process begins with pelletization of the samples using CAVER model manual palletizing machine at a pressure of 6 - 8 torr to give 13mm pellets. Proton Induced X-ray Emission (PIXE) spectrometer component of 1.7 MV NEC model Tandem Nuclear Accelerator was used for the metal determination. Dried plant and soil samples were analyzed for heavy metal contents using a procedure similar to one described by [13] This was done by irradiating the target sample in PIXE spectrometer for 3 minutes in a vacuum chamber with 3 MeV protons (beam currents of 10-70 nA and beam diameter of 4 mm). Bombardment with ions of sufficient energy (MeV protons) produced by an ion accelerator, will cause inner shell ionization of element atoms in the sample. Outer shell electrons drop down to replace inner shell vacancies and only certain transitions are allowed. X-rays of a characteristic energy of the element are emitted. An energy dispersive detector is used to record and measure these X-rays [14]. The X- rays generated from the target were measured with two Si/Li detectors and the corresponding metal concentration was ascertained by means of incorporated computer device.

In addition to concentration of metals in the soil samples, soil pH; percentages of sand, silt and clay as well as percentages of organic matter were determined using Orion 920A pH meter Hydrometer method and Walkley and Black method respectively [15-18].

2.3 Statistical Analysis and Calculation of PI, BAF and TF

Composite samples of the investigated soils and plants were assayed and analyzed individually in triplicates. Data generated from PIXE were reported as mean + Standard Deviation. One way analysis of variance (ANOVA) and Fisher's Least Square Difference (LSD) were used to determine significant difference within and between groups, considering a level of significance of less than 5% ($p < 0.05$) by using Minitab 2007 version 13.6 statistical software [19]. PI, BAF and TF were calculated from the generated data [20-45].

Single Pollution Index (PI) was used to determine the level of the pollutant in the environment by comparing the concentration of the pollutant to the allowable maximum limit of regulatory bodies such as World Health Organisation (WHO) [46]. Values of PI < 1 indicate that the soil or plant material is not yet contaminated whereas PI < 1 indicates pollution. On the other hand, PI = 1 reveals a critical state which makes the involved plant useful for environmental monitoring [7]. Mathematically, PI is expressed as

$$PI = C_{soil} / C_{USEPA\text{-}standard}$$

Where PI is the individual pollution index of study metal; C_{soil} is the Concentration of the metal in soil or plant. $C_{USEPA\text{-}standard}$ is the value of the regulatory limit of the heavy metal by USEPA [47]. The overall Pollution status of the investigated soil was determined with the formula

$$PI_{soil} = \sqrt{\frac{(PIave)^2+(PImax)^2}{2}}$$

Where PI_{soil} is the overall Pollution Index of the soil, PI_{ave} is the average Pollution indices of different soil samples analysed while PI_{max} is the maximum PI recorded [48].

Bioaccumulation factor (BAF) is the ability of the plant to accumulate the heavy metals with respect to the metal concentration in the ecosystem [9]. For a plant to be an efficient phytoremediation tool in the contaminated soil, the BAF have to be higher than one. Bioaccumulation factor is defined as a ratio of metal concentration in plant shoot to extractable concentration of metal in the soil. It is the progressive increase in the amount of metal in a living plant because the rate of intake exceeds the plant's ability to remove the substance from the body [49]. Mathematically, BAF is expressed as

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

Translocation factor is the plant's ability to translocate heavy metal from root to harvestable aerial part. When TF >1 is obtained, it indicates a preferential partitioning of metals from soil to root and from root to shoot respectively. Mathematically, TF is expressed as

$$TF = C_{shoot} / C_{root}$$

Where C_{shoot} and C_{root} is the concentration of metal in shoot and root respectively.

3. RESULTS

Tables 1 and 2 present the mean concentration of heavy metals and the properties of the soils of Enyigba derelict respectively. Table 3 presents the mean concentration of heavy metals in the investigated tubers while Tables 4 and 5 showed their corresponding translocation and bioaccumulation factors respectively.

4. DISCUSSION

4.1 Heavy Metals in Soil

From Tables 1 and 2, the mean concentrations of the heavy metals in the soil samples were observed to decrease with depth. It is a known fact that when concentrations of pollutants are significantly higher in top soil than sub soil, it suggests that the source of the pollutants is of anthropogenic origin [15]. The obtained results revealed that heavy metal concentration in the Enyigba derelict mine decrease in the order Pb > Zn > Cu > Mn > Cd > Ni > As> Cr in topsoil and Zn > Cu > Mn > Pb > Ni > Cd > As > Cr in the subsoil but only Pb, Cu and Zn were significant at $p < 0.05$. These results are at variance both in trend and values obtained for each metal with the earlier results of previous work [7,21]. The difference is due to the sensitivity of PIXE technique used in this present study compared to AAS technique used by other researchers in the previous work. In addition, environmental dependent parameters such as metal concentrations and pH, are never static due to changes in temperature, volume and nature of human perturbation on the natural ecosystem. Three of the heavy metals namely Pb, Ni and Cd exceeded the US-EPA Regulatory Limits [22] and their pollution indices were in the order, Pb (2.7) > Cd (1.5) > Ni (1.1). The overall pollution status of soils was 1.96 which confirms that Enyigba soils are already polluted with heavy metals [7,21]. Soils with PI > 1are known to affect plants, animal and ultimately distort the food chain they support [15].

The percentage of organic matter content of Enyigba soil was 1.34 % while the soil pH was 6.5. The extent of soil pollution with heavy metals and subsequent uptake by crops depend on organic matter content among other factors. Organic matter and pH values have been reported to independently and associatively influence the concentrations of heavy metals in soils. Soils with pH around 7.0 have higher availability of nutrient elements such as Mg, Ca, K, N and S, while metals such as Pb, Cu, Mn and Zn are less soluble and therefore less available at about this pH [23]. The soil parameters results were comparable to another similar research on lead in soil and vegetations [24]. On the basis of organic matter and pH, the results however indicate that the soils from the study areas can support agricultural activities efficiently in agreement with similar findings [21,25,26]. However on the basis of heavy metal indices, Enyigba soil was already polluted.

4.2 Heavy Metal in Tubers

Lead: The concentration of Pb in the roots of white yam (0.41 mg/kg) and cassava (0.32 mg/kg) exceeded the WHO maximum limit (Table 3). Lead is the main cause for concern in this work because it is highly toxic at minute concentration and can be harmful to man who may consume the effected tubers. Presence of lead affects the gastrointestinal tract, kidneys, and central nervous system. Children exposed to lead are at risk for impaired development, lower IQ, shortened attention span, hyperactivity, and mental deterioration while, while adults usually experience decreased reaction time, loss of memory, nausea, insomnia, anorexia, and weakness of the joints

Table 1. PIXE Mean Concentrations (mg/kg) of heavy metals in soil of Enyigba derelict and their pollution indices

	Enyigba (n = 3)				
Metal	**Topsoil**	**PI**	**Subsoil**	**PI**	**US-EPA**
As	4.8 ± 1.8	0.06	2.12 ± 1.6	0.03	75*
Cd	126.0 ± 42	1.5	28.8 ± 6.2	0.34	85
Cu	812.2 ± 141.2	0.19	322.2 ± 12.2	0.07	4300
Cr	2.12 + 0.2	–	1.02 + 0.2	–	
Mn	424.0 ± 50.4	–	120.0 ± 44.0	–	
Ni	82.6 ± 22.0	1.1	34.8 ± 8.2	0.46	75
Pb	1116.8 ± 43.2	2.7	91.7 ± 16.7	0.22	420
Zn	995.2 ± 82.4	0.13	322.0 ± 62.4	–	7500

** Values refer to metal concentration in typical soils [20], PI = Pollution index*

Table 2. Properties of soil from Enyigba mine

Properties	**Enyigba Mine (n = 3)**
Sand (%)	61.28 ± 5.2
Silt (%)	7.12 ± 0.8
Clay (%)	31.60 ± 2.6
Organic Matter (%)	1.34 ± 0.5
pH	6.5 ± 0.29

when exposed to lead (ATSDR, 1993). In many plants, Pb accumulation can exceed several hundred times the threshold of WHO ML (Wong, [47]). Tubers usually show ability to accumulate large amounts of lead without visible changes in their appearance or yield. In this work, Pb was observed to have accumulated more in the roots than in the leaves unlike the result of Muhammad et al. (2008) where higher concentration of Pb was observed in the leaves of studied plants than in other roots. The reason may have to do with the difference in pH of the soils and the percentage of organic matter which are known to promote Pb uptake [27].

Arsenic: Arsenic was detected in all the investigated tubers except in the leaves of cocoyam and white yam from Enyigba mine (Tables 3). The concentration of As exceeded WHO maximum Limit (WHO ML) in white yam and sweet potato. Low BAF and TF of As were observed in all the studied plants (Tables 4 and 5). This suggests that though As level was high in white yam and potato but they are ineffectiveness in accumulating As from the soil to the aerial part of the plants. Immobilizations As and other metals depend on soil pH, amount of organic matter, and texture Soil. It is a known fact [28] that arsenate [As(V)] absorbed to soil at pH 4-7 while arsenite [As(III)] at pH 7-10. But since speciation study was not carried out in this work, pH may be responsible for the low uptake of As by the tubers.

Cadmium: Cadmium accumulated more in the leaves than in the root of the some of the tubers (Table 3). Cadmium in cassava (0.01 mg/kg) is at critical point which means that the concentration was of the same value as WHO ML but it exceeded in cocoyam (0.12 mg/kg). Environmentally speaking cassava can be effectively used for biomonitoring of Cd [29]. Although Low BAF values of Cd were observed generally, however high TF of Cd observed were in the order: sweet potato (2.0) > cocoyam (1.2) > cassava (1.0) (Tables 4 and 5). A key trait of metal hyperaccumulators is the efficient metal transport from roots to shoots, characterized by the TF being greater than one [30]. Based on the foregoing, the TF >1 observed in sweet potato and cocoyam means they are potential Cd-hyperaccumulators [31].

Copper: Concentration of Cu in roots were more than in the leaves (Table 3). Low BAF values were observed in all the investigated tubers which suggest that none of the tubers was contaminated by Cu (Table 4). Normal concentration of copper in plant tissues is approximately 5-25 mg/kg [32]. The variation in copper accumulation may be related to soil pH, soil moisture, the season of the year, individual genotypic variability and varying degrees of soil contamination [33]. High TF values were observed in sweet potato (1.47) and white yam (1.05) which showed that the transport of copper from root to leaves was effective and hence sweet potato and white yam are potential Cu-accumulators [30,32].

Table 3. Concentrations of heavy metals in common tubers in Enyigba derelict (n=3)

			Heavy metals concentration (mg/kg)							
Botanical name	**Common name**	**Plant parts**	**Pb**	**As**	**Cd**	**Cu**	**Cr**	**Zn**	**Mn**	**Ni**
Colocosia &	Cocoyam	Leaves	0.01	ND	0.12	12.4	0.02	56.22	34.44	12.24
Xanthosama		Root	0.22	0.02	0.10	24.10	0.12	82.12	54.62	22.82
Manihot	Cassava	Leaves	0.12	0.38	0.10	0.82	0.40	122.12	342.18	34.66
esculentus		Root	0.32	0.54	0.10	6.42	0.32	108.32	624.26	34.02
Dioscorea	White yam	Leaves	0.21	ND	ND	42.12	0.01	24.18	18.46	12.42
rotundata		Root	0.41	0.12	0.02	40.12	0.01	62.43	82.62	14.86
Ipomoea	Sweet	Leaves	0.04	0.02	0.04	62.12	0.21	46.12	28.12	8.24
batatas	potato	Root	0.12	0.14	0.02	42.24	0.18	74.60	84.90	10.24
WHO/ FAO	Maximum	Limit	0.3	0.1	0.1	73	0.05	100	500	67

Table 4. Translocation factor of heavy metals of common tubers from Enyigba derelict

Botanical name	**Common name**	**Pb**	**As**	**Cd**	**Cu**	**Cr**	**Zn**	**Mn**	**Ni**
Colocosia and Xanthosama	Cocoyam	0.05	-	1.2	0.52	0.17	0.68	0.63	0.54
Manihot esculentus	Cassava	0.38	0.70	1.0	0.13	1.25	1.13	0.55	1.01
Dioscorea rotundata	White yam	0.51	-	-	1.05	1.0	0.39	0.22	0.84
Ipomoea batatas	Sweet potato	0.33	0.14	2.0	1.47	1.17	0.62	0.33	0.81

Table 5. Bioaccumulation Factor of Heavy Metals of Common Tubers from Enyigba Derelict

Botanical name	**Common name**	**Pb**	**As**	**Cd**	**Cu**	**Cr**	**Zn**	**Mn**	**Ni**
Colocosia and Xanthosama	Cocoyam	0.000	0.003	0.001	0.032	0.045	0.105	0.164	0.299
Manihot esculentus	Cassava	0.000	0.132	0.001	0.006	0.229	0.734	1.777	0.585
Dioscorea rotundata	White yam	0.000	0.017	0.000	0.073	0.006	0.077	0.186	0.231
Ipomoea batatas	Sweet potato	0.000	0.023	0.001	0.092	0.127	0.086	0.208	0.158

Chromium: Levels of Cr was higher than WHO ML in all the investigated tubers except white yam (Table 3). While low BAF was generally observed, the TF was found to decrease in the order: cassava > sweet potato > white yam > cocoyam in the tubers (Table 4 and 5). High TF values of Cr in cassava (1.25) and sweet potato (1.17) suggest that translocation of Cr was effectively made to the shoot from root and hence they are Cr hyper-accumulators [31,34,35,36].

Zinc: Mean concentration of Zn was observed to have exceeded WHO ML only in cassava (Table 3). The TF value of cassava from Enyigba mine was 1.25 and cocoyam from Abakaliki urban was 1.43. TF>1means that translocation of Zn effectively from root to shoot was effective [31,34,35,36]. Among all the studied metals, Zn is the least toxic and it is an essential element in the human diet as it is required to maintain the proper functions of the immune system, normal brain activity and is fundamental in the growth and development of the foetus [37]. Zinc deficiency in the diet may be more detrimental to human health than excess Zinc in the diet [38,39]. Zinc shortage causes birth defect and anaemia, stomach cramps and vomiting and Skin irritation etc. Although the average daily intake of Zinc is 7-16.3 mg Zn/day, the recommended dietary allowance for it is 15 mg Zn/day for men and 12 mg Zn/day for women [40]. Concentrations of Zn in the roots were higher than in the leaves. The TF value of Zn was above 1 in cassava which suggests preferential accumulation of zinc in the leaves than in the root.

Manganese: Levels of Mn was higher than WHO ML in all the investigated tubers except cassava (Table 3). Manganese ions function as cofactors for a number of enzymes in higher organisms, where they are essential in detoxification of superoxide free radicals. Manganese is also a required trace mineral (essential trace nutrient) for all known living organisms. In larger amounts, it can cause a poisoning syndrome in man, with neurological damage which is sometimes irreversible [41]. Human body [42] contains about 10 mg of manganese, which is stored mainly in the liver and kidneys. In the human brain the manganese is bound to manganese metalloproteins most notably glutamine synthetase in astrocytes [43]. Low BAF was observed and TF >1 was observed in cassava which makes cassava a potential Mn-hyperaccumulator

Nickel: Nickel concentrations in all the studied tubers were below WHO ML (Table 3). This suggests that all the studied tubers were free of Ni contamination and therefore are safe for human consumption. Nickel at this level is not a known toxic metal to human health [44]. Excess and deficiency of Ni in tubers are detrimental to human health [45]. Deficiency of Nickel have been linked with hyperglycemia, hypertension, depression, sinus congestion, fatigue, reproductive failures and growth problems in humans, while excess intake of Ni leads to hypoglycemia, asthma, nausea, headache, and epidemiological symptoms like cancer of nasal cavity and lungs. The prescribed safety limit of Nickel is 3 to 7 mg/day in humans. Low BAF and TF were observed except TF of Zn in cassava (1.13) which is greater than one (Tables 4 and 5). Cassava based on TF > 1, is a potential Ni-hyperaccumulator [31].

5. CONCLUSION

From this present work, Concentrations of Cd and Cr in cocoyam; Pb, As, Cr, Zn and Mn in Cassava; Pb and As in white yam in addition to As and Cr in sweet potato all exceeded the WHO ML and therefore are unfit for human consumption. Their simple pollution indices (the ratio of their concentrations to WHO ML) were all above one [45,46,47]. To avoid food crisis, edible plants such as the studied tubers are not considered best to be employed as phytoremediation agents. From this study and on the basis of TF >1, cocoyam hyperaccumulated of Cd just as cassava is a potential hyperaccumulator of Cd, Cr, Zn and Ni. Similarly, white yam is a potential hyperaccumulator of Cu and Cr while sweet potato is a hyperaccumulated Cd, Cu and Cr. On the basis of BAF > 1, cassava is a potential hyperaccumulator of Mn. The implication of all these is that while consumption of these plants may likely result to metal toxicity in man, these plants can be useful in cleaning up the contaminated mine derelict.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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