



Distribution of Some Trace Metals in Bitter Leaf (*Vernonia amygdalina*) Obtained in Oil Exploration Area of Ibeno, Nigeria

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Abstract: Levels of some trace metals (Fe, Zn, Cu, Mn, Cr, Ni, Pb and Cd) in *Vernonia amygdalina* (bitter leaf) obtained in Ibeno, Akwa Ibom State, Nigeria were quantified using standard procedures. Soil samples as well as roots and leaves samples of the plant were collected from three locations (Okoroitak, Inua Eyet Ikot, Iwuo Okpom) in Ibeno, where intensive oil exploration activities are carried out. Samples were equally collected from Mbiabam Ibiono where there is no oil exploration, to serve as the controls. The results revealed that the metal levels in the soil ranged from < 0.06 mg/kg Cd to 8316.72±3.09 mg/kg Fe, those in the roots of the plant ranged from < 0.001 mg/kg Cd to 450.48±3.08 mg/kg Fe and those in the leaves, ranged from < 0.001mg/kg Cd to 1650.36±5.46 mg/kg Fe. The corresponding metal levels in the control samples were generally low compared to those of the study area. This could be attributed to the absence of oil exploration activities in the control area. Analysis of the metals pollution status of the plant using transfer factor (TF) revealed the trend: Cu > Pb > Zn > Cr > Mn > Ni > Cd > Fe. The levels of all the investigated metals in the analyzed *V. amygdalina* samples were lower than the WHO maximum permissible limits of the metals in plants and hence, the suitability of the analyzed *V. amygdalina* for human consumption in whatever form, at the time of the study.

Keywords: Quantification, Trace Metals, Contamination, *Vernonia amygdalina*, Oil Exploration, Ibeno

1. Introduction

In recent years, metal pollution of soils has posed a serious threat to environmental ecosystems and human health [1, 2]. This issue has received increasing attention because of the bioaccumulation, persistence, and toxicity of metals [3, 4]. Trace metal pollution and the resulting health effects present some of the biggest challenges currently affecting the world. Activities of oil exploration and other industries result in pollution through gas flares, constant oil spills, and industrial effluence which affect both the aquatic and terrestrial ecosystem and has impacted negatively on surface water quality around the area resulting in the release of large amounts of hydrocarbons and trace metals into the terrestrial

and aquatic environment [5, 6]. The major pollutants from industrial discharge have been shown to be trace metals and organic substances. Soil pollution due to oil exploration in the Niger Delta of Nigeria has created consciousness of the pollution of the environment. Soil whether in urban or agricultural areas represents a major sink for metals released into the environment from a variety of anthropogenic sources. Once in soil, some of these metals will be persistent because of their fairly immobile nature. Plant uptake is one of the major pathways by which metal in the soil enter the food chain. Moreover, metals can be transported long distances and transferred to soils by atmospheric deposition, wastewater, and the discharge of solid wastes containing metals [7]. Thus, soils around industrial sites are particularly prone to act as sinks for metals. The degree of soil metal

contamination can pose a hidden danger to human health via many different ways (e.g., the oral ingestion pathway, dermal contact pathway, and inhalation pathway) [8, 9].

A person's age is also a significant factor that should be given more consideration. Compared with adults, children and infants are more likely to be affected because of their behavioural characteristics (e.g., outdoor activities, mouthing non-food objects, and sucking their hands or fingers) and are at greater risk of exposure to contaminants in soils [10–12]. However, for industrial soils, oral ingestion of soil particles plays an important role in exposing humans to metals [9, 13]. Thus, it is more appropriate to evaluate the human health risk posed by metals in industrial soils based on direct exposure pathways because there are significant differences in the bioaccessibility of toxic elements via different exposure routes [14, 15]. The understanding of the behaviour of trace metal in soil-plant system seems to be particularly significant. The sources of trace metal in plants are their growth media (air, soil, nutrients) from which trace metals are taken up by roots or foliage. Plants growing in a polluted environment can accumulate the toxic metals at high concentration causing serious risk to human health when consumed [16]. There is considerable variability in actual uptake by plants of these elements from soil depending on such properties as pH and organic matter content, cationic exchange capacity, binding to different soils components and the plant species involved [17].

It has been the interest of the public to know whether vegetables, fruits and food crops cultivated in polluted soils are safe for human consumption especially now that the environmental quality of food production are of major concern [18]. The food-chain plants might absorb large quantities of trace metal to become a potential health hazard to consumers. The uptake of metal levels by roots depends on the nature of the metal, soil physicochemical properties and plant species [19]. Consequently, metal mobility and plant availability are very important when assessing the effect of soil contamination on plant metal uptake, as well as translocation and toxicity or ultra-structural alterations [20, 21]. Trace metal contamination of food items is one of the most important considerations in food quality assurance [22, 13]. Trace metal contamination in vegetables cannot be underestimated as these food items are highly nutritious, largely consumed and form important components of human diet. International and national regulations on food quality have lowered the maximum permissible levels of toxic metals in food items due to an increased awareness of the risk, these metals pose to food chain contamination.

Vernonia amygdalina (bitter leaf) is one of the most widely consumed perennial leafy vegetables and herbal plants in Ibeno. *V. amygdalina* is a very important protective food and useful for the maintenance of health, prevention and treatment of various diseases. Some principal chemical constituents found in bitter leaf are a class of compounds called steroid glycosides-type vernonioside B1—these

chemical substances possess a potent anti-parasitic, anti-tumor, and bactericidal effects. Bitter leaf is mainly employed as an agent in treating schistosomiasis, which is a disease caused by parasitic worms. It is also useful in the treatment of diarrhea and general physical malaise. Bitter leaf helps to cleanse vital organs of the body like the liver and the kidney, and is used in the treatment of skin infections such as ringworm, rashes and eczema. However, bitter leaf and other vegetables contain both essential and toxic metals over a wide range of concentrations [23].

Anthropogenic activities such as oil exploration in the area, may introduce trace metals into the plant and the soil where it grows. Trace metals may occur naturally in soils. Some are essential micronutrients for plants and animals and are thus important for human health and food production. At elevated levels, all trace metals, however, become potentially toxic. The uptake and bioaccumulation of metals in the plants are influenced by a number of factors such as climate, atmospheric depositions, background geology and soil composition, the vicinity of roads and industry, as well as the degree of maturity of the plants at the time of harvest. Certain trace metals are known to have long half-lives and majorities are not easily biodegradable. They can accumulate in different parts of the body, remain for long periods and exert harmful effects, especially at certain critical stages of life, such as pregnancy or childhood. The consumption of plants rich in trace metals can cause various clinical and physiological adverse conditions in the consumers. For example, exposure to high doses of trace metals like Pb, Cd and Cr is related to an increased prevalence of upper gastrointestinal cancer. The determination of the levels of some trace metals in *V. amygdalina*, a widely consumed perennial plant in an oil exploration area of Ibeno, in order to ascertain the suitability of the plant for human consumption is therefore quite necessary.

2. Materials and Methods

2.1. Study Area

Ibeno is located in the southern part of Akwa Ibom State. It is bounded in the West by Eastern Obolo Local Government Area, to the North by Onna, Esit Eket and Eket, to the South by Atlantic Ocean. Ibeno town lies on the eastern side of the Qua Iboe River about 3 kilometres (1.9 miles) from the river mouth and is one of the largest fishing settlements on the Nigerian coast. Ibeno lies in the Mangrove Forest Belt of Niger Delta region of Nigeria. The area has rain throughout the year with the peak between May and September. The climatic condition in Ibeno is favourable all year round for agriculture. The prime occupation of the people is fishing, farming and petty trading. The presence of oil exploration activities by oil giant Exxon Mobil and other service companies influence agricultural activities in both upstream and downstream [24]. The study area is shown in Figure 1.

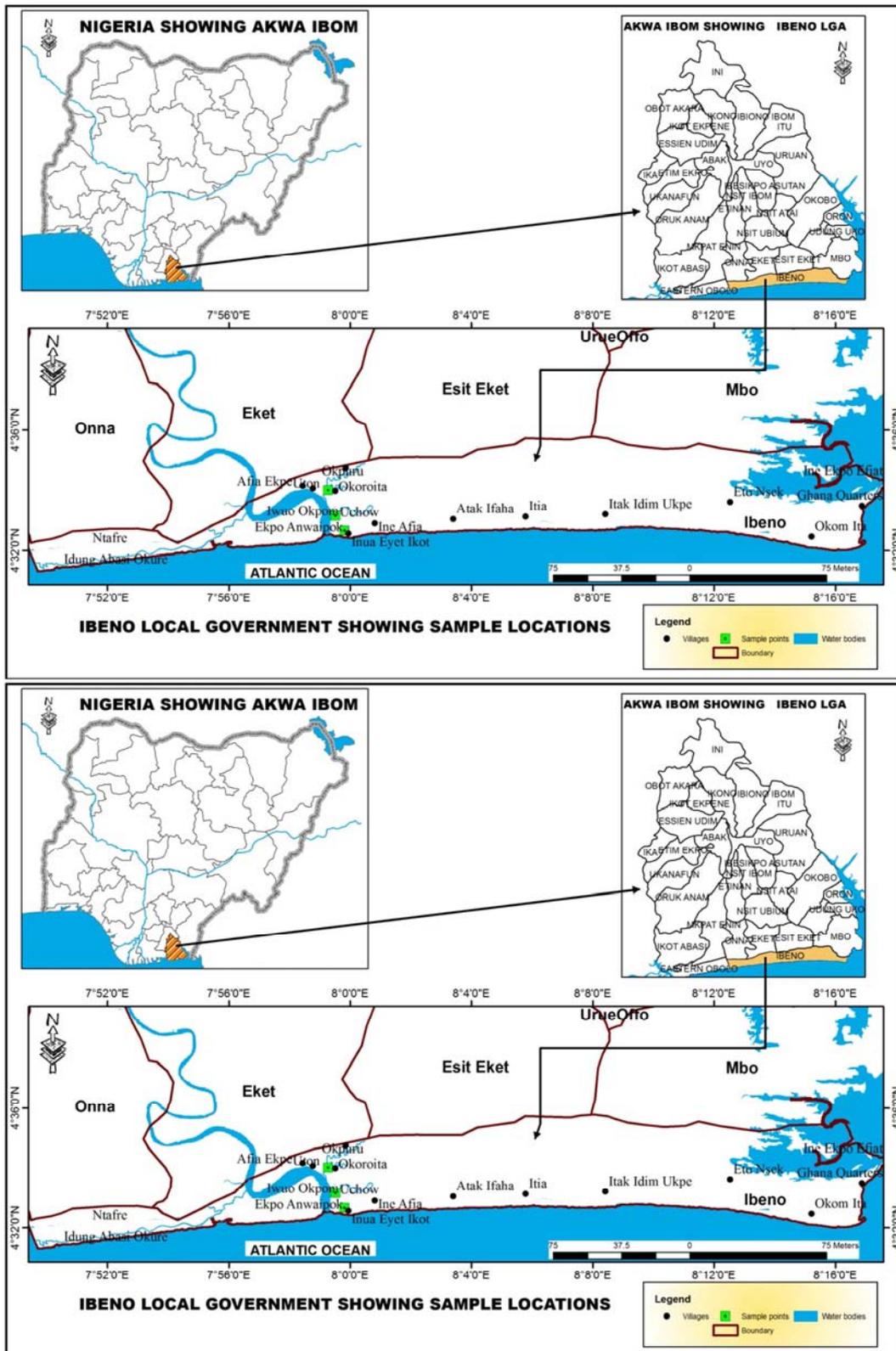


Figure 1. The study area.

2.2. Samples Collection

The samples (roots and leaves of *Vernonia amygdalina* as well as soil from the vicinity of the plant) were collected from three locations (Okoroitak, Inua Eyet Ikot, Iwuo Okpom)

in Ibiono, where intensive oil exploration activities are carried out. Samples were equally collected from Mbiabam Ibiono in Ibiono Ibom Local Government Area, Akwa Ibom State, where there is no oil exploration, to serve as the controls At each sampling location, samples were randomly collected

and then pooled together to obtain composite samples. The samples were appropriately labeled, stored in a clean polythene bags and transported to the laboratory.

2.3. Samples Preparation and Digestion

The soil samples were dried in oven, crushed and sieve through 100 μm nylon mesh to remove extraneous matter such as pieces of brick, paving stone and other debris. An exact amount (1 g) of the sieved soil samples was weighed into digestion flash and 20 ml of nitric acid added, together with the addition of 10 ml of perchloric acid and a drop of concentrated sulphuric acid. The mixture was allowed to stand for 30 minutes in the digestion chamber until the colour turns white. The samples were allowed to cool and 30 ml of distilled water was added and filtered with filter paper. The filtrate was then diluted to 50 ml solution with distilled water. The digest solution was stored in a sample bottle ready for analysis. Equally, the plant samples (leaves and roots) were chopped into small pieces and oven dried at 80°C till constant weight was achieved. The dried samples were ground with a mortar and sieved into a polythene bottle previously rinsed with deionized water. The plant samples were digested with HNO_3 and HClO_3 in the ratio of 5:1 until a transparent solution was obtained. The digest was cooled to room temperature, filtered and transferred quantitatively to 50 and 25 ml volumetric flasks respectively and made up to volume with deionized water and kept in a clean plastic vials before analysis.

2.4. Determination of Trace Metals in the Soil and *Vernonia amygdalina* Samples

The trace metals (Fe, Zn, Cu, Mn, Cr, Ni, Pb and Cd) levels in the soil and *V. amygdalina* Samples were determined by atomic absorption spectrophotometer (AAS) (model: Varian spectra 100, Australia). The AAS works in the physical process involving absorption of light at a wavelength specific to that element by free atoms of that element. The AAS was calibrated with standard solution for each element. The appropriate lamps and correct wavelength of each element were used.

2.5. Determination of Physicochemical Parameters of Soil Samples

The physicochemical parameters of the soil samples were determined using standard procedures. Electrical conductivity was determined using conductivity meter. The meter was switched on and the conductivity electrode immersed into the partly settled suspension of the each soil sample, the "CND" button pressed and the readings recorded.

Nitrate determination was done by brucine colometric method. A total of 10 ml aliquot of the soil extract was transferred into a 25 ml volumetric flask, 2 ml of brucine reagent were added and 10 ml of concentrated sulphuric acid (H_2SO_4) were also added. The flask was set in cold water for about 5 minutes and the volume made up to the mark with distilled water. The absorbance was measured at 470 nm [25].

Sulphate determination was done by turbidimetric method.

A total of 10 ml of the sample aliquot was transferred into a 25 ml volumetric flask. Distilled water was added to bring the volume to approximately 20 ml. Exact amount of 1 ml of gelatin BaCl_2 reagent was added and the solution made up to the mark with distilled water, mixed and allowed to stand for 30 minutes. The percentage transmittance (% T) and absorbance was measured at 420 nm within 30 minutes [26].

Chloride (Cl) in the soil was determined by taken exact amount of 2.5 g of the sample in a conical flask and 50 ml of distilled water added and shaken on a mechanical stirrer for 2 hours and then filtered and 25 ml of the filtrate transferred into another flask and 1 ml of potassium dichromate added and then titrated with 0.026N AgNO_3 to a pink end point [27].

Exchangeable acidity (EA) was determined by taken exact amount of 2.5 g of the soil sample in a shaking bottle and 50 ml of 1N KCl added and shaken on a mechanical stirrer for 1 hour, filtered and the extract titrated with 0.02N NaOH to a permanent pink end point.

The pH of the soil sample was determined using pH meter.

The pH meter was calibrated using two pH standard solution (pH 4 and pH 10). A total of 20 g of the prepared soil sample was taken in a 50 ml beaker and 20 ml distilled water added and allowed to stand for 30 minutes while stirring occasionally with a glass rod. The pH electrodes were immersed in the partly settled suspension and the readings were recorded.

2.6. Data Analyses

The generated data were processed using Microsoft Excel 2007 and Statistical Programme for Social Science (SPSS) Software Package 2013. Correlation between toxic metals in the plant and soil was established using Pearson Correlation Coefficient method. The level of significance was placed at 95%.

3. Results and Discussion

3.1. Levels and Distribution of Trace Metals in the Soil and *Vernonia amygdalina* Samples

The levels of the investigated trace metals in the soil and plant samples are presented in Figures 2 to 4. The levels of the metals in the soil samples are presented in Figure 2. That of the roots and leaves samples of the plant are as presented in Figures 3 and 4, respectively. The metal levels were higher in the soil samples than those in the roots and leaves samples of the plant, with the trend: trace metals levels in soil > trace metals levels in roots of *V. amygdalina* > trace metals levels in the leaves of *V. amygdalina*. Generally, the levels of all the metals in the soil and plant samples were higher in the study sites than in the control site. This could be attributed to the oil exploration activities taken place in the study area.

The results showed that the levels of the trace metals in the analyzed soil samples followed the pattern: $\text{Fe} > \text{Zn} > \text{Mn} > \text{Cu} > \text{Ni} > \text{Pb} > \text{Cr} > \text{Cd}$, with the values ranging from 1.22 ± 0.03 mg/kg Cd to 8316.72 ± 3.09 mg/kg Fe. The levels of most of the metals reported in the soil samples in this study

were higher than those of the corresponding metals reported in similar studies by [28–34]. However, the observed trace metals levels in the soil samples across all the locations reported in this study are all below the maximum permissible limits set by the WHO and European Union (EU).

In the roots samples of the studied plant, the levels of the investigated metals were in the order: Fe > Zn > Cu > Mn > Pb > Ni > Cr > Cd. The values ranged from 0.11±0.01 mg/kg Cd to 450.48±5.98 mg/kg Fe. As observed for the soil samples, the levels of most of the metals reported in the roots samples of the studied plant in this study were higher than those of the corresponding metals reported in similar studies by [35-38, 29]. Again, the observed trace metals levels in the roots samples across all the locations reported in this study are all below the maximum permissible limits set by the WHO and EU.

The metals levels in the leaves samples of the plant in all

the study locations ranged from 0.53±0.02 mg/kg Cd to 1650.36±5.46 mg/kg Fe. They followed the sequence: Fe > Zn > Mn > Cu > Ni > Cr > Pb > Cd. As noted for the soil samples and the roots samples of the plant, the levels of most of the metals reported in the leaves samples of the studied plant in this study were higher than those of the corresponding metals reported in similar studies by [36, 29, 38, 39]. Accordingly, the observed trace metals levels in the leaves samples across all the study locations were all below the maximum permissible limits set by WHO and EU. Thus the suitability of the plant in whatever form for human consumption.

3.2. Physicochemical Parameters of Soil Samples

The results of physicochemical parameters analyses of the soil samples obtained in this study are as presented in Table 1.

Table 1. Physicochemical parameters of the soil samples.

Sample site	pH	EC (sm ⁻¹)	SO ₄ ²⁻ (mg/kg)	NO ₃ ⁻ (mg/kg)	Cl (mg/kg)	EA (mg/kg)
OK	6.22	3.41	2064.40	608.42	4.43	1.20
IN	6.35	3.63	1838.65	700.07	2.22	1.10
IW	6.70	3.74	1690.08	624.21	2.88	0.81
MB	6.90	3.79	1187.21	589.01	3.42	1.10

OK=Okoroitak, IN=Inua Eyet Ikot, IW=Iwuo Okpom, MB=Mbiabam Ibiono (Control), EC=Electrical conductivity, EA=Exchangeable acidity.

The soil samples pH ranged from 6.22 to 6.90 which signify slightly acidic. It is known that pH serves as useful index for availability of nutrients in the soil. The potency of toxic substances presents in the soil and the physical properties of the soil have shown that the availability of trace metals is pH dependent, and so the solubility of metal cations generally increases with a decrease in pH. The pH values obtained in this study are slightly higher than the values reported by [40]. They were in agreement with those reported by [41]. Soil pH and other soil properties are especially important in soil processes responsible for solubility of trace metals in soil and their transportation. Since at low pH (acidic), metals are more bioavailable in the soil solution, the range of pH values obtained in this study will favour plant uptake of trace metal and hence toxicity problem is possible.

Electrical conductivity (EA) offers very quick and convenient way for determination of total amount of ionisable salts in soil. The EA in the soil samples recorded in this study ranged from 3.41 to 3.79 Sm⁻¹. The values are higher than those reported by [41]. The electrical conductivity may be ascribed to the presence of salts, trace metals ions or ionisable materials in the soil.

Nitrate occurs in various dissolved forms as dissolved molecular nitrogen, inorganic nitrogen as ammonia nitrite, nitrate and organic nitrogen as amino acid etc. The major source of nitrate in the soils could be bacteria and cyanobacteria which fix atmospheric nitrogen emitted from fossil fuel combustion. The nitrate levels in soil samples in this study varied from 589 to 700 mg/kg. This can enhance plant growth, quality of yield, seeds and fruits production.

Exchange acidity is the amount of hydrogen and aluminium that can be replaced from the adsorption complex by a neutral salt solution. It is usually expressed in mill equivalent per 100 g of soil. The exchange acidity in soil samples reported in this study ranged from 0.81 to 1.20 mg/kg. These values are lower than those reported by [42]. Sulphates are naturally occurring anions found in soil. About 95% of sulphur contents in the soil are contained in organic matter. Most fuels that are burnt for heat, power and transportation contain some sulphur. The sulphur escapes as sulphur dioxide gas which later dissolves in rain water and reaches the soil as sulphate-sulphur. The sulphate levels reported in this study ranged from 1187.21 to 2064.40 mg/kg. These values are higher than those reported by [42].

Chloride ions are found in great levels in soils. When chlorine picks up an electron to form an anion, it is also known as chloride ion. The chloride levels reported in soil samples in this study ranged from 2.22 to 4.43 mg/kg. The soluble chloride ions account for nearly all the chlorine available in the soils and remains mainly in solution in which the plant can take up. Chloride ions are essential micronutrients that are available in small quantities for certain photochemical reactions during photosynthesis.

3.3. Transfer Factor Analysis

Analysis of the metals pollution status of the plant using transfer factor (TF) revealed the trend: Cu > Pb > Zn > Cr > Mn > Ni > Cd > Fe. TF is the ratio of the level of a given trace metal in a plant to the level of the trace metal in soil. The physicochemical properties of soil could influence the

transfer or mobility of the metals from soil to plant. The transfer factor analysis of the investigated trace metals from soil to the plant in the three locations of the study area is as presented in Figure 5.

3.4. Correlation Analysis

Correlation analysis between pairs of the investigated metals in order to ascertain their sources in the soil revealed that some of the metals exhibited strong positive or negative correlations with each other, indicating that they are being influenced by the same sources (anthropogenic activities) of pollution in the same or opposite direction. Accordingly, some of the metals exhibited weak positive or negative correlations with each other, indicating that they are being influenced by different sources (anthropogenic activities) of pollution in the same or opposite direction. The correlation analyses of the metals are as presented in Table 2.

4. Conclusion

Based on the analyses and results, it can be concluded that the roots and leaves samples of *Vernonia amygdalina* (bitter leaf) obtained in the oil exploration area of Ibeno in Nigeria,

contained variable levels of the investigated trace metals (Fe, Zn, Cu, Mn, Cr, Ni, Pb and Cd). Similarly, the soil samples from the vicinity of the plant contained variable levels of the trace metals. The corresponding metal levels in samples from the control location in Mbiabam Ibiono where there is no oil exploration were lower compared with those in samples from the study area. The high levels of these metals in the samples obtained in the study area could be attributed to the oil exploration activities in the area. Correlation analyses indicated that the metals were being influenced by the same sources of pollution (anthropogenic activities) in the same or opposite direction, as well as by different sources in the same or opposite direction. The metals pollution status of the plant from the soil analyzed by transfer factor indicated that $Cu > Pb > Zn > Cr > Mn > Ni > Cd > Fe$. Variable levels of the investigated physicochemical parameters were equally recorded. The physicochemical properties of soil could influence the transfer or mobility of the metals from soil to plant. The good news however is that the levels of all the investigated metals in the analyzed *V. amygdalina* samples were lower than the WHO maximum permissible limits of the metals in plants and hence, the suitability of the analyzed *V. amygdalina* for human consumption in whatever form, at the time of the study.

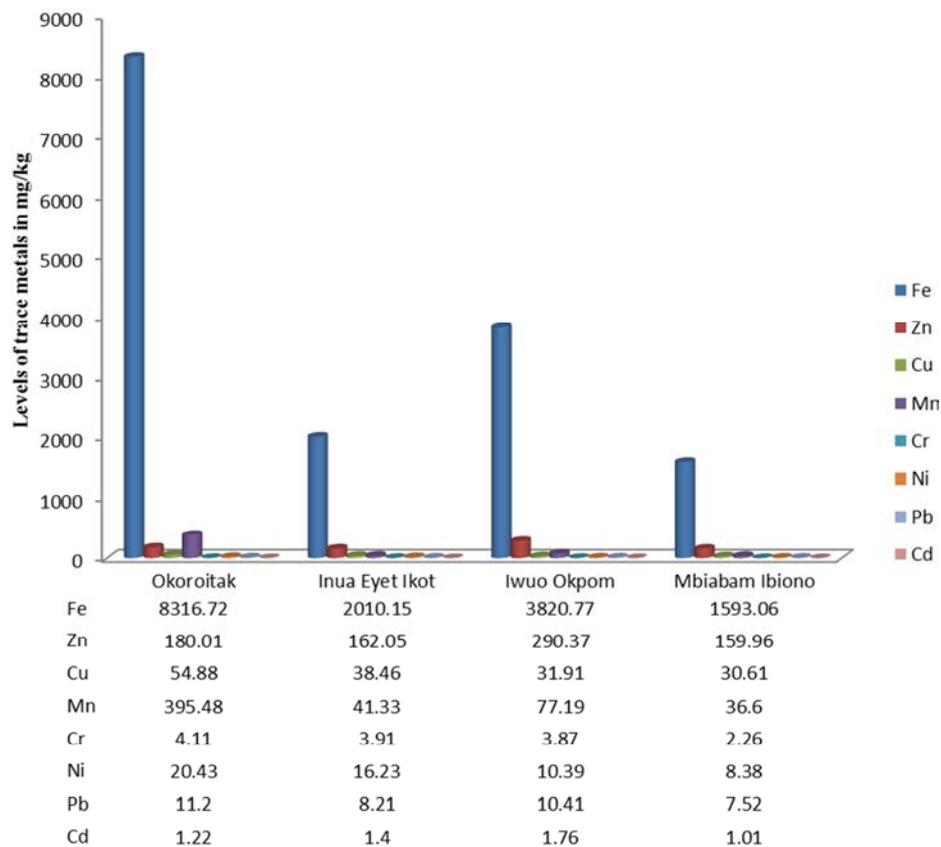


Figure 2. Levels of some trace metals in the soil samples.

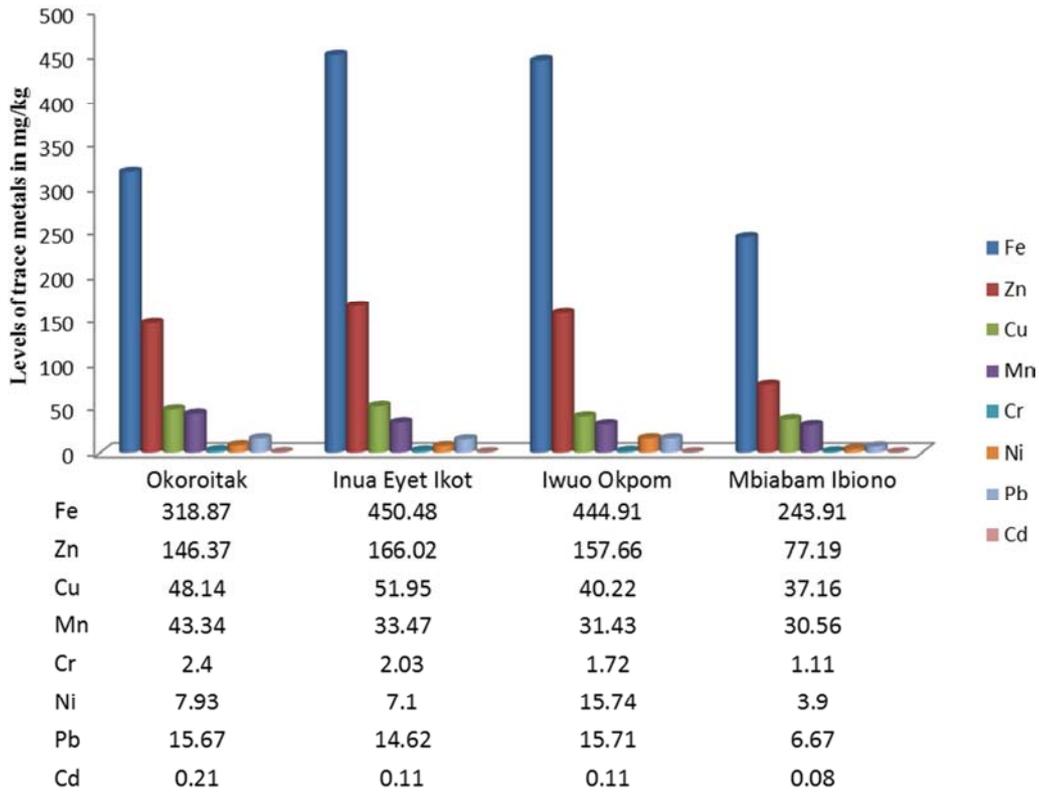


Figure 3. Levels of some trace metals in the root samples of *V. amygdalina*.

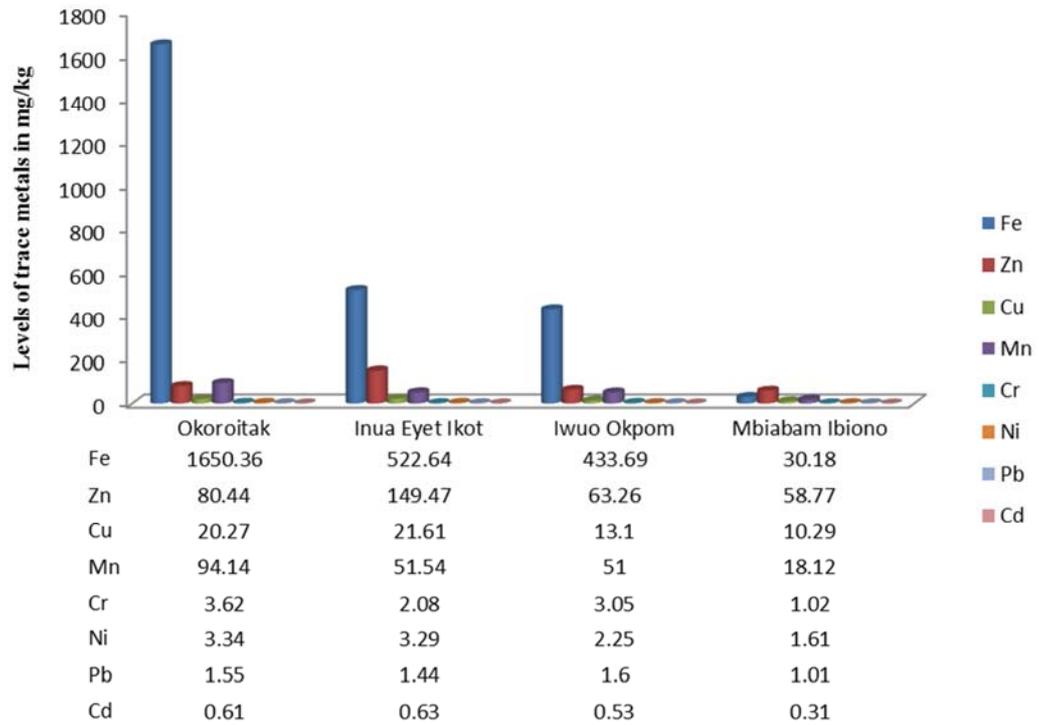


Figure 4. Levels of some trace metals in the leaves Samples of *V. amygdalina*.

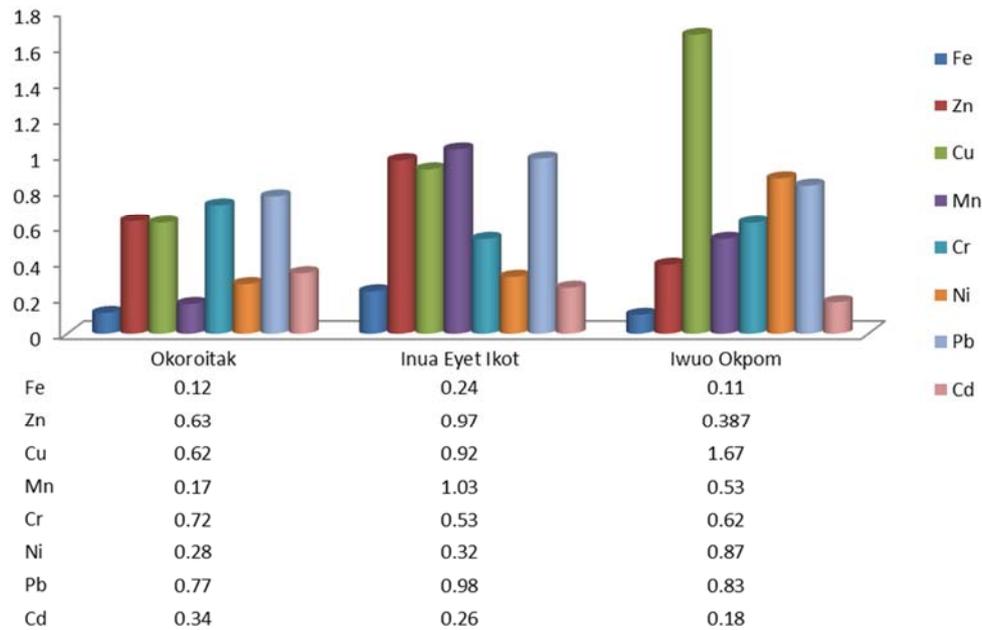


Figure 5. The Transfer Factor analysis of metals from soil to *V. amygdalina*.

Table 2. Pearson's correlation of trace metals in soil.

	Fe	Zn	Cu	Mn	Cr	Ni	Pb	Cd
Fe	1							
Zn	.120	1						
Cu	.885	-.289	1					
Mn	.977*	-.90	.938*	1				
Cr	.599	.348	.601	.496	1			
Ni	.737	-.307	.943*	.781	.741	1		
Pb	.895	.537	.636	.780	.738	.547	1	
Cd	.028	.866	-.202	-.175	.629	-.056	.455	1

*Correlation is Significant at the 0.05 level (1-tailed).

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