



Risk Assessment of Trace Metals in Roadsides *Vernonia amygdalina* Obtained in Abak, Nigeria

Emmanuel Isaac Uwah^{1,2,*}, Itohowo Ime Jonathan¹, Iboroakam Essien Udosen³

¹Department of Chemistry, University of Uyo, Uyo, Nigeria

²International Centre for Energy and Environmental Sustainability Research (ICEESR), University of Uyo, Uyo, Nigeria

³Department of Zoology, Akwa Ibom State University, Ikot Akpaden, Akwa Ibom State, Nigeria

Email address:

emmanueluwah@uniuyo.edu.ng (E. I. Uwah), aitychem@gmail.com (I. I. Jonathan), piccolosen@yao.com (I. E. Udosen)

*Corresponding author

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Abstract: Levels of some trace metals (Cu, Zn, Cd, Pb and Mn) were investigated in roadside soil and the leaves of a common perennial vegetable (*Vernonia amygdalina*). Soil and vegetable samples were collected from roadsides of three major roads in Abak (Ikot Ekpene road, Ikot Abasi road and Uyo road). The samples were digested using standard methods and analysed using Unicam 939/959 atomic absorption spectrophotometer. The results revealed that the levels of all the metals in soil samples were higher than the levels of metals in the corresponding vegetable. The metal levels in both the soil and vegetable samples were in the order: Cu > Zn > Pb > Mn > Cd. Cu, Cd and Pb with the levels of 106.71, 0.25 and 0.54 mg/kg, respectively, in plant samples respectively were higher than the WHO maximum permissible limits of 73.30 mg/kg for Cu; 0.20 mg/kg for Cd and 0.30 mg/kg for Pb. Zn (45.25 mg/kg) and Mn (0.47 mg/kg) were below the maximum permissible limits of 99.40 mg/kg for Zn and 500 mg/kg for Mn. Further analysis of the *V. amygdalina* pollution status using transfer factor (TF) revealed that Zn had the highest TF value of 0.5 while Cd recorded the lowest of 0.019. The toxic risk (hazard quotient) for adults and children consuming the *V. amygdalina* leaves showed that Cu was greater than one (1) and other metals were generally low and within safe limits. The Hazard indices were greater than 1 for both adults (1.69E+0) and children (1.94E+0). This implies that the trace metal contamination was capable of posing health hazard to consumers of this plant.

Keywords: Risk, Assessment, Trace Metals, Contamination, *Vernonia amygdalina*, Roadsides

1. Introduction

The herb known as bitter leaf (*Vernonia amygdalina*) is a shrub or small tree that can reach 23 feet in height when fully grown. Bitter leaf has a grey or brown coloured bark, which has a rough texture and is flaked. The herb is an indigenous African plant; which grows in most parts of sub-Saharan Africa. It is a medicinal plant and fresh bitter leaf is of great importance in human diet because of the presence of vitamins and mineral salts [1]. It 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 [2].

Heavy or toxic metals are trace metals which are detrimental to human health and having a density of at least five times that of water. Large quantities of pollutants such as trace metals have continuously been introduced into the ecosystems as a result of urbanization and industrial processes [3]. Once liberated into the environment through the air, drinking water, food, or countless varieties of man-

made chemicals and products, trace metals are taken into the body via inhalation, ingestion and skin absorption. If trace metals enter and accumulate in body tissues faster than the body's detoxification pathways can dispose of, then a gradual build-up of these toxins occurs. High concentration exposure is not a necessity to produce a state of toxicity in the body, as trace metal accumulation occurs in body tissues gradually and, over time, can reach toxic concentration levels, much beyond the permissible limits [4]. Higher levels of even the essential metals may lead to toxicity [5].

Trace metal contamination is a major problem of the environment (biosphere) especially of growing medium sized cities in developing countries primarily due to uncontrolled pollution levels driven by causative factors like industrial growth and heavy increase in traffic using petroleum fuels. Trace metal contamination may occur due to factors including irrigation with contaminated water, the addition of fertilizers and metal based pesticides, industrial emissions, transportation, harvesting process, storage and/or sale [2, 6].

Atmospheric depositions are captured by the plant canopy and accumulated in or on the leaves. As the accumulation is roughly a function of the deposited amount of pollutants, the exposure time and the effects of climatic factors are of prime importance. As such, many plant species are useful for bio-monitoring atmospheric deposition of pollutants. In the case of trace metal containing aerosols, the elements are mainly accumulated on the leaf surface. Trace metal pollution not only affects the production and quality of crops, but also influences the quality of the atmosphere and water bodies, and threatens the health and life of animals and humans within a food chain. Most severely is that this kind of pollution is covert, long term and non-reversible [7]. Trace metals are also one of the major contaminating agents in our food supply [8, 9]. Vegetables are vital to the human diet and in particular provide the well-known trace elements and trace metals. Minor or trace elements are essential for good health if they come from an organic or plant source [10]. In contrast, if they come from an inorganic or metallic source, they become toxic. The processes of plant growth depend on the cycle of nutrients including trace elements, from soil to plant [11]. Vegetables, especially the leafy ones, accumulate higher amounts of trace metals because they absorb these metals in their leaves.

Food safety is a major concern at present. The increasing demand of food safety has accelerated research regarding the risk associated with food consumption contaminated by trace metals [12]. The rate at which trace metals are accumulated in the soil depends on the physicochemical properties of the soil and the relative efficiency of crops to remove the metals from the soil. Trace metals accumulated in cultivated soils can be transferred to humans through various exposure pathways causing adverse effects on human health [13].

Different vegetables are cultivated on the roadsides of major roads in Abak. These vegetables are exposed to trace metal contaminations as a result of atmospheric deposition due to anthropogenic activities like vehicular emission, industrial emission and burning of all forms of wastes along

the roadsides. These are in addition to the plants uptake from the soil, as a result of the agricultural practices adopted by the farmers to increase productivity. This study was conducted to quantify the levels of some trace metals in leaves of a common perennial vegetable (*V. amygdalina*) grown along the roadsides of three major roads in Abak and to assess the health risks associated with the consumption of the vegetable.

2. Materials and Methods

2.1. Study Area

The study was carried out in Abak, Akwa Ibom State, Nigeria. The area lies between latitudes 4°59'N and 4°983'N and longitudes 7°47'E and 7°783'E with an area of 190 km² (70 m²). The major occupation of the people is farming. There are also automobile mechanics, welders, petty traders, timber dealers, as well as production industries like paint and fertilizer blending.

2.2. Sample Collection

Leaves samples of roadsides *V. amygdalina* grown along three major roads (Abak/Ikot Ekpene, Abak/Ikot Abasi and Abak/Uyo) in Abak were collected. Samples were randomly collected at a height of 10 cm above the soil surface with the help of a stainless knife and pooled together to obtain composite samples for each road. Corresponding composite soil samples were also collected in the area, as described by [14]. The vegetable and soil samples were equally collected at Ikot Ekeang village, about 10 km from the study area. The collected samples were kept in clean polyethylene bags, properly labeled and transported to the laboratory.

2.3. Sample Pretreatment and Preparation

The vegetable samples were washed with clean tap water according to the normal household technique. After draining the excess water, the samples were chopped into small pieces and oven dried at 50 – 60°C, ground into powder. Exactly 1.0 g was weighed into a crucible and ashed in a SXL muffle furnace at a temperature range of 750°C - 850°C for 4 hours. The crucible with the ash was removed from the furnace and allowed to cool. The ash was leached with 5 cm³ of 6M HCl and transferred into a 50 cm³ volumetric flask. The volume was made up to 50 cm³ with distilled water. The solution was stored in a plastic reagent bottle for trace metal analyses.

The soil samples were air-dried for 48 hours, ground and homogenised. The homogenised matrix was sieved using a 2 mm mesh sieve. Two (2) g of the ground soil were digested with 15 cm³ aqua regia (2HNO₃: 1HClO₄) and heated to near dryness, leached with 5 cm³ of 20% HNO₃. The solutions were filtered into acid-washed 100cm³ volumetric flask after being rinsed with deionised water. Further, the solution was made up to 50 cm³ with deionised water prior to trace metal analysis.

2.4. Instrumental Analysis

Trace metals (Cu, Zn, Cd, Pb, and Mn) in soil and plant samples were determined as described by [15] using atomic absorption spectrophotometer (AAS) (UNICAM939/959 model). The sample solutions were aspirated into the instrument and the absorbance obtained was used to determine the concentrations of the metals in the different samples from calibration curves.

2.5. Transfer Factor (TF)

The transfer factor (TF) of metals from soil to vegetables was calculated using Equation 1 described by [16].

$$TF = \frac{C_{plant}}{C_{soil}} \quad (1)$$

Where, C_{plant} and C_{soil} represent the levels of the toxic metal in the plant and soil, respectively.

2.6. Health Risk Assessment

For the assessment of health risks through consumption of the roadsides *Vernonia amygdalina* leaves, the daily intake of metal (DIM), hazard quotient (HQ), and the hazard index (HI) were evaluated.

2.6.1. Daily Intake of Metals (DIM)

Toxic trace metals enter the human body through different path-ways. It may be oral intake (through consumption foods), inhalation, and or dermal contact [17]. Equation 2 was used to evaluate the daily intake of metals in the human body.

$$DIM = \frac{(C_{metal} \times C_{factor} \times D_{food})}{B_{average\ weight}} \quad (2)$$

Where C_{metal} , C_{factor} , D_{food} and $B_{average\ weight}$ represent the trace metal level (mg/kg) in plants, conversion factor, and daily intake of vegetable by human and average body weight, respectively [18]. Fresh green vegetable weight was converted to dry weight by using the conversion factor 0.085 as described by [19 - 21]. The average daily vegetable intakes were taken as 0.345 and 0.232 kg/person/day for adults and children, respectively, while the average body weights were taken as 55.9 and 32.7 kg for adults and children, respectively [18, 22]. The health risk for adults and children are considered separately since the contact pathway with each exposure way changes with age.

2.6.2. Risk Characterisation

The health risks associated with metals ingested through vegetable consumption was assessed using hazard quotient (HQ) as expressed in equation 3 as modified by [23].

$$HQ = \frac{DIM}{Rfd} \quad (3)$$

Where DIM is the daily intake of metals (kg person⁻¹day⁻¹) through vegetable consumption and Rfd represents reference oral dose (mg kg⁻¹ day⁻¹). The Rfd value for Cu, Pb, Cd, Mn, and Zn are 0.04, 0.004, 0.001, 0.033, and 0.30 (mg kg⁻¹ bw

day⁻¹), respectively [24]. The Rfd is an estimation of the daily exposure to which the human population is likely to be without any appreciable risk of deleterious effects during a lifetime. The exposed population is assumed to be safe when HQ is < 1 [18].

In a situation where there are toxicants or multiple exposure routes, it will be best that their possible interactions are taken into consideration [19]. It is assumed that the toxic risk due to potentially hazardous chemicals in the same medium is cumulative; therefore, the summation of the HQs was done to obtain the overall toxic risk; the hazard index (HI) is shown in Equation 4 according to [19 and 25].

$$HI = \sum HQ_i; i = 1 \dots n \quad (4)$$

Where n is the number of trace metals. This computation was done to determine the level of risk posted. If the calculated HI is greater than one, then the toxic adverse effect due to the exposure pathway or toxicant will be assumed to be hazardous and negligible when HI is less than one.

2.7. Statistical Analysis

Statistical analysis of data was carried out using Microsoft Excel 2007 and the SPSS 16.0 statistical package program.

3. Results and Discussion

3.1. Levels and Distribution of Trace Metals in Soil and Vegetable Samples

The levels of the investigated trace metals in soil and plant samples are presented in Tables 1 and 2, respectively. Generally, the values of all the metals in soil and plant were higher in the study sites than in the control site and the levels of the metals in soil samples were higher than those in plant for all the sites. The results agreed with the report of [26]. The metal levels in the soil samples were significantly higher in samples from Ikot Abasi Road and lower in samples from Ikot Ekpene Road. The metal levels in the soil were in the order: Cu > Zn > Pb > Mn > Cd. The only exception is that Cd (31.56 mg/kg) was higher than Mn (27.94 mg/kg) in soil from Ikot Abasi Road. The metal levels in the vegetable samples were also in the order: Cu > Zn > Pb > Mn > Cd. This could be attributed to different anthropogenic activities that affect the availability, mobility of metals and physicochemical parameters. The levels of Cu and Pb obtained in the vegetable in this study were higher than the FAO/WHO maximum permissible levels of 73.30 and 0.3 for Cu and Pb, respectively in the vegetable as provided by [27 and 28]. Those of Zn and Mn lower than the FAO/WHO maximum permissible levels of 99.40 and 500 mg/kg in the vegetable as provided by [27 and 28]. Cd levels were lower than the 0.2 mg/kg provided by [27 and 28] except for the vegetable samples from Abak/Ikot Abasi Road and Abak/Uyo Road whose Cd levels were 0.32 ± 0.002 and 0.34 ± 0.004 mg/kg, respectively.

Table 1. Level (mg/kg) of trace metals in soil samples.

Sites	Cu	Zn	Cd	Pb	Mn
AIE	243.78 ± 0.02	104.26 ± 0.10	16.45 ± 0.03	46.07 ± 0.02	31.56 ± 0.02
AIA	360.30 ± 0.02	161.94 ± 0.04	38.02 ± 0.03	60.22 ± 0.03	27.94 ± 0.02
AU	254.47 ± 0.002	156.84 ± 0.02	23.63 ± 0.01	50.08 ± 0.03	29.29 ± 0.02
CTS	185.53 ± 0.04	92.30 ± 0.10	0.51 ± 0.21	18.38 ± 0.21	19.50 ± 0.03

AIE = Abak/Ikot Ekpene Road, AIA = Abak/Ikot Abasi Road, AU = Abak/Uyo Road, CTS = Control Sample

The high levels of Cu recorded in both soil and the plant samples in this study, could be attributed to the discharge of domestic waste, wind-blown dust, decaying vegetation and forest fire [5]. The fact that Pb and Cd are traffic-related metals that originated mainly from the combustion of leaded

fuel, lubricating motor oil, vehicle tire wear, and brake pads, makes it possible to also link their levels on the roadside plants from the fumes emitted by the automobile plying the high ways [29].

Table 2. Levels (mg/kg) trace metals in *Vernonia amygdalina* leaves samples.

Site	Cu	Zn	Cd	Pb	Mn
AIE	100.93 ± 0.004	48.37 ± 0.002	0.08 ± 0.001	0.56 ± 0.005	0.44 ± 0.000
AIA	116.76 ± 0.004	61.14 ± 0.002	0.32 ± 0.002	0.52 ± 0.001	0.54 ± 0.002
AU	102.44 ± 0.009	31.25 ± 0.003	0.34 ± 0.004	0.55 ± 0.000	0.43 ± 0.000
CTS	89.79 ± 0.004	15.82 ± 0.002	BDL	0.11 ± 0.003	0.42 ± 0.000

AIE = Abak/Ikot Ekpene Road, AIA = Abak/Ikot Abasi Road, AU = Abak/Uyo Road, CTS = Control Sample, BDL = Below Detection Limit.

High Cu levels in the soil could reduce the uptake of Mn to below adequate level for plant [30]. High Cu levels in the soil could also be attributed to high Iron (Fe) and molybdenum (Mo) fertilizer applications [31].

3.2. Transfer Factor (TF) of Trace Metals

The calculated TF of trace metals from soil to the *V. amygdalina* are as presented in Table 3.

Table 3. Transfer factor (TF) of trace metals from soil to *V. amygdalina*.

Site	Trace metals				
	Cu	Zn	Cd	Pb	Mn
AIE	0.500	0.710	0.015	0.010	0.017
AIA	0.360	0.450	0.024	0.040	0.031
AU	0.580	0.350	0.019	0.080	0.026

AIE = Abak/Ikot Ekpene Road, AIA = Abak/Ikot Abasi Road, AU = Abak/Uyo Road

From the trace metals (Cu, Zn, Cd, Pb and Mn) studied, the highest TF was recorded by Zn (0.500) while the lowest was recorded by Cd (0.019). The higher the TF values, the more

mobile and available the metals are in the soil [18]. As the level of trace metals in the soil increased, the plant uptake also increased. The results recorded in this study, was in agreement with findings by [32 and 33]. Soil-to-plant transfer is one of the key processes of human exposure to toxic metals through food chain, and it reveal bioavailability of trace metals in investigated soils.

3.3. Risk Assessment

The estimated daily intake of metals (DIM) for ingestion of *V. amygdalina* obtained for adults and children. The results revealed that intake values for trace metals via the consumption of the investigated vegetable collected from the studied sites were of the trend: Cu > Zn > Pb > Cd > Mn for both adults and children. The values from Table 4 revealed that Cu contributed most to the dietary intake of trace metals through consumption of bitter leaf while Mn contributed least for all the three roadsides in the area. The result also indicate that the metal intake for children were higher than those for adults.

Table 4. Estimated daily intake of metals (DIM) via consumption of *V. amygdalina*.

Location	Trace Metals					
	Cu	Zn	Cd	Pb	Mn	CDI
Adults						
AIE	5.28E-02	2.53E-02	4.19E-05	2.93E-04	2.31E-04	7.88E-02
AIA	6.12E-02	3.20E-02	1.68E-04	2.72E-04	2.83E-04	9.39E-02
AU	5.36E-02	1.64E-02	1.18E-04	2.88E-04	2.25E-04	7.07E-02
Children						
AIE	6.09E-02	2.92E-02	4.82E-05	3.38E-04	2.65E-04	9.07E-02
AIA	7.04E-02	3.69E-02	1.93E-04	3.14E-04	3.26E-04	1.08E-01
UA	6.18E-02	1.88E-02	2.05E-04	3.32E-04	2.59E-04	8.14E-02

AIE = Abak/Ikot Ekpene Road, AIA = Abak/Ikot Abasi Road, AU = Abak/Uyo Road, CDI = Cumulative daily Intake

3.4. Evaluation of Toxic Risk

The hazard quotient (HQ) and hazard index (HI) helps in the evaluation of the magnitude of harm posed to the consumers of *V. amygdalina* contaminated with trace metals. The result summarized in Table 5 showed that the HQ of Cu was all greater than one (1) while other metals (Zn, Cd, Pb and Mn) were less than one (1). As noted by [34 and 5], this suggests significant risk to the consumers of the vegetable as

a result of the high level of Cu.

The summation of the HQs gave the overall toxic risk, which is the hazard index (Table 5). From the general observation, all the computed HI values were above unity for both adults and children. This indicates that the consumers of *V. amygdalina* plant from the roadsides of these major roads are at potential health risks.

Table 5. Estimated hazard quotients (HQ) and hazard index (HI) for adults and children due to daily intake of metals in *V. amygdalina*.

Location	HQ					HI
	Cu	Zn	Cd	Pb	Mn	
Adults						
AIE	1.32E+00	8.45E-02	4.19E-02	7.34E-02	6.99E-03	1.53E+00
AIA	1.53E+00	1.07E-01	1.68E-01	6.81E-02	8.58E-03	1.88E+00
AU	1.34E+00	5.46E-02	1.78E-01	7.21E-02	6.83E-03	1.65E+00
Children						
AIE	1.52E+00	9.72E-02	4.82E-02	8.44E-02	8.04E-03	1.76E+00
AIA	1.76E+00	1.23E-01	1.93E-01	7.84E-02	9.87E-03	2.16E+00
AU	1.54E+00	6.28E-02	2.05E-01	8.29E-02	7.86E-03	1.90E+00

AIE = Abak/Ikot Ekpen Road, AIA = Abak/Ikot Abasi Road, AU = Abak/Uyo Road,

4. Conclusion

Based on the analyses and results, it was concluded that the roadsides soil and vegetable (*V. amygdalina*) samples contain variable levels of the investigated trace metals. The levels of these metals were lower in the control samples than samples from the study area. The contamination sources could be linked to the anthropogenic activities like indiscriminate dumping of domestic wastes, metallic waste, used lubricating oil spillage and combustion of fuel as well as wearing of vehicular tires and brake pads and all kinds of agricultural activities in the area. The health risk assessment indicated that the hazard quotient (HQ) for Cu was all greater than one (1) which suggests significant risk to the consumers of the vegetable as a result of the high level of Cu, while those of Zn, Cd, Pb and Mn were less than one (1). The computed hazard index (HI) values were above unity for both adults and children, indicating that the consumers of *V. amygdalina* from the roadsides of these major roads are at potential health risks. Further research should be carried out on remediation of trace metals in the roadsides soil of the study area to reduce their bioaccumulation in plants and possible health risk associated with their consumption.

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