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Human Health Risk Assessment of Heavy Metals Contamination in Bitter Leaf Grown in an Oil Exploration Area

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Abstract Concentrations of some heavy metals (Fe, Zn, Cu, Mn, Cr, Ni, Pb and Cd) in bitter leaf (*Vernonia amygdalina)* grown in an oil exploration area of Ibeno, AkwaIbom State, Nigeria, were assayed using standard procedures. Human health risks associated with the consumption of the metals in the plant were equally assessed using risks assessment models. Roots and leaves samples of bitter leaf were collected from three locations prone to intensive oil exploration activities. Control samples were equally collected from Mbiabam Ibiono, with no oil exploration activities. Results revealed that the concentrations of the metals in the roots ranged from < 0.001 mg/kg Cd to 450.48 ± 3.08 mg/kg Fe while those in the leaves ranged from < 0.001mg/kg Cd to 1650.36 ± 5.46 mg/kg Fe. The concentrations of the metals in the controls were generally low. These could be attributed to the absence of oil exploration in the control area. Ingestion pathway assessed to ascertain the risks associated with the consumption of the metals in the plant, gave hazard quotient and hazard index each less than unity (1) for every metal investigated. So, it could be argued that consuming the plant either as medicine or as food may not contribute to non-carcinogenic or carcinogenic risk in humans as the levels of contamination may not cause toxicity in the humans. Since the bitter leaf plants were actually contaminated with the investigated heavy metals, constant monitoring of the heavy metals in bitter leaf grown and consumed in Ibeno is recommended.

Keywords Risk, assessment, heavy metals, contamination, bitter leaf, oil exploration

Introduction

Vegetables are readily available. They form and constitute a major part of human diets since they contain carbohydrates, proteins, vitamins, minerals, trace elements as well as essential nutrients to the body [1, 2]. Indeed consumption of vegetables is regarded as one of the main pathways for the intake of essential nutrients. However, the presence of hazardous chemicals (toxic heavy metals, pesticide residues and organic environmental contaminants) in vegetables has been reported [3, 4].

Heavy metals pollution of agricultural soil and vegetables is one of the most severe ecological problems on a world scale because of their toxicity in plants, animals and humans as well as their lack of biodegradability [5, 6]. Heavy metals pollution has potential threats to the environment and can damage human health through various absorption pathways such as direct ingestion, dermal contact and diet through the soil-food chain, inhalation and oral intake [7].

This is true as dietary intakes of vegetables containing toxic heavy metals may pose potential health risks to the consumers of such vegetables [8]. Chronic accumulation of heavy metals in the organs of living things caused by high exposures, possessed different deleterious effects on human health (including cardiovascular, bone and nervous diseases, carcinogenic and developmental effects) [9].

Naturally, plants are able to take up metals which are essential and some few others (Hg, Cd, Ni and Pb) which are toxic to them [10]. Potentially harmful heavy metals in soil may not come solely from the soil (bedrock), but also from human activities such as solid or liquid waste deposits, agricultural inputs and fallout of industrial and urban emissions [11]. According to Liu et al. [4], heavy metals contamination in vegetables can be attributed to multiple pathways such as industrial emissions, sewage discharge, mining, over application of agro-chemicals and fertilizers in farm lands. Indeed, heavy metals can be absorbed and accumulated on edible and non-edible parts of vegetables when they are dispersed into the environment due to their unique characteristics (persistence and non-biodegradability in environmental media, high environmental stability, bio-accumulation, and bio-magnification in organism and food chains) [6]. According to Lokeshwari and Chandrappa [12], heavy metals pollution of the environment is covert, persistent and irreversible. This kind of pollution not only degrades the quality of the atmosphere, water bodies, and food crops, but also threatens the health and well-being of animals and humans through the food chain [13].

Bitter leaf **(***Vernonia amygdalina*) is one of the most widely consumed perennial leafy vegetables and herbal plants in Ibeno. Bitter leafis a very important protective vegetable and useful for the maintenance of good 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 potent anti-parasitic, antitumor and bactericidal effects [14–16]. Bitter leaf is mainly employed as an agent in treating schistsomiasis, 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. This is addition to its leaves being used as an integral component in the preparation of soup. However, bitter leaf like other vegetables contains both essential and toxic metals over a wide range of concentrations [14–16].

In Ibeno, the presence of oil exploration activities by oil giant, Exxon Mobil and other service companies influences agricultural activities in both upstream and downstream [16-17]. There are no data and information on human health risk of heavy metals contamination in bitter leaf grown in Ibeno, a major oil exploration area in Nigeria. Considering the significant and economy important of biter leaf to the populace, there is no doubt that this study is necessary. The objectives of this study are to quantify the concentrations of some heavy metals (Fe, Zn, Cu, Mn, Cr, Ni, Pb and Cd) in bitter leaf grown in Ibeno and to assay the human health risk associated with the consumption of these heavy metals as a result of consuming this important vegetable in whatever form (either as medicine or as food).

Materials and Methods

The 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 coastal area [16, 17]. 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 [16, 17]. The study location is shown in Figure 1.

Samples Collection

Roots and leaves samples of biter leaf 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 in Ibiono Ibom Local Government Area, where there are no oil exploration activities, to serve as the controls.

At each sampling location, samples were randomly collected and pooled together to obtain composite samples. The samples were appropriately labeled, stored in a clean polythene bags and transported to the laboratory.

Samples Preparation and Treatment

The collected roots and leaves were carefully washed with water remove a soil and dust particles, chopped into small pieces and oven dried at 80°C. The dried samples were separately ground with a mortar and sieved into polyethene bottles previously rinsed with deionized water and digested with HNO_3 and $HClO_3$ in the ratio of 5:1 until transparent solutions were obtained. The digests were cooled to room temperature, filtered and transferred quantitatively to 50 ml volumetric flasks, each made up to volume with deionized water and kept in a clean plastic vials before analyses.

Determination of Heavy Metals in the Biter Leaf Samples

The heavy metals (Fe, Zn, Cu, Mn, Cr, Ni, Pb and Cd) concentration levels in the digested biter leaf samples were determined by atomic absorption spectrophotometer (AAS) (model: Varian spectra 100, Australia). The AAS works in the physical process involving absorption of light by atoms of a given element at a wavelength specific to that element. The AAS was calibrated with standard solution for each element. The appropriate lamp and correct wavelength of each element were used.

Quality Control

Quality control of the AAS was guaranteed through the implementation of laboratory quality assurance and laboratory methods, including the use of standard operating procedures, calibrations with standards and the use of reagent blanks. In addition, all chemicals and reagents used were of analytical grade and **s**amples were analysed in triplicates. The detection limit of the AAS for each of the all the metals investigated in both the roots and leaves samples was 1.0×10^{-4} mg/kg. In addition, interference corrections were performed by the addition of appropriate reagents that convert interfering ions into precipitates which were easily filtered off. Care was taken to ensure that the investigated metals were not lost since precipitates tend to adsorb substances from solution.

Health Risks Assessment

Humans may be exposed to heavy metals contamination in a medium through three exposure pathways (ingestion, inhalation, and dermal contact). The ingestion pathway was used to assess the health risks associated with the consumption of heavy metals through biter leaf (either as food or as medicine). This was calculated based on the average daily intake (ADI_{ing}) of metals in biter leaf using Equation 1.

$$ADI_{ing} = \frac{C \times IR_{ing} \times CF \times EF \times ED}{BW \times AT} \quad (1)$$

Where: ADI_{ing} is the average daily intake of heavy metals ingested from biter leaf in mg/kg/day, C = concentration of heavy metal (mg/kg) in biter leaf, IR_{ing} in mg/day is the ingestion rate = 200 for children and 100 for adults, EF in days/year is the exposure frequency = 350, ED is the exposure duration in years = 6 for children and 30 for adults, BW is the body weight of the exposed individual in kg = 16 for children and 60 for adults,AT is the average time (day) = 70 years x 360, CF is the conversion factor in kg/mg = 10^{-6} [18].

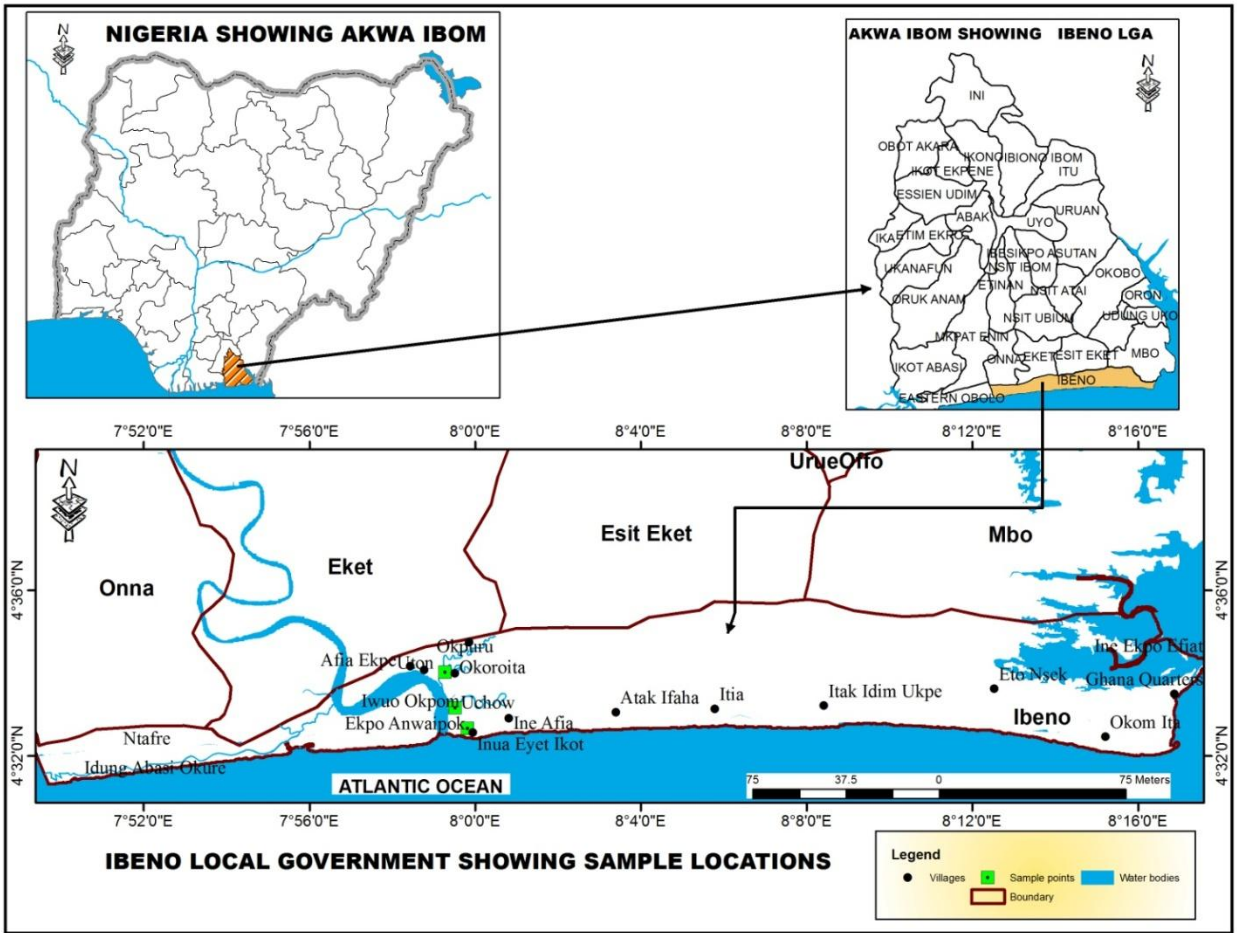


Figure 1: Location of the Study

Non-Carcinogenic Risk Assessment

The method provided by USEPA [18] was used to evaluate the health risk associated with non carcinogenic effects of metals in the biter leaf. Hazard quotient (HQ) was calculated as the ratio of the ADI_{ing} and the ingestion reference dose (RfD_{ing}) in mg/kg/day of the metals for a given metal using Equation 2.

$$HQ = \frac{ADI_{ing}}{RfD_{ing}} \quad (2)$$

Hazard index (HI) which is the sum of the HQ values of all metals in the biter leaf was used to assess the overall non carcinogenic effects posed by multiple metals, according to Equation 3.

$$HI = \sum HQ \quad (3)$$

If the HI value is < 1, the exposed individual is unlikely to experience obvious adverse health effects, but if the HI value is > 1, there could be a risk of non carcinogenic effects. The RfD_{ing} (mg/kg/day) of all the investigated metals are presented in Table 1.

Table 1: RfD_{ing} (mg/kg/day) of the Investigated Heavy Metals

Heavy metals	RfD_{ing}	References
Fe	0.70	[19]
Zn	3.0×10^{-1}	[19]
Cu	4.0×10^{-2}	[19]
Mn	1.4×10^{-1}	[20]
Cr	3.0×10^{-3}	[19]
Ni	2.0×10^{-2}	[21]
Pb	3.5×10^{-3}	[19]
Co	2.0×10^{-2}	[22]
Cd	1.0×10^{-3}	[20]

Carcinogenic Risk Assessment

This is the probability of an individual developing any type of cancer [carcinogenic risk (CR)] throughout his or her lifetime due to exposure to carcinogen. For a given metal, it was calculated according to Equation 4.

$$CR = \sum ADI \times SF \quad (4)$$

Where: ADI = average daily intake of the metal and SF is the carcinogenicity slope factor (mg/kg/day). Risks < 1.0×10^{-6} were ignored, risks lying between 1.0×10^{-4} and 1.0×10^{-6} were generally considered acceptable, and those > 1.0×10^{-4} imply a lifetime carcinogenic risk. For multiple metals, the CR was obtained from the sum of the CR values of different metals according to Equation 5, as described by USEPA [18].

$$TCR = \sum CR \quad (5)$$

Statistical Analysis

Statistical analyses were performed using the SPSS version 20.0. $P < 0.05$ was considered the level of statistical significance.

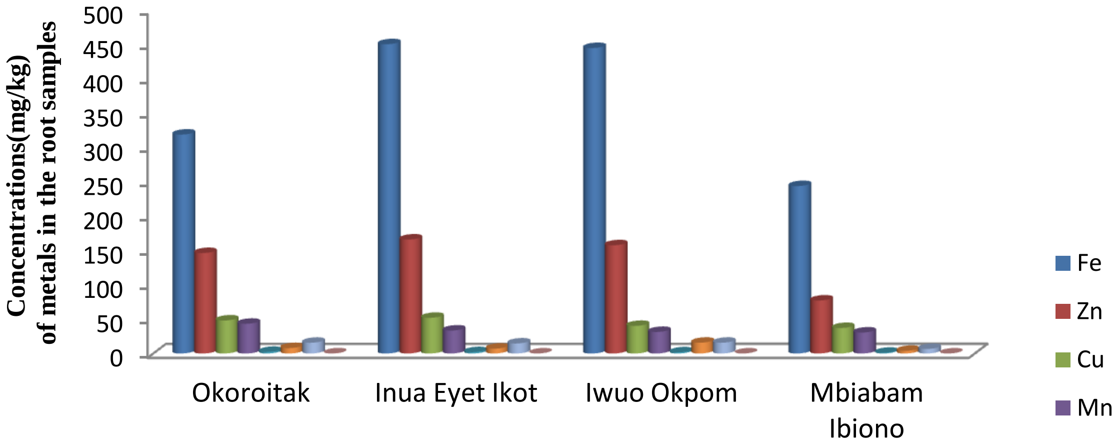
Results and Discussion

Concentration Levels of Heavy Metals in the Biter Leaf

Heavy metals concentrations in roots samples of the biter leaf are presented in Figure 2 while those of the leaves are presented in Figure 3. The concentrations of the heavy metals were higher in the roots samples than in the leaves (with the exception of Fe and Mn whose concentration levels were higher in the leaves than in the roots samples) for all the samples, except the controls. This could be due to metals - plant absorption from soil, through soil to roots transfer.Interestingly, the concentration levels of the heavy metals were higher in samples from the study area than in samples from the control site. This could be attributed to the oil exploration activities taken place in the study area.

In the roots samples, the concentrations 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. The concentrations of most of the metals reported in the roots samples of biter leaf in this study were higher than those of the corresponding metals reported by Jasha and Petevino [23]; Abdulkadir et al. [24]; Nathan et al. [25] and Dzomba et al. [26]. The heavy metals concentrations in the roots samples of the plant across all the study locations in this study were all below the maximum permissible limits set by the WHO.

The metals concentrations in the leaves samples of the plant in all the study locations ranged from 0.53±0.02 mg/kg Cd to1650.36 ±5.46 mg/kg Fe. The sequence of variation was:



	Okoroitak	Inua Eyet Ikot	Iwuo Okpom	Mbiabam Ibiono
Fe	318.87	450.48	444.91	243.91
Zn	146.37	166.02	157.66	77.19
Cu	48.14	51.95	40.22	37.16
Mn	43.34	33.47	31.43	30.56
Cr	2.4	2.03	1.72	1.11
Ni	7.93	7.1	15.74	3.9
Pb	15.67	14.62	15.71	6.67
Cd	0.21	0.11	0.11	0.08

Figure 2: Concentrations of Heavy Metals in the Roots Samples

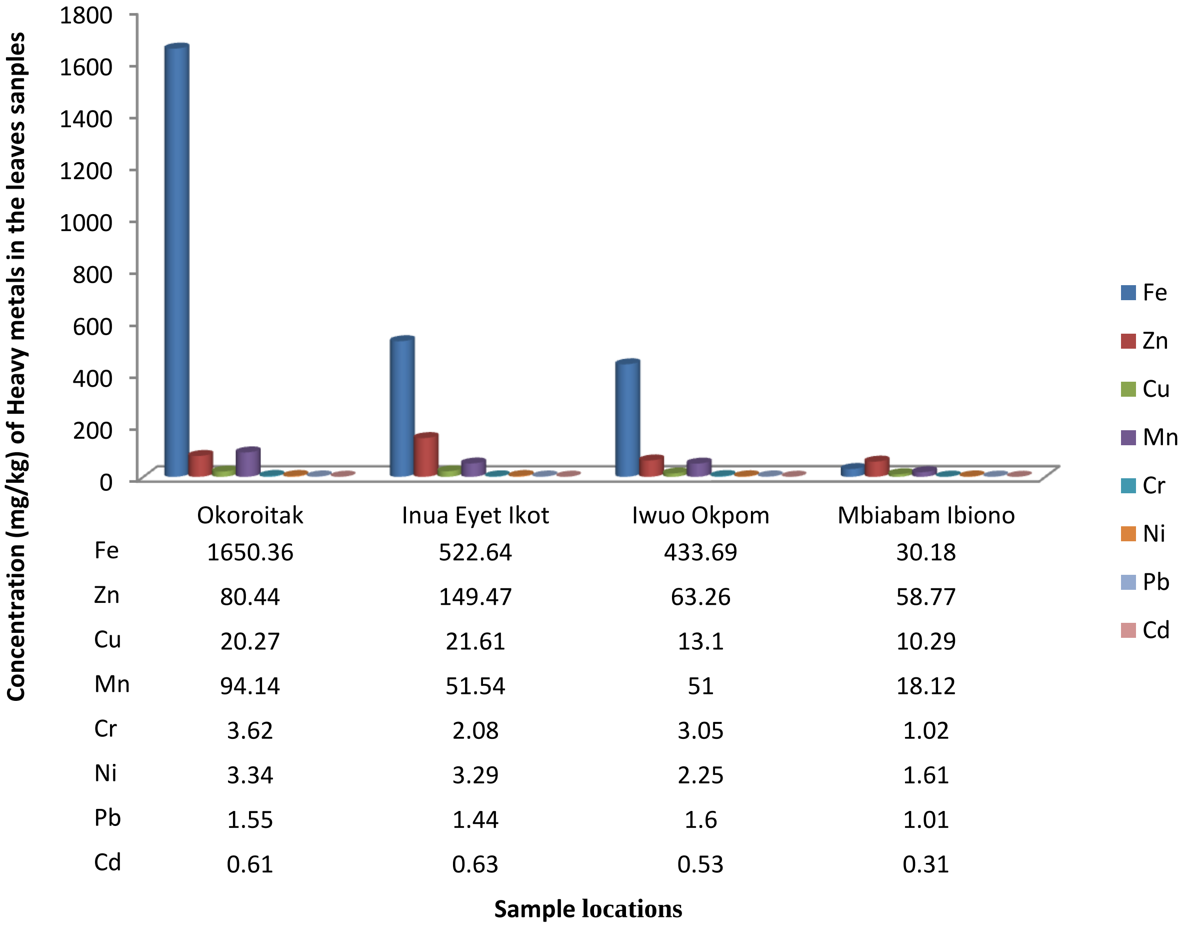
Fe > Zn >Mn> Cu > Ni > Cr >Pb> Cd. Again, the concentrations of most of the metals reported in the leaves samples of the studied plant in this study were higher than those of the corresponding metals reported in similar studies by Abdulkadir et al. [24]; Dzomba et al. [26]; Bhanisana and Sarma [27]. Accordingly, the observed heavy metals concentrations in the leaves of the plant across all the study locations were below the maximum permissible limits set by WHO. These rendered the plant suitable for human consumption in whatever forms (either as medicine or as food).

Non-Carcinogenic Risk of Heavy Metals for Adults and Children by Ingestion through the Leaves

For the adults' and children's populations in the studied locations, the ingestion of each of Fe, Zn, Cu, Mn, Cr, Ni, Pb and Cd through the leaves (either as food or as medicine) had HQ and HI values less than one. Meaning that for each of the heavy metals considered, the adults and children were not at risk of non-carcinogenic effects. The pattern of variation of the metals was in the order: Fe > Cr > Cd >Mn> Cu >Pb> Zn > Ni for the adults. For the children, the pattern of variation of the metals was in the order: Fe > Ni > Cr > Cd >Mn> Cu >Pb> Zn. The HI values for the metals due to ingestion through consumption of the leaves of biter leaf (either as food or as medicine) are presented in Figure 4.

Non-Carcinogenic Risk of Heavy Metals for Adults and Children by Ingestion through the Roots

Again, for the adults' and children's populations in the studied locations, the ingestion of each of Fe, Zn, Cu, Mn, Cr, Ni, Pb and Cd through the roots (either as food or as medicine) had HQ and HI values less than one. These also mean that for each of the heavy metals considered, the adults and children were not at risk of non-carcinogenic effects. The sequence of variation of the metals was in the order: Pb> Cu > Cr > Ni > Fe > Zn >Mn> Cd for the adults. For children, the sequence of variation of the metals was in the order: Pb> Fe > Ni > Cu > Cr > Zn >Mn> Cd. The HI values for the metals due to ingestion through consumption of the roots of biter leaf (either as food or as medicine) are presented in Figure 5.



	Okoroitak	Inua Eyet Ikot	Iwuo Okpom	Mbiabam Ibiono
Fe	1650.36	522.64	433.69	30.18
Zn	80.44	149.47	63.26	58.77
Cu	20.27	21.61	13.1	10.29
Mn	94.14	51.54	51	18.12
Cr	3.62	2.08	3.05	1.02
Ni	3.34	3.29	2.25	1.61
Pb	1.55	1.44	1.6	1.01
Cd	0.61	0.63	0.53	0.31

Figure 3: Concentrations of Heavy Metals in the Leaves Samples

Carcinogenic Risk (CR) of Heavy Metals for Adults and Children by Ingestion through the Roots

The calculated average daily doses (ADD) of the heavy metals ingestion through consumption of the roots of biter leaf either as food or as medicine are presented in Table 2. Based on this, the lifetime carcinogenic risk (CR) values of the consumers are presented in Figure 6**.** In Okoroitak sample location, the total carcinogenic risk (CR_{tot}) due to Cd, Pb, Cr and Ni for children was 1.493×10^{-7} while for adults, it was $9.950 \times 10^{-8.}$ In InuaEyetIkot sample location, CR_{tot} due to Cd, Pb, Cr and Ni for children was 1.382×10^{-7} while for adults, it was 9.204×10^{-8}. In IwuoOkpom, the CR_{tot}due to Cd, Pb, Cr and Ni for children was 1.461×10^{-7} while for adults, it was 9.739×10^{-8}. From all the sample locations, these values were below the limits that could cause cancer in the consumers due to consumption of the affected metals in the plant's roots. According to USEPA [18], the standard CR values of the affected metals are in the range of 1.0×10^{-6} to 1.0×10^{-4}.

Carcinogenic Risk (CR) of Heavy Metals for Adults and Children by Ingestion through the Leaves

The calculated ADD values of the heavy metals ingestion through consumption of the leaves of biter leaf either as food or as medicine are presented in Table 3. The lifetime CR values of the consumers are presented in Figure 7**.** In Okoroitak sample location, the CR_{tot}due to Cd, Pb, Cr, and Ni for children was 3.643×10^{-8}. For adults, it was 2.543×10^{-8}. In InuaEyetIkot sample location, CR_{tot} due to Cd, Pb, Cr, and Ni were 2.585×10^{-8} and 1.787×10^{-8} for children and adults, respectively. In IwuoOkpom, the CR_{tot} due to Cd, Pb, Cr and Ni was 1.743×10^{-7} for children and 1.271×10^{-8} for adults. Again these values were below the limits that could cause cancer in the consumers due to consumption of the affected metals in the leaves of the biter leaf from all the sample locations.

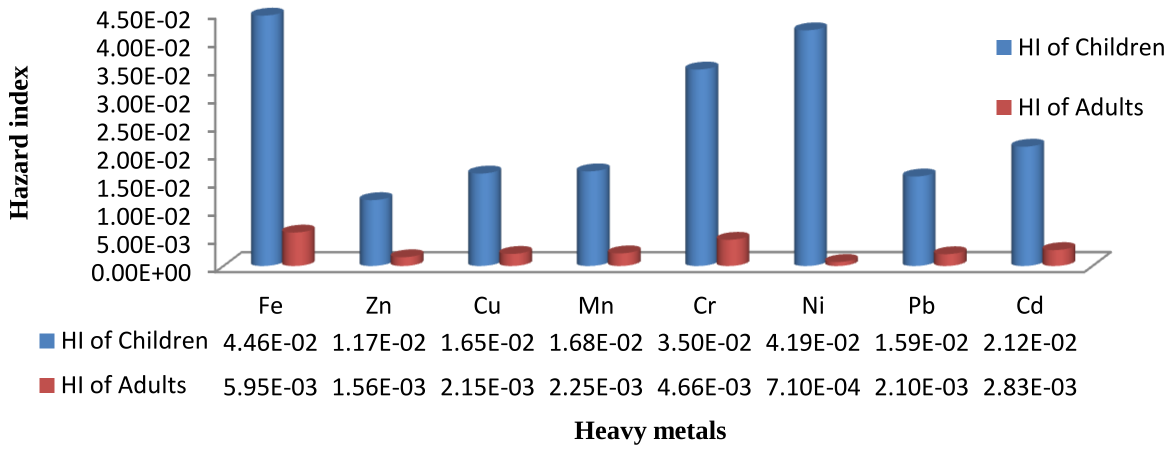


Figure 4: Hazard Index (HI) for the Metals due to Ingestion through Consumption of the Leaves

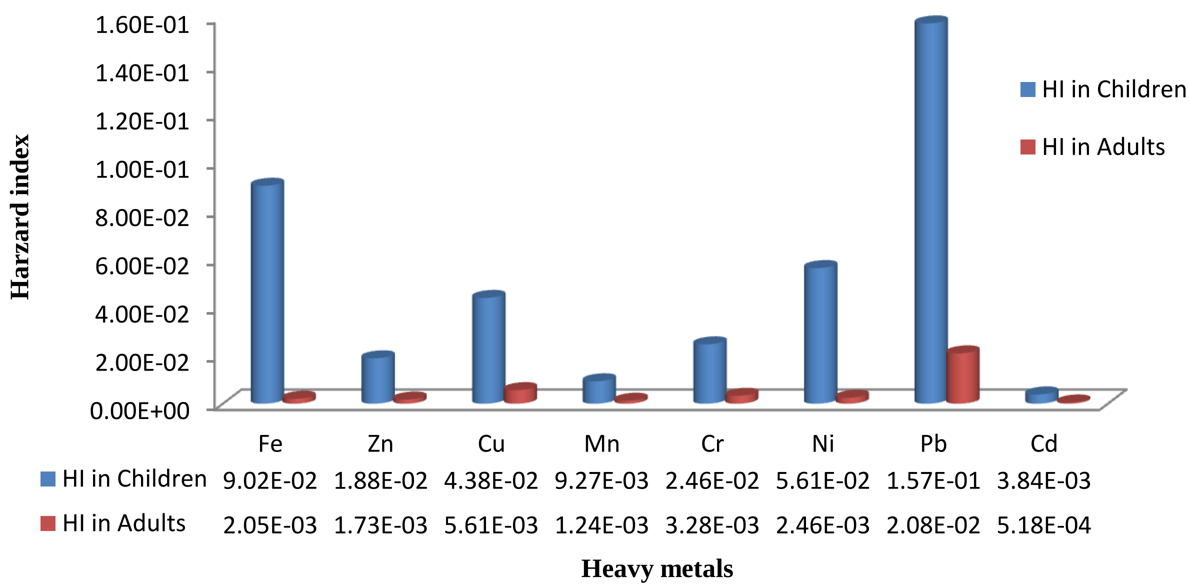


Figure 5: Hazard Index (HI) for the Metals due to Ingestion through Consumption of the Roots

Table 2: Average Daily Doses (ADD) of Heavy Metals Ingestion through Consumption of the Roots

Sample Location	Heavy metal	Children ADD_{ing}	Adults ADD_{ing}
OK	Cd	2.16E-07	1.44E-07
	Pb	1.61E-05	1.07E-05
	Cr	2.47E-06	1.64E-06
	Ni	8.15E-06	5.43E-06
IN	Cd	1.13E-07	2.51E-09
	Pb	1.50E-05	1.00E-05
	Cr	2.09E-06	1.39E-06
	Ni	7.29E-05	4.86E-06
IW	Cd	1.13E-07	7.53E-08
	Pb	1.61E-05	1.08E-05
	Cr	1.77E-06	1.18E-06
	Ni	1.62E-05	1.08E-05

ADD_{ing} = Average daily dose of ingestion, OK =Okoroitak, IN =InuaEyetIkot, IW =IwuoOkpom

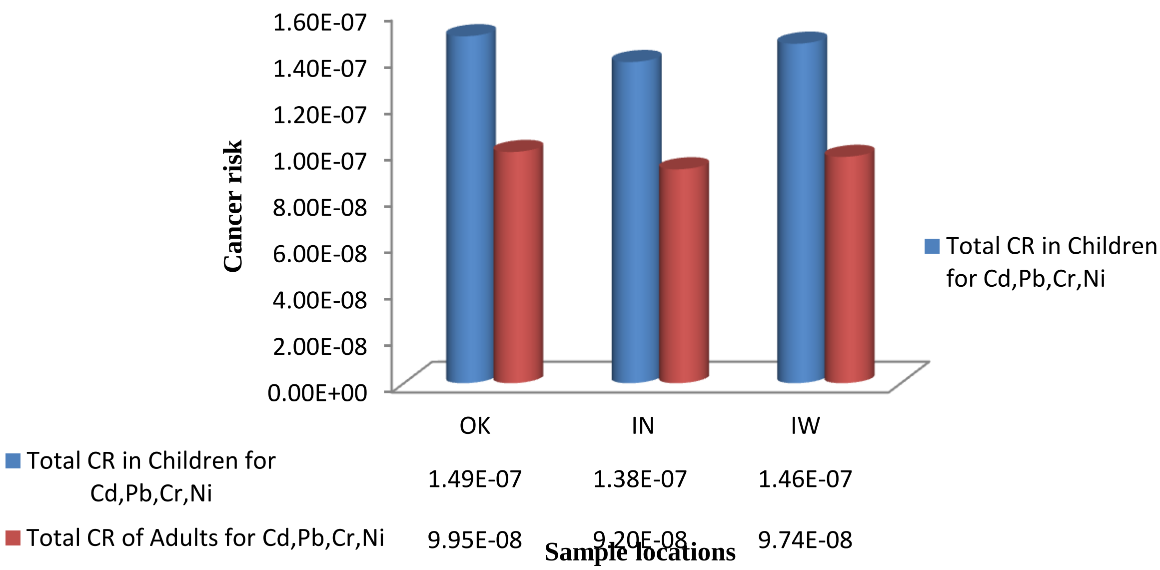


Figure 6: Carcinogenic Risk (CR) Values for the Metals due to Ingestion through Consumption of the Roots of Biter Leaf.OK = Okoroitak, IN = InuaEyet Ikot, IW = IwuoOkpom

Table 3: Average Daily Doses (ADD) of Heavy Metals Ingestion through Consumption of the Leaves

Sample Location	Heavy metal	Children ADD_{ing}	Adults ADD_{ing}
OK	Cd	6.27E-07	4.18E-07
	Pb	1.59E-06	1.06E-06
	Cr	3.72E-06	2.48E-06
	Ni	3.43E-06	2.29E-06
IN	Cd	6.47E-07	4.32E-07

	Pb	1.48E-06	9.86E-07
	Cr	2.14E-06	1.43E-06
	Ni	3.38E-06	2.25E-06
IW	Cd	5.45E-07	3.63E-07
	Pb	1.64E-06	1.10E-06
	Cr	3.13E-06	2.09E-06
	Ni	2.31E-06	1.54E-06

ADD_{ing} = Average daily dose of ingestion,OK = Okoroitak, IN = InuaEyetIkot, IW = IwuoOkpom

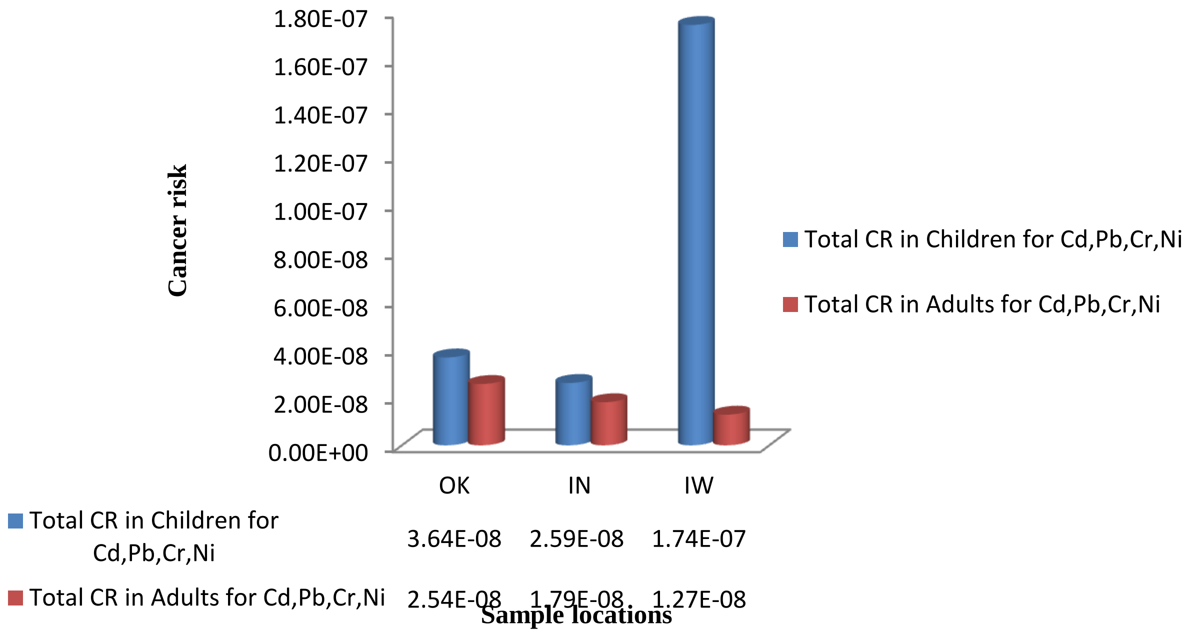


Figure 7: Carcinogenic risk (CR) values for the metals due to ingestion through Consumption of the leaves of biter leaf. OK = Okoroitak, IN = InuaEyetIkot, IW = IwuoOkpom

Conclusion

Based on the analyses and results, it was concluded that the roots and leaves samples of the biter leaf contain variable concentrations of the investigated heavy metals. The concentrations of the heavy metals were higher in the roots than in the leaves (with the exception of Fe and Mn whose concentrations were higher in the leaves than in the roots) for all the samples, except the controls. This could be due to metals - plant absorption from soil as a result of soil to roots transfer.The concentrations of the heavy metals were higher in samples from the study area than in samples from the control site. This could be attributed to the oil exploration activities taken place in the study area. Interestingly, the heavy metals concentrations in both the roots and leaves of the plant across all the study locations were below the maximum permissible limits set by the WHO. These suggested that the biter leaf plant is suitable for human consumption in whatever form (either as medicine or as food).

The carcinogenic and non carcinogenic risks assessment indexes revealed that the biter leaf plant from the study area was not polluted with the investigated heavy metals as a result of oil exploration activities in the area. This is because the investigated ingestion pathway through consumption of the plant in whatever form (either as medicine or as food) did not contribute to carcinogenic and non carcinogenic risks in both the children and adults that consumed the vegetable. This indicated that consumption of the biter leaf in whatever form (either as medicine or as food) by human may not pose any health hazard to the consumers at the time of the study. Routine investigation of the concentrations and further health risk assessment of the investigated metals and other heavy metals in the biter leaf and other vegetables cultivated and consumed in the oil exploration area of Ibeno are highly recommended.

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