



Human Health Risk Assessment of Cadmium (Cd), Lead (Pb) and Mercury (Hg) levels in organs of fish obtained from Iko River, Eastern Obolo Local Government Area (L.G.A), Akwa Ibom State, Nigeria

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Abstract: The levels of cadmium, lead and mercury in the organs of three marine fish species in Iko River, Eastern Obolo, L.G.A. in Akwa Ibom State, Nigeria were determined using atomic absorption spectrometry (AAS). Assessment of the concentrations of cadmium, lead and mercury in the muscles (flesh), gills and liver of *Micropogonians undulate* (MU), *Ethmalosa fimbriata* (EF) and *Oreochromis niloticus* (ON) showed that the lowest Pb mean concentration (0.6 mg/kg) was found in liver of EF while liver of MU recorded the highest Pb concentration of 5.0 mg/kg. Cd concentration in fish organs ranged from 1.2 mg/kg (gills of MU and EF) to 4.1 mg/kg (liver of MU). Generally, these metal values were above the WHO and NSDQW limit, posing a serious human health hazard. Statistical analysis was carried out for the metal contents in the fish samples and there were significant difference between them. Human health risk assessment in this study indicated a high hazard quotient and hazard index, especially in children; implying hazardous effects in humans from consuming contaminated fish.

Keywords: Heavy metals; *Micropogonians undulate*; *Ethmalosa fimbriata*; *Oreochromis niloticus*

1. Introduction

Fish, an aquatic organism, is a common table food which is usually consumed by humans for protein nourishment. Compared to other sources of protein such as beef and pork, fish is easily more digestible and inexpensive. Fishes are desirable and nutritional source of high quality protein, as well as supply generously supply minerals and vitamins (Elnimr, 2011) to humans. Metals entering the aquatic ecosystem can be stored in fish and other aquatic organisms through the process of bioaccumulation and biological magnification via the food chain, becoming potentially toxic when accumulation reaches a substantially high level (Huang *et al.*, 2016). Fish which occupy a top level in the aquatic food chain are notorious for its ability to bio-concentrate heavy metals in its organs, according to (Raja *et al.*, 2009).

Marine pollution is a critical environmental issue of concern across the globe. Increasing human population intensifies the anthropogenic threat exerted on the environment (Alaqarbeh *et al.*, 2022; Raja *et al.*, 2009). Metals such as Mercury, cadmium and lead have no biological function in the human

system and are potentially toxic even at trace concentrations. Thus, there is a growing concern that metals accumulated in fish tissues may represent a health risk, especially for high consuming population (Karim *et al.*, 2016a; 2016b). The increased use of metal-based fertilizer in agricultural revolution of government could result in continued rise in concentration of metal pollution in fresh water reservoir due to water run-off (Marwa *et al.*, 2020; Ubong *et al.*, 2015). As human population increases, the intensity of anthropogenic threat exerted on the environment increases, as a result of industrialization and agricultural activities (Ubong *et al.*, 2020). Apart from soil environment and aquatic ecosystem, atmospheric inorganic contaminants of natural origin or anthropogenic sources with heavy metals at high concentrations could lead to serious ecological consequences and pose human health risks (Ite *et al.*, 2016). Heavy metals are potentially hazardous to humans and various ecological receptors because of their toxic, persistent, bioaccumulative and nonbiodegradable nature (Alaqrbeh *et al.*, 2022). Therefore, monitoring and evaluation of heavy metal concentrations in environmental compartments is imperative in order to identify human health hazards and prevent bioaccumulation in the food chain and further degradation of the ecosystem.

Human health risk assessment (HHRA) of consuming heavy metal contaminated fish is key to assessing the risk posed by the presence of toxic compounds in the environment. HHRA is made up of four steps, namely, hazard identification, dose-response assessment, exposure assessment and risk characterization (Ordenez *et al.*, 2003). Health risk assessment classifies elements as carcinogenic or non-carcinogenic. The classification determines the procedures to be followed when potential risks are calculated. Non-carcinogenic chemicals are assumed to have a threshold; a dose below which no adverse health effects will be observed where an essential part of the dose-response portion of a risk assessment includes the use of a reference dose (RfD). Also, carcinogens are assumed to have no effective threshold. This assumption implies that there is a risk of cancer developing with exposures at low doses and therefore, there is no safe threshold for exposure to carcinogenic chemicals. Carcinogens are expressed by their Cancer Potency Factor (Lushenko, 2010). Therefore this study was undertaken to compare the level of hazardous heavy metal, (Lead, Cadmium and Mercury) accumulated in the organs of some edible fish species obtained from Iko River in Eastern Obolo L.G.A and assess the human health risk (non-carcinogenic) posed by their consumption..

2.0 Materials and methods

2.1 Description of the Study Area

Iko River in Eastern Obolo Local Government Area, is located within the Petroleum belt of the Niger Delta, thus rivers in this area are exposed to additional risk of Pollution from Petroleum and related sources. Iko River is located in the Eastern part of the Niger Delta between latitude 4°30'N and 4°45'N and longitude 7°35'E and 7°40'E, the river has a shadow depth ranging from 1.0 metre to 7.0 metres at flood and ebb tide and an average width of 16 metres. Iko River takes its rise from the Qua Iboe River catchment and drains directly into the Atlantic Ocean at the Bight of Bonny (Ekpo, 1995).

2.2 Sample Collection and Preparation

Before sample collection, all quality control precautions were observed regarding sample containers, handling and preservation. All the samples were collected fresh using a cast net from Iko River in Eastern Obolo L.G.A. The fish caught were placed in an ice cooled box for transportation. In the laboratory, the scales were removed carefully, and muscles, gills and liver were scraped out or removed. Subsequently, the fish were dissected and the muscles, liver and gills were dried. Two (2) grams of each fish sample was weighed and separated into different parts (muscles, gills and liver).

Digestion of the fish samples were carried out using Hydrochloric (HCl)/ Nitric acid (HNO₃) following the method of American Society for testing and materials (ASTM, 2009). The digested samples were adjusted to 30 mL with double distilled water and analyzed for Pb, Cd and Hg using the Atomic Absorption Spectrophotometer (Unicam 969 thermo elemental).

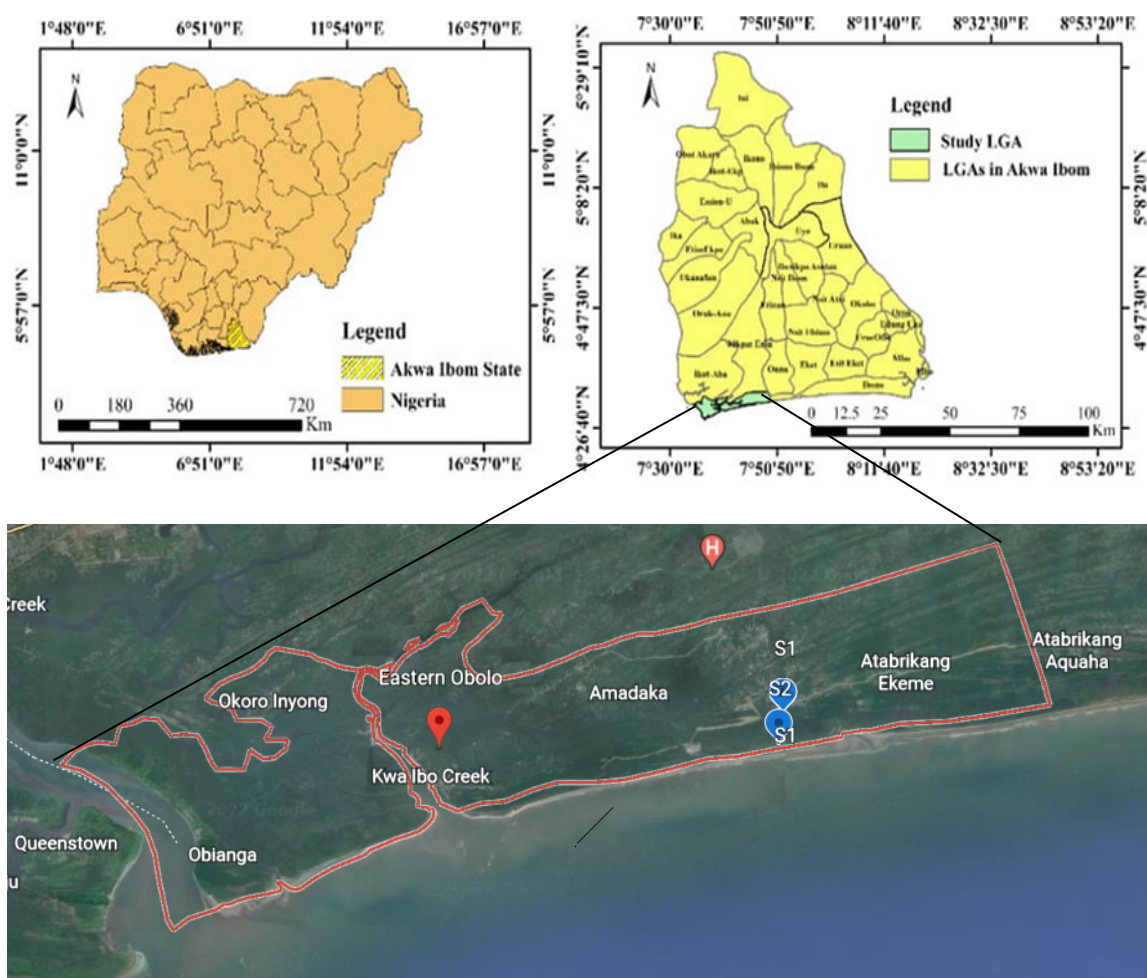


Figure 1. Map showing the study site

The water samples were also obtained in the study river for physicochemical heavy metal analysis. They were placed in pre-cleaned plastic bottles and nitric acid (10mL) was immediately added to prevent any microbial action on the water. They were then taken to the laboratory for digestion. The digestion was done using hydrochloric acid (HCl), Nitric acid (HNO₃), and Perchloric acid (HClO₄) following the American Society for testing and materials method (ASTM, 1986). All the treated samples were analyzed for selected heavy metals; Pb, Cd, and Ni using Atomic Absorption Spectrophotometer. Total dissolved solids (mg/l), Conductivity (μs/cm), and water temperature (°C), were measured using the conductivity meter (DDST-3084), and salinity (%), current (nA) and dissolved oxygen were measured using the DO oxygen meter DC analyzer JPS-605.

2.3 Risk Assessment Procedure

The human health risk assessment (HHRA) of heavy metals content in fish obtained from Iko River was carried out. HHRA is considered as the characterization of potential adverse effects on humans as a result of exposures to environmental hazards (USEPA, 2005). This process employs the tools of science, engineering and statistics to identify and measure a hazard, determine possible routes of exposure and finally use that information to calculate a numerical value to represent the potential risk

(Lushenko, 2010). In this study, hazard identification was accomplished through the collection of fish samples from Iko River and subsequent analysis to quantify the contamination level of trace metals. In order to estimate the non-carcinogenic risks, the dose-response assessment was carried out using standard reference dose (RfD) values for the trace metals considered in this study, as outlined below.

2.3.1 Exposure Assessment

The exposure assessment is the determination or estimation (qualitative or quantitative) of the magnitude, frequency, duration and route of exposure with respect to the chemical contaminant in question (Means, 1989). According to (Lee *et al.*, 2008), it is important to analyze contaminant release, identify populations and potential pathways of exposure as well as estimating intakes for specific pathways. The exposure assessment for ingestion of contaminated fish according to (Means, 1989) may be evaluated using a modified expression below:

$$\text{Estimated Daily Intake (mg}^{-1}\text{kg}^{-1}\text{day}^{-1}\text{)} = \frac{(CF \times IR \times FI \times EF \times ED)}{BW \times AT} \quad (1)$$

Where CF is the chemical concentration in fish (mg/kg), IR is ingestion rate (kg/day), FI is the fraction ingested (unitless), EF is the exposure frequency (365 days/ year), ED is exposure duration, BW is body weight and AT is the average time (period over which exposure is average in days). Specific values for each of the exposure parameters are provided in Table 1:

Table 1. Summary of risk assessment parameters

Symbol	Description	Unit	Value(s)
CF	Metal concentration in fish	Mg/kg	Presented in figures 3.1
*IR	Ingestion rate	Kg/day	Adults = 0.024, children = 0.019
FI**	Fraction ingested	Unitless	1, assuming the whole fish organ is consumed.
EF	Exposure frequency	Days /years	365 days / year
^a ED	Exposure duration	Years	Adults = 70, children = 10
^a BW	Body weight	Kg	Adults = 70, children = 10
AT	Average time	Days	ED x EF
^b RFD	Reference dose	mg/kg/day	Cd =0.001 and Pb = 0.004

*Derived from a similar study conducted in Nigeria on consumption of muscles of cow meat (Ihedioha & Okoye, 2013), (Udosen *et al.*, 2014), and having considered that the ingestion rate proposed by (Means, 1989) for fish and meat consumption are essentially the same; ** (Urban *et al.*, 2009), ^a(Inyang, 2013); ^b (Barlow & Schlatter, 2010)

2.3.2 Evaluation of non-carcinogenic (toxic) risk

Toxic risks can also be called non-carcinogenic effects that would arise as a result of the exposure to contaminant (Lee *et al.*, 2008). The magnitude of the effects is estimated in terms of hazard quotients (HQ) (Yap and Al-Mutairi, 2022; Shah *et al.*, 2012). According to Means, (1989), this is the ratio of the single substance exposure level (i.e. intake) over a specific period to a reference dose for that substance derived from a single exposure period. This may be evaluated by employing the equation below:

$$HQ = \frac{\text{Intake}}{\text{RfD}} \quad (2)$$

The values for the reference dose (RfD) of trace metal considered in this study are listed alongside the risk assessment parameters in Table 1. A hazard quotient (HQ) value that is below one implies that the

level of exposure is not likely to cause any obvious adverse effects (Wang *et al.*, 2005). According to Means (1989) and Lee *et al.* (2008), in a situation where there are multiple toxicants and /or multiple exposure pathways, it is important that their possible interaction be considered. The assumption is that the toxic risk due to potentially hazardous chemicals in the same medium is cumulative. The HQs would then be added up to obtain overall toxic risk, the hazard index (HI) as indicated in the expression below:

$$HI = \sum_{i=1}^n HQ_i \quad (\text{where } i = 1, \dots, n) \quad (3)$$

Where n is the number of trace metals. This computation was carried out for all the three species of fish. If the calculated HI is less than one, then the non-carcinogenic adverse effect due to the exposure pathway or toxicant will be assumed to be negligible.

3.0 Results

3.1 Physico-chemical parameters

The physico-chemical analysis of water obtained from the study site reveal a high salt concentration, especially in site 1. This can be attributed to the closeness of the river to the Atlantic Ocean, especially for site 1 which has the closest proximity to the ocean. For site 2, salinity was 0.03%, TDS (324 mg/l), Conductivity (645 μ S/cm) while site 1: salinity (0.08%), TDS (762 mg/l), Conductivity (1529 μ S/cm). Other physico-chemical parameters are shown on Table 2. Generally, most of these indicators were higher in Site 2 than Site 1, which implies that Site 2 had more dissolved minerals. Mizeb *et al.* (2022) has stated that high EC in water may be due to marine intrusion the sea, which agrees with Table 2 where a higher EC value is observed in Site 1, due to its relatively closer proximity to the ocean.

Table 2. Physico-chemical parameters of water sample

Parameters	(Site1)	(Site 2)
Salinity	0.08%	0.03%
TDS (mg/l)	762	325
Temperature (°C)	27.1	27.1
Conductivity (us/cm)	1529	645
Current I (nA)	1343	1295
pH	6.5	6.9

Warlina *et al.* (2022) has stated that TDS is an important parameter of water quality. TDS indicates the content of inorganic and organic salts contained in aqueous solutions. The main constituents are calcium, magnesium, sulphate, hydrogen carbonate, carbonate cations, potassium, chloride and nitrates (Warlina *et al.* 2022). TDS can be attributed to mineralization from geological formation (Mizeb *et al.*, 2022) or anthropogenic sources. The mean metal concentration in water obtained from site 1 were Pb (15.413 mg/l), Cd (11.927 mg/l), Ni (1.642 mg/l) while for site 2: Pb (12.107 mg/l), Cd (8.649 mg/l), Ni (1.126 mg/l). Generally, site 1 and lead gave the highest metal concentration, followed by cadmium while the least metal concentration was nickel. Lead is transportation-related arising from fuel combustion and rainfall, which washes particles out of the air and stops re-entrainment of particles (Ubong *et al.*, 2011). The origin of the high lead and cadmium levels in the sample area can be linked to the numerous oil spillage and bunkering activities in the region; this should be a major cause for concern, since these metals are very harmful to exposed humans.

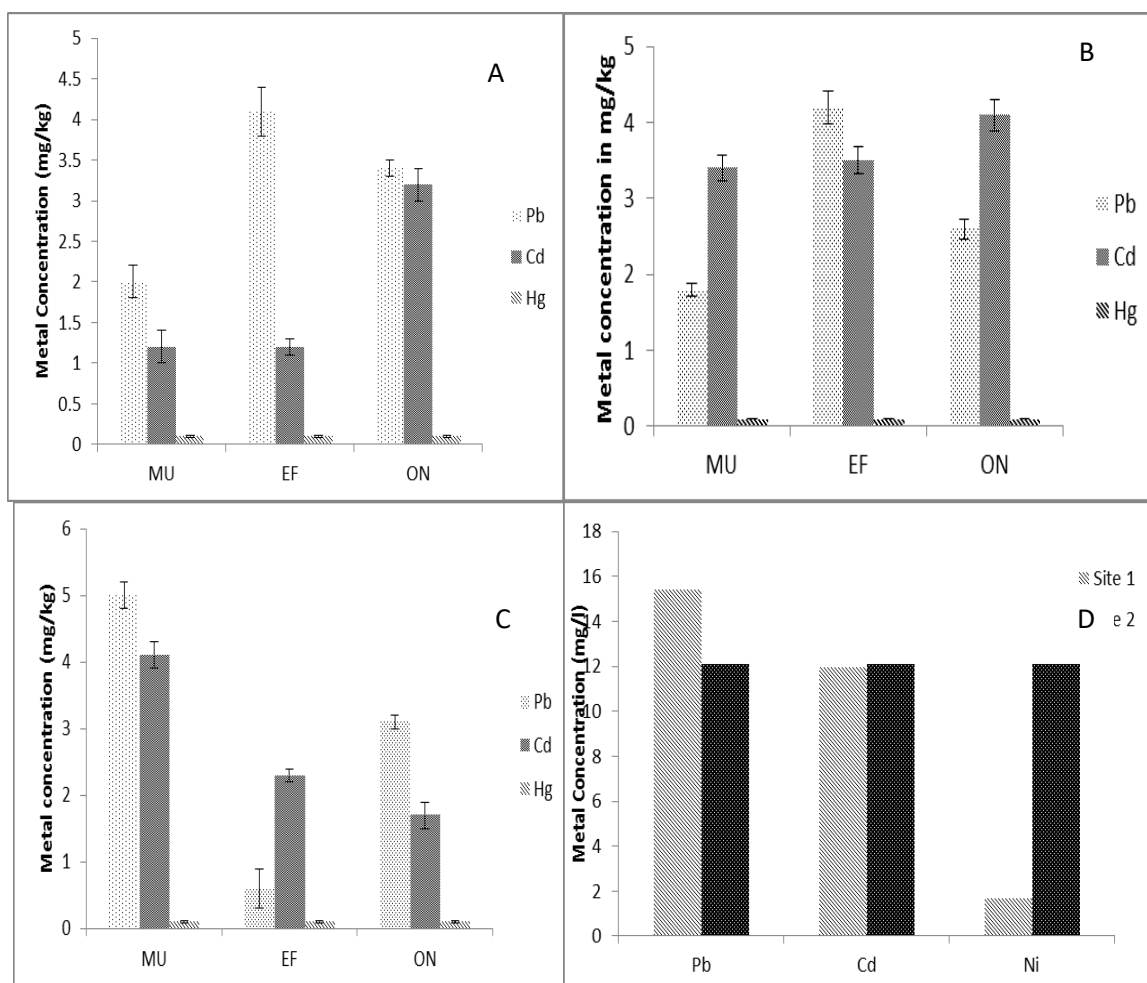


Fig. 2. Metal distribution in fish tissues and water (A) Gills (B) Muscle (C) Liver (D) Water

3.2 Heavy Metal Concentration in Fish Organs

Fish are exposed to waterborne heavy metal fractions when they consume considerable amount of sea water. Therefore, gills serve as the important route of heavy metal cationic exposure from surrounding sea water. The large surface area of gills further facilitates the adsorption of Cd and Pb onto the surface of gills during respiration osmoregulation processes. Metallothionens binding proteins were also found in fish gills which trapped heavy metals compounds (Dallinger *et al.*, 1987; Hasyimah *et al.*, 2011).

3.1.1 Lead

Lead content in the gills of MU ranged between 1.5 – 2.5mg/kg (2.0 ± 0.7 mg/kg), EF ranged from 3.5 – 4.6 mg/kg (4.1 ± 3.0 mg/kg), and while ON ranged between 2.3 – 4.6 mg/kg (3.4 ± 0.1 mg/kg). Muscles of EF had a Pb value ranging between 3.9-4.7 mg/kg (4.2 ± 1.8 mg/kg) which indicated the highest value compared to the muscles of ON with a range of 1.9-3.7 mg/kg (2.6 ± 1.2 mg/kg) and MU ranging from 1.2 - 2.3 mg/kg (1.8 ± 4.0 mg/kg). The highest levels of Pb in the liver of the fish samples was seen in MU (5.0 ± 1.5 mg/kg), when compared to the range (and mean) values of ON and EF which corresponds to 2.9 - 3.3 mg/kg (3.1 ± 0.5 mg/kg) and 0.2 - 1.0 mg/kg (0.6 ± 0.2 mg/kg) respectively (Figure 2). In comparison, the Pb content in the fish tissues were in conformity with the concentration reported by Kong *et al.* (2005). However, the observed values were above the standard limit set by (WHO, 2017) which was 1.0 mg/kg and NSDWQ was 1.0 mg/kg but was below the limit set by FMENV (5.0 mg/kg). Generally, the Pb concentration in the tissues of the fish species were above the standard limit set by W.H.O (2008) as 1.0 mg/kg and NSDWQ as 1.0 mg/kg; while they were below FMENV limit of 5.0

mg/kg. Lead, when consumed by humans, is a well-known neurotoxin; the impairment of neurodevelopment in children is the most critical effect. Exposure in uterus, during breastfeeding and in early childhood may all be responsible for the effects. Lead accumulates in the skeleton and its mobilization from bones during pregnancy and lactation causes exposure to fetuses and breastfed infants (ATSDR, 2007). Silbergeld (2003) has suggested that lead on a cellular and molecular level may permit or enhance carcinogenic events involved in DNA damage, DNA repair, and regulation of tumour suppressor and promoter genes.

3.1.2 Cadmium

Liver of MU had the highest Cd concentration ranging from 3.9-4.4 mg/kg (4.1 ± 2.1 mg/kg), while Cd in liver of EF ranged from 2.0 - 2.5 mg/kg (2.3 ± 0.4 mg/kg) and ON 1.3 - 2.2 mg/kg (1.7 ± 0.1 mg/kg). These concentrations were above the recommended limits set by W.H.O (0.3 mg/kg) and NSDWQ (0.3 mg/kg). Gills of MU and EF had similar Cd values ranging between 1.0 - 1.3 mg/kg (1.2 ± 0.1 mg/kg), while ON recorded a higher mean value of 3.2 ± 0.9 mg/kg (Figure 2). However, the Cadmium content in the muscles of ON had a range value between 3.9-4.4 mg/kg (4.1 ± 0.3 mg/kg) which indicated the highest value, in comparison to EF (3.5 ± 0.9 mg/kg) and MU (3.4 ± 1.6 mg/kg). However, these concentrations were above the recommended limits set by W.H.O and NSDWQ. A similar study by Seddek *et al.* (1996) and Celik & Oehlenschlaeger (2007), reported high levels of Cadmium in fish in the range of 2.0 to 15.8 ppm, which exceeds the levels recorded in most of the samples in this study. Adil *et al.* (2015) pointed out that metal concentrations in the Moroccan canned tuna fish were assessed for human uses according to provisional tolerable weekly and daily intake. The highest metal intake through fish consumption corresponded to Hg ($0.67 \mu\text{g/kg bw/week}$), Cd ($0.09 \mu\text{g/kg bw/week}$) and Pb ($0.064 \mu\text{g/kg bw/week}$). According to Elnimir (2011), some fish in polluted regions may accumulate substantial amounts of metals in their tissues which sometimes exceeded the maximum acceptable levels. Thus, the high incidence of Cadmium in organs in some fish species in this study may be due to the contamination of the aquatic ecosystem by industrial waste of exploration activities in the study area, as well as domestic sewage disposal. According to the International Agency for Research on Cancer (IARC), cadmium is a probable human carcinogen, highlighting the need to ensure that their concentration in the environment is very minimal.

3.1.3 Mercury

Mercury level in the organs of the three fish samples were below the detection limit of the instrument used, indicating that mercury contamination in the study area was very minimal or negligible and that the study area was under no threat of mercury pollution. This should be sustained through continuous monitoring and advocacy to ensure that the aquatic resources are safe for human consumption.

3.2 Human Health Risk Assessment

3.2.1. Exposure assessment for fish consumption

The assessment of human exposure to trace metals in fish species was obtained for adults and children. The estimated dietary intake of trace metals in the edible fishes are summarized in Table 3. The results show that intake values for Pb due to consumption of the investigated fish species collected from the study area had the following trend for the muscles $\text{EF} > \text{ON} > \text{MU}$ for both the adult and children. In the gill and liver, the reverse trend of $\text{ON} > \text{EF} > \text{MU}$ was observed for both adult and children. The values of Pb in the liver were of the trend in which $\text{ON} > \text{MU} > \text{EF}$ for the adult and children (Table 3). The intake values for Cd in the muscles had a trend of $\text{ON} > \text{EF} > \text{MU}$ for the adult and children

while that of the gills was $ON > EF > MU$ for the adult and children. The liver recorded a trend of $MU > EF > ON$ for adult and children, this was similar to the observed intake of Pb in the gills of the fish. The result of Hg was below the detection limit of the equipment used.

The estimated dietary intake of trace metals due to consumption of investigated fish species for both adult and children, collected from the study areas were of the trend $Cd > Pb > Hg$ in the flesh of MU and ON while in the muscles of ON the trend was $Pb > Cd > Hg$. A remarkable change was observed in the gills of all the fish species in which the trend was $Pb > Cd > Hg$. In the liver, a trend of $Cd > Pb > Hg$ was observed for MU and EF while ON had a trend of $Pb > Cd > Hg$ as shown on Table 3.

Table 3. Estimated dietary intake (mg/kg/day) of heavy metals in fish species from Iko River

Fish species	Pb		Cd		Hg	
	Adult	Children	Adult	Children	Adult	Children
MU muscles	0.00062	0.00342	0.00117	0.00646	0.00000	0.00000
EF muscles	0.00144	0.00798	0.00120	0.00663	0.00000	0.00000
ON muscles	0.00089	0.00494	0.00141	0.00779	0.00000	0.00000
MU Gills	0.000686	0.0038	0.00041	0.00228	0.00000	0.00000
EF Gills	0.00141	0.00779	0.00041	0.00228	0.00000	0.00000
ON Gills	0.00117	0.00646	0.00110	0.00608	0.00000	0.00000
MU Liver	0.00051	0.00285	0.00072	0.00399	0.00000	0.00000
EF Liver	0.00021	0.00114	0.00065	0.00361	0.00000	0.00000
ON Liver	0.00106	0.00589	0.00058	0.00323	0.00000	0.00000

3.2.2 Evaluation of non-carcinogenic (toxic) risks

This study examined the different species of fishes contaminated with trace metals in the study area and evaluated the magnitude of toxic harm posed by the fish consumption through the use hazard quotient (HQ) (Table 4).

Table 4. Estimated hazard quotients (HQ) and hazard indices (HI) for adults and children due to dietary intake of heavy metals in fish species from Iko River

Fish Species	Hazard Quotient (HQ)			Hazard indices (HI)
	Pb	Cd	Hg	
Adults				
MU muscles	0.15	1.17	0.00	1.32
EF muscles	0.36	1.20	0.00	1.56
ON muscles	0.22	1.41	0.00	1.63
MU Gills	0.17	0.41	0.00	0.58
EF Gills	0.35	0.41	0.00	0.76
ON Gills	0.29	1.10	0.00	1.39
MU Liver	0.13	0.72	0.00	0.85
EF Liver	0.05	0.65	0.00	0.70
ON Liver	0.27	0.58	0.00	0.85
Children				
MU muscles	0.86	6.46	0.00	7.32
EF muscles	2.00	6.65	0.00	8.65
ON muscles	1.24	7.79	0.00	9.03
MU Gills	0.95	2.28	0.00	3.23
EF Gills	1.95	2.28	0.00	4.23
ON Gills	1.62	6.08	0.00	7.70
MU Liver	0.71	3.99	0.00	4.70
EF Liver	0.29	3.61	0.00	3.90
ON Liver	1.47	3.23	0.00	4.70

All the HQ values calculated for the muscles, gills and liver of the fishes for the Pb consumption by adult and Cd in the liver had value of less than one, while Cd in the muscles and ON of the gills were higher than one for adult. In the children, the Pb content in the MU of the flesh and the MU, EF of the liver were less than one. The HQ values for Cd and Pb in the gills, liver of ON and Cd of all the organs under study were greater than one (Table 4). Hazard index (HI) values were computed and are presented in Table 4. HI values for adult show that the muscles and the gills of ON had values that were higher than one, which implies it can pose harm to humans. All other organs had values that were lower than one for adult. HI values for children exposure were higher when compared to those of adult. All the computed HI values for fish species for children were higher than one, implying a high risk of adverse health effect in children due to their consumption. Statistical analysis showed $P_{0.05} < 0.05$, implies that there was significant difference between the different species of fishes studied.

Conclusion

In conclusion, the fish obtained from the study area (Iko River, Eastern Obolo LGA, Akwa Ibom State) is contaminated with lead and cadmium. However from the study, the level of mercury was within recommended limits, which indicates that the fish species studied were not contaminated with mercury. The continuous consumption of the fish by the populace has disastrous and long lasting effect on human health, since Pb and Cd are known to be hazardous to humans. The lowest Pb mean concentration (0.6 mg/kg) was found in liver of EF while liver of MU recorded the highest Pb concentration of 5.0 mg/kg. Cd concentration in fish organs ranged from 1.2 mg/kg (gills of MU and EF) to 4.1 mg/kg (liver of MU). Generally, these metal values were above the WHO and NSDQW limit. The risk assessment of human exposure to the heavy metals in the fish organs revealed that some of the hazard index values were above safe limit, especially in children exposure. The findings of this study showed that the water body is contaminated with heavy metals such as Pb, Cd, and Ni and hence can contaminate seafood and humans as a whole. The heavy metals concentrations in water were above the permissible limits of national and international standards and hence pose a serious environmental and human health risk. Most of the physicochemical parameters were slightly above the permissible limits of regulatory agencies. Therefore, this equally supports the conclusion that Iko River is heavily contaminated, making the fish and water it holds unfit for human consumption.

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