

**HUMAN HEALTH RISK ASSESSMENT OF TRACE METALS IN WATER,
SEDIMENTS AND EDIBLE FISH SPECIES COLLECTED FROM IDU-URUAN
BEACH, AKWA IBOM STATE, NIGERIA**

ABSTRACT

Aim: This study evaluates human health risk assessment of trace metals particularly (Cr, Cu, Mn, Pb, and Zn) in water, sediments and edible fish species collected from Idu-Uruan Beach, Akwa Ibom State, Nigeria.

Study design: Sediments, water and fish were collected from Esuk River Idu Uruan.

Methodology: The water samples were collected in 1 liter sterile polyethylene plastic container in triplet. Sediments samples were also collected in triplicates from the same site where water samples were sampled and preserved in polyethylene bags. A total of 25 fish samples (5 of each species) were also randomly collected from Esuk River. The total samples collected were 29. The samples were digested with aqua regia (HCl and HNO₃; 3:1) and analysed with an Atomic Absorption Spectrophotometer for Cu, Mn, Cr, Zn and Pb.

Results: Results showed that trace metals were accumulated more in the gills than in the muscles as such consumption of the gills is more detrimental to health than the muscle. Trace metals were accumulated more in water collected at the bank of the river (UWB) than in water collected at the middle of the river (UWA) as such UWB was more polluted than UWA. Also trace metals were accumulated more in sediment collected at the bank of the river (USB) than in sediment collected at the middle of the river (USA), implying that USB was more polluted than USA. The estimated daily intake, hazard index and hazard quotient via ingestion pathway for all the investigated metals were less than unity.

Conclusion: Water and sediments in Esuk River, Idu Uruan is not highly polluted. Frequent monitoring of the aquatic environment is advocated to detect and prevent cumulative effect of trace metal pollutants in edible fishes, which may result in health risk in humans.

Keywords: trace metals, health risk, exposure, Fishes, water, sediment

1. INTRODUCTION

Fish plays a vital role in human diet owing to its high nutritional quality. In recent years, prospective nutritional and therapeutic benefits have made fish a preferred protein source to consumers [1]. Consequently, fish is a vital source of high quality protein contributing about 17% of animal protein and 6.7% of all protein consumed by the world population. Moreover, the polyunsaturated n-3 fatty acids in fatty fish species are biologically important. It contains low cholesterol, and its consumption could reduce the risk of coronary heart disease, decrease mild hypertension and prevent certain cardiac arrhythmias.

In the aquatic food chain, fishes are good biomarkers for long term monitoring of metal pollution due to their accumulation of high concentrations of metals in tissues [2]. Using fishes as biological indicators can help detect the temporal and spatial changes in trace metals and help understand pollution trends in water bodies [3]. Furthermore, fish plays key roles in ecosystems because they are vertebrates, which are at the top of the food chain. Trace metal intakes by edible fish species in polluted aquatic environment depends on ecological requirements, metabolisms and other factors such as salinity, water pollution level, food and sediment. Trace metals are common components of natural water, but the increase in their concentrations due to different human activities have resulted in the contamination of these aquatic environments and pose problems to man through the food chain.

It is said that water is the “life-blood of the biosphere.” Since water is a universal solvent, it dissolves different organic and inorganic chemicals and environmental pollutants. Aquatic ecosystems, both freshwater and marine, are vulnerable to pollution. Contamination of water resources by heavy metals is a critical environmental issue which adversely affects plants, animals, and human health [4]. Environmental pollution has become an extensive and dangerous problem as a consequence of industrial and human activities [5]. A very important fact about heavy metal poisoning is that they are not easily excreted out of the body, and their effect in the body is not immediate; therefore, individuals can accumulate the heavy metal over a long period of time without knowing, only to manifest much later in life [6]. One source of heavy metals in the aquatic ecosystems is effluents from mining operations. Other sources of water contamination with heavy metals include different industrial effluents, domestic sewage, and agricultural run-off. After release from both natural and anthropogenic sources, trace metals contaminate natural water bodies, sediments, and soils. Since most of the inhabitants in most rural communities do not have access to modern facilities, some communities that are located along streams and rivers discharge their waste directly into such water bodies [7]. The contamination of aquatic system by heavy metals, especially in sediments has become one of the most challenging pollution issues owing to its toxicity, abundance, persistence and subsequent bioaccumulation in benthic organisms [8,9]) Serious check on water sources must be made by ensuring appropriate treatment of the water sources and a total avoidance of indiscriminate dumping of garbage near these sources in order to ascertain their portability [10]. Contamination of biota and groundwater

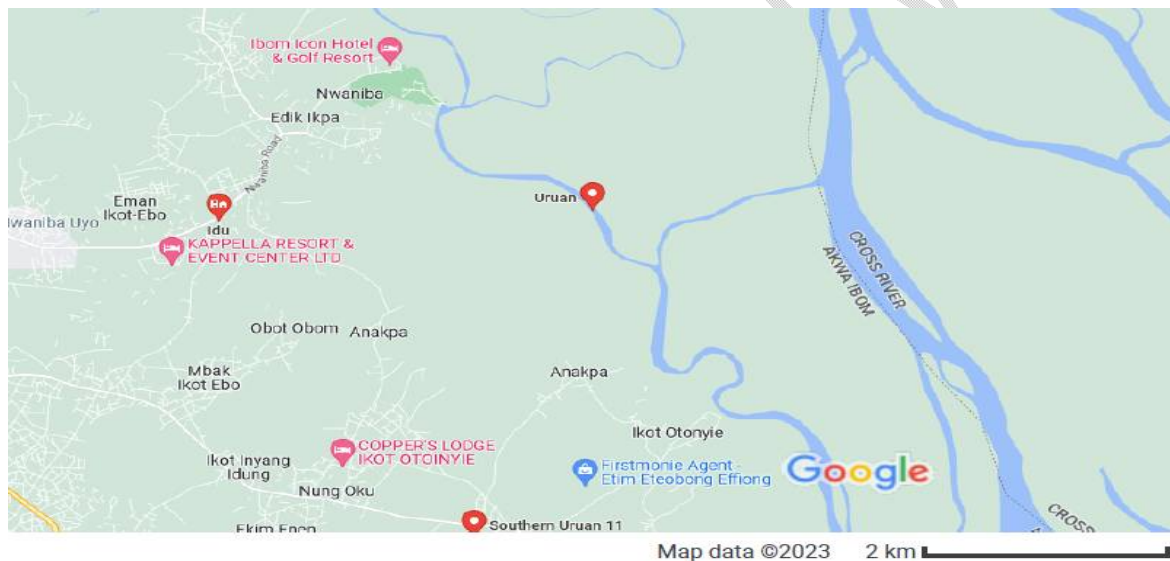
with potentially toxic heavy metals has important implications for human health. It is important to assess the degree of heavy metal pollution in riverine ecosystems by investigating the concentrations of these elements and their distribution.

The health risk assessments are based on assumptions that most chemicals with non-carcinogenic effects exhibit a threshold response. It may be seen as a systematic procedure for making estimate of all possible significant risk factors that exist over an entire range of failure mode or exposure scenarios associated with some type of hazards. In accomplishing a comprehensive risk assessment procedure, four distinct phases of procedure are generally considered; hazard identification, exposure assessment, dose response assessment and risk characterization. Several methods have been proposed for estimation of the potential risks to human health of trace metals in fishes. The risks may be divided into carcinogenic and non-carcinogenic effects. Risks assessment is one of the fastest method which is needed to evaluate the impact of the hazards on human health and also needed to determine the level of treatment which tend to solve the environmental problem that occur in daily life [11]. In addition, estimated daily intake (EDI), Hazard quotient (HQ) and hazard index (HI) are used to assess human health risks posed by these trace metals. Trace metals contaminations of food is considered to be one of the most significant exposure to human health risk. The risk of metal contamination through food is attributed to trace metals toxicity, non-biodegradability, biological amplification in the food chain, and the capability of metals to bioaccumulate in organic tissues. Some researchers have worked on heavy metals in fish and its effect on human consumption such as [9,12,13,14,15,16,17,18,19,20,21,22,23,24]. In fact, this has been considered as the main route of exposure to trace metals for human beings. Since they pose serious health problem to human health. Increase in particulate matter air contamination and their negative impacts on human health have resulted in efforts to monitor and identify the various fractions [25]. The aim of this study is to evaluate human health risk assessment of trace metals particularly (Cr, Cu, Mn, Pb, and Zn) in water, sediments and edible fish species collected from Idu-Uruan Beach, Akwa Ibom State, Nigeria, as well as evaluate the non-carcinogenic risk caused by these trace metals.

2. MATERIAL AND METHODS

2.1 Description of the study area

Esuk Odu is a village in Uruan Local Government Area of Akwa Ibom State in Nigeria. Esuk Odu major language is Ibibio and they are enriched with culture. The most prominent occupation among the Esuk Odu residents is agriculture, trading, clay moulding, arts and crafts, canoe/boat building and fishing. Esuk River is a freshwater ecosystem with several aquatic resources. The capital of Uruan is Idu. The area lies in the rain forest belt with extensive arable land and the region abounds with the wildlife, raffia palm and timber. The rich coastal plains support the cultivation of crops such as cassava and maize. The mineral deposit includes; crude oil, silicon/glass sand, natural gas, sulphur nitrate, limestone and clay deposits. The Figure and table showing the study area and sampling sites are seen in Fig. 1 and Table 1.



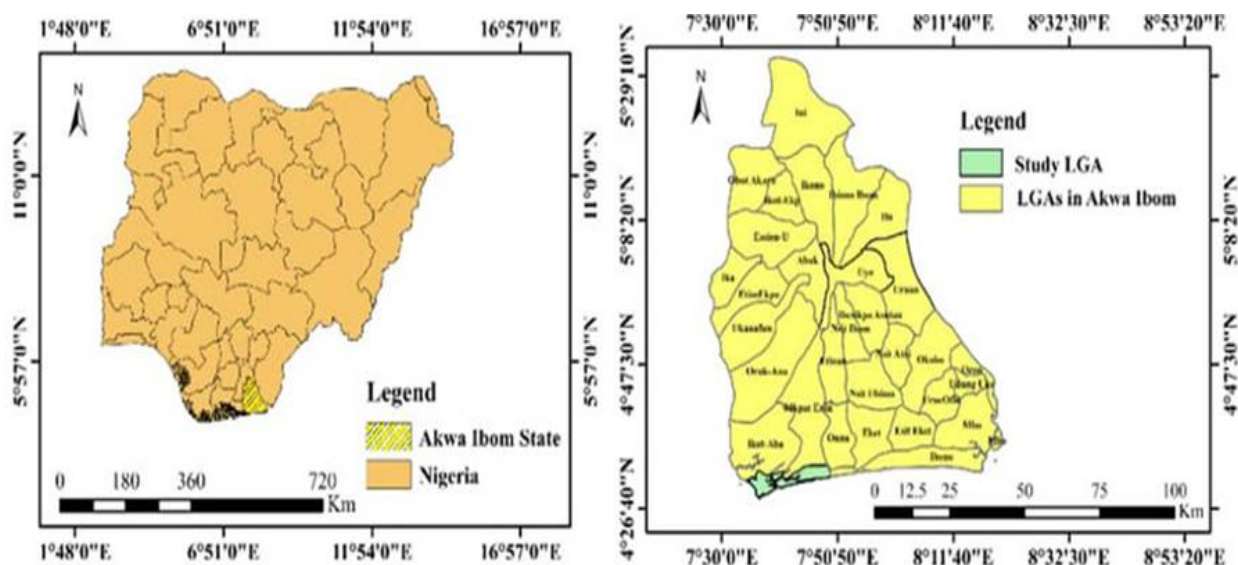


Fig. 1: Map of the Study Area Showing Sampling Stations Compiled Using Open Street Map Database and Fieldwork

Table 1: Global Positioning System (GPS) Coordinates for the Sampling Site and Station Code

Stations	Sampling Location	Latitudes	Longitudes
Sf	Idu River (Esuk)	N 5°2' 53.106"	E 8°2' 48.834"
Ss1	Idu River (Esuk)	N5°2' 44.616"	E 8°2' 47.946"
Ss2	Idu River (Esuk)	N 5°2' 46.188"	E 8°2' 48.174"
Sw1	Idu River (Esuk)	N 5°2' 47.796"	E 8° 2' 48.63"
Sw2	Idu River (Esuk)	N 5°2' 52.98"	E 8° 2' 48.762"

2.2 Sample Collection

The sediment, water and fish were collected from Esuk River, Idu Uruan. The water samples were collected in 1 liter sterile polyethylene plastic container in triplet. Sediment samples were also collected in triplet from the same site where water samples were collected and preserved in polyethylene bags. A total of 25 fish samples (5 of each species) were also collected from Esuk River. The total samples collected were 31. The fish species were randomly collected with local

wooden traps and nets to avoid sample contamination with the aid of a fisherman. The species collected were *Carangoides fulvaguttatus* (CF), *Poropuntius laoensis* (PL), *Crenimugil seheli* (CS), *Xenomystus nigri* (XN), and *Sardina pilchardus* (SP). After collection, fish samples were iced in an insulated box and immediately transported to the laboratory. The fish sample species were identified in the zoology laboratory of Akwa Ibom State University.

2.3 Sample Pre-Treatment

The fish samples were washed with distilled water and the scales were removed. The gills and muscles of each fish were removed using a plastic knife and oven dried at 80-85 °C to a constant weight. At this stage, adequate care was taken to avoid any source of contamination especially for micro nutrient analysis. The samples were removed, allowed to cool, and then the dried fish tissues were individually grounded and homogenized into tiny powder with a clean mortar and pestle and sieved with a 100 mesh sieve; thereafter, stored in polythene bags prior to digestion. The water samples were filtered through Whatman filter paper with 0.45 mm pore size to remove suspended particles. Some drops of nitric acid were added to each of the water sample immediately after collection at the sampling site to lower the pH below 2 in order to preserve the water sample until it is digested. The sediment sample was air dried, ground with mortar and pestle and sieved through a 100 mesh sieve before they were digested.

2.4 Sample digestion

Fish and sediment samples (2 g each), as well as water sample (2 ml) were digested with 20 ml of aqua regia (HCl and HNO₃) in the ratio 3:1 (375 ml of HCl and 125 ml of HNO₃) in a fume hood. Anti-bumping agent was added to each beaker to avoid bumping during the digestion. The digested samples were allowed to cool, filtered and then transferred into a 100 ml volumetric flask and diluted to the mark with distilled water; thereafter transferred into sample bottles for Atomic Absorption Spectrophotometer (AAS) analysis.

2.5 Sample analysis

Trace metals determination in the samples were performed using Atomic Absorption Spectrophotometer (AAS, Perkin Elmer-2280, USA). The analysis of the standard, reagent blanks

and replicates were all run in the same way and concentrations were determined using standard solutions prepared in the same acid matrix. All samples were analyzed for Cu, Mn, Cr, Zn, and Pb using Atomic Absorption Spectrophotometer (AAS, Perkin Elmer-2280, USA).

2.6 Health risk estimation

Human health risk has been estimated considering the metal concentrations in the fish species, in relation with the estimated daily intake (EDI) of the studied trace metals by human through oral reference dose [26]. Assessment of non-carcinogenic risk was also conducted through the hazard quotient (HQ) equation.

2.6.1 Estimated daily intake (EDI)

Health risk was estimated considering the average concentrations of all fish tissues and daily heavy metal intake (EDI) following equation (3.1) below, as reported by [27].

$$EDI = \frac{CF \times IR \times FI \times ED \times EF}{BW \times AT} \quad (1)$$

Where: CF is the chemical concentration of fish (mg/kg), IR is ingestion rate (0.024 kg/day for adult and 0.019 kg/day for children), FI is the fraction ingested (1, assuming that the whole fillet is consumed), EF is exposure frequency (365 days/year), ED is exposure duration (70 years for adult and 10 years for children), BW is the body weight (70 years for adult and 10 years for children) and AT is the average exposure time in days (ED × EF) [9,27].

2.6.2 Evaluation of non-carcinogenic health hazard

The magnitude of the effects is estimated in terms of hazard quotient (HQ). This according to the USEPA is the ratio of the single substance exposure level (that is, EDI) over a specified period to a reference dose for that substance derived from a single exposure period [28]. This may be evaluated by employing the equation below:

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

The values for the reference dose, (RfD) of trace metals considered in this study are Cr = 1.50, Cu = 0.04, Mn = 0.14, Zn = 0.30 and Pb = 0.004 [29, 30]. Hazard quotient below 1 implies that the level of exposure is not likely to cause any obvious adverse effects [7, 26].

Furthermore, in a situation where there are multiple toxicants or multiple exposure pathways, it is important that their possible interactions be considered [28]. The assumption is that the toxic risk due to potentially hazardous chemicals in the same medium is cumulative. The THQs would then be added up to obtain overall toxic risk, the hazard index (HI) as indicated in the expression below;

$$HI = \sum_{i=1}^n HQ \quad (3)$$

Where n is the number of trace metals. This computation would be carried out for all the five 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 [7].

3. RESULTS AND DISCUSSION

3.1 Levels of trace metals in fish species

Cr is an essential metal in humans and some animals, but the occurrence of excessive levels of it is regarded as a potential hazard which can endanger both fish and human health. The mean concentrations of Cr in the muscles of fish species ranged from 0.00 to 10.80 mg/kg with highest amount found in the muscle of *Sardina pilchardus* (10.80 mg/kg) and the lowest in *Crenimugil seheli* and *Poropuntius laoensis* (0.00 mg/kg). These values were below the proposed limit by the FAO/WHO [31] (2.0 mg/kg) for human consumption except for *Sardina pilchardus* species. Mean Cr concentrations in the muscle tissues of *Xenomystus nigri* in this study were found to be higher than the data reported by [27, 32] and in the selected literature presented in Table 3. However, the concentrations of Cr in the gills of the fish species ranged from 0.00 to 28.81 mg/kg with highest in *Xenomystus nigri* (45.68 mg/kg) and the lowest in *Poropuntius laoensis*, *Sardina pilchardus* and *Sardina pilchardus* species (0.00 mg/kg). Cr concentrations in the gills decrease in the following order: *Xenomystus nigri* > *Carangoides fulvaguttatus* > *Sardina Pilchardus* > *Crenimugil seheli* > *Poropuntius laoensis*. The mean concentrations of Cr in the gills tissue of all

fish species considered in this study were higher than the concentrations reported by [33] except of *Crenimugil seheli* and *Poropuntius laoensis*.

Pb is not essential for fish, and excessive amounts can cause deficits or decreases in the survival, and growth rates, as well as development and metabolism, in addition to increased mucus formation. The levels of Pb found in the muscles and gills of the different fish species considered in this study were below the detection limits (0.00 mg/kg). This indicates that all the fish species in this study are considered safe for human consumption since they do not contain lead which is very dangerous to human health. Furthermore, the levels of Lead in the fish species organs considered in this study were below the values reported in some selected literature (Table 3).

As seen in Table 2, mean concentrations of Cu in the muscles of fish species ranged from 11.40-13.40 mg/kg with highest amount in *Carangoides fulvaguttatus* (13.40 mg/ kg) and the lowest amount in *Poropuntius laoensis* (11.40 mg/kg). Cu concentrations in the muscles of the fish species decrease in the following order: *Carangoides fulvaguttatus* > *Crenimugil seheli* > *Sardina pilchardus* > *Xenomystus nigri* > *Poropuntius laoensis*. Mean Cu concentrations in the muscle tissues of *Xenomystus nigri* in this study were found to be higher than the data reported for *Xenomystus nigri* with 0.03 µg/g in Enyong Creek, Itu [27] but lower than the reported data for *G. chapra* in Baghdad by [33] with 21.80 mg/kg and higher than those of some selected literature (Table 3). The mean Cu concentrations in the gills of fish species ranged from 24.52-41.53 mg/kg. Cu concentrations in the gills decrease in the following order: *Sardina pilchardus* > *Poropuntius laoensis* > *Xenomystus nigri* > *Carangoides fulvaguttatus* > *Crenimugil seheli*. Copper concentrations in the gills of the fish species investigated in this study were higher than the WHO/FAO [31] limits except of the gills of *Crenimugil seheli*.

Zinc is a trace element that can be accumulated in fatty tissues of fish. According to FAO/WHO [31], the maximum permissible limit for zinc in fish muscle is 50 mg/kg. In this study, Zn concentrations in the examined fish muscles ranged from 0.00-2,101.00 mg/kg dry weight, with the highest concentration recorded in *Poropuntius laoensis* (2,101.00 mg/kg) and the lowest concentration observed in *Crenimugil seheli*(0.00). The levels of Zn recorded in the muscles of examined fish in this study were higher than those reported in some selected literature (Table 3). Based on the values obtained, Zn concentrations found in muscles of all fishes are below the

proposed limit by the FAO/WHO[31] for human consumption of toxic compounds except for *Poropuntius laoensis* and *Carangoides fulvaguttatus* species. Moreover, the level of Zinc in the gills of the analyzed fish species were below the detection limits except of *Sardina pilchardus*(10.51 mg/kg). The highest amount of Zn was found in the muscles of *Poropuntius laoensis* (2,101.00 mg/ kg). The levels of Zn recorded in the gills of the examined fish in this study were higher than those reported in some selected literature (Table 3) except for the report of [34] in *Labeo rohita* collected at Kolkata wetland, India. Based on the values obtained, Zn concentrations found in gills of all fishes are below the proposed limit by the FAO/WHO [31] for human consumption of toxic compounds.

Table 2: Mean Concentration of the Trace Metals in the Fish Organs in mg/kg and FAO/WHO Permissible limits

S/N			Cu	Zn	Pb	Cr	Mn
1	MUSCLES	CFM	13.40	558.00	0.00	1.35	45.30
		PLM	11.40	2101.00	0.00	0.00	40.75
		CSM	13.25	0.00	0.00	0.00	52.55
		XNM	12.15	19.80	0.00	0.65	4.35
		SPM	12.70	38.60	0.00	10.80	5.60
2	GILLS	CFG	30.27	0.00	0.00	28.40	2.13
		PLG	39.49	0.00	0.00	0.00	75.90
		CSG	24.52	0.00	0.00	2.33	120.82
		XNG	36.14	0.00	0.00	45.68	256.82
		SPG	41.53	10.51	0.00	27.97	0.51
WHO/FAO[31]			30.00	50.00	1.30	2.00	1.00

Carangoides fulvaguttatus muscles (CFM), *Poropuntius laoensis* muscles (PLM), *Crenimugil seheli* muscles (CSM), *Xenomystus nigri* muscles (XNM), *Sardina pilchardus* muscles (SPM), *Carangoides fulvaguttatus* gills(CFG), *Poropuntius laoensis* gills (PLG), *Crenimugil seheli* gills (CSG), *Xenomystus nigri* gills (XNG), and *Sardina pilchardus* gills (SPG). Guideline values obtained from Udosen *et al.*, (2014)

Mn is naturally present in the environment, which has played a significant role in the biological system to maintaining metabolic regulation and certain homeostatic. It is also an essential co-factor for pyruvate carboxylase and superoxide dismutase. The Mn deficiencies may cause poor reproductive performance, growth retardation, congenital malformations in offspring, and abnormal function of bone and cartilage. Manganese tends to have accumulated to high

concentrations in muscles and gills in most of the fish species considered in this study than other trace metals. Showing highest levels of 52.55 mg/kg for the muscles of *Crenimugil seheli* and lowest levels of 4.35 mg/kg for *Xenomystus nigri*. Mn concentrations in the muscles of the fish species decrease in the following order: *Crenimugil seheli* > *Carangoides fulvaguttatus* > *Poropuntius laoensis* > *Sardina pilchardus* > *Xenomystus nigri*. The mean concentrations of Mn in the muscle tissues of *Xenomystus nigri* in this study were found to be higher than the data reported for *Xenomystus nigri* (0.74 µg/g) in Enyong Creek, Itu [27]. The levels of Mn in the fish species analyzed in this study were higher than those of some selected literature (Table 3). Considering the gills, the highest amount of Mn was found in *Xenomystus nigri* (256.82 mg/kg) and the lowest amount of Mn concentrations was observed in *Sardina pilchardus* (0.51 mg/kg). The mean concentrations of Mn in the gills of fish species ranged from 0.34 to 257.50 mg/kg. Generally, Fig. 2 & 3 reveals that trace metal was accumulated more in the gills than in the muscles as such consumption of the gills is more detrimental to health than the muscle.

Table 3: Levels of Trace Metals in the Fish Organs reported in the literature (mean values in mg/kg dry weight)

Sample location/ References	Fish species	Organs					
			Cu	Zn	Pb	Cr	Mn
Kolkata wetland, India [34]	<i>Labeo rohita</i>	Muscles	3.93	24.26	0.16	0.63	0.47
		Gills	3.39	19.73	0.26	0.59	1.51
Shirinsu wetland, Iran [35]	<i>Cyprinus carpio</i>	Muscles	0.01	0.007	0.015		
		Gills	0.26	0.53	0.009		
Enyong creek, Itu [27]	<i>Xenomystus nigri</i>	Muscles	0.03	0.32	0.04	0.18	0.74
Baghdad [33]	<i>B. sharpeyi</i>	Muscles	1.10	1.05	1.50	2.20	
		Gills	0.50	0.90	2.05	2.50	

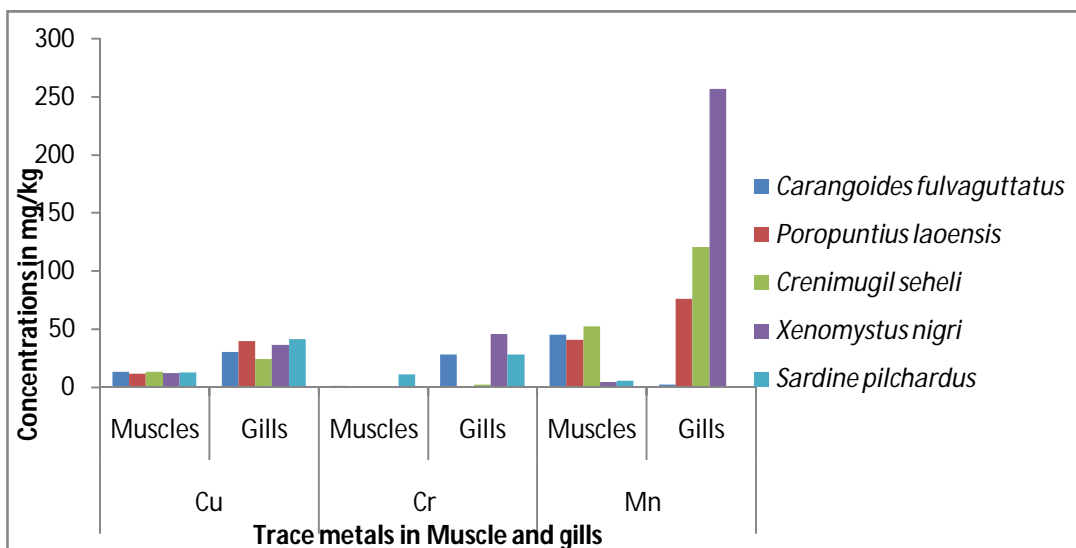


Fig 2: Comparison of Mean trace metal Concentrations (mg/kg dry weight) in the Muscles and gills of the different Fish Species

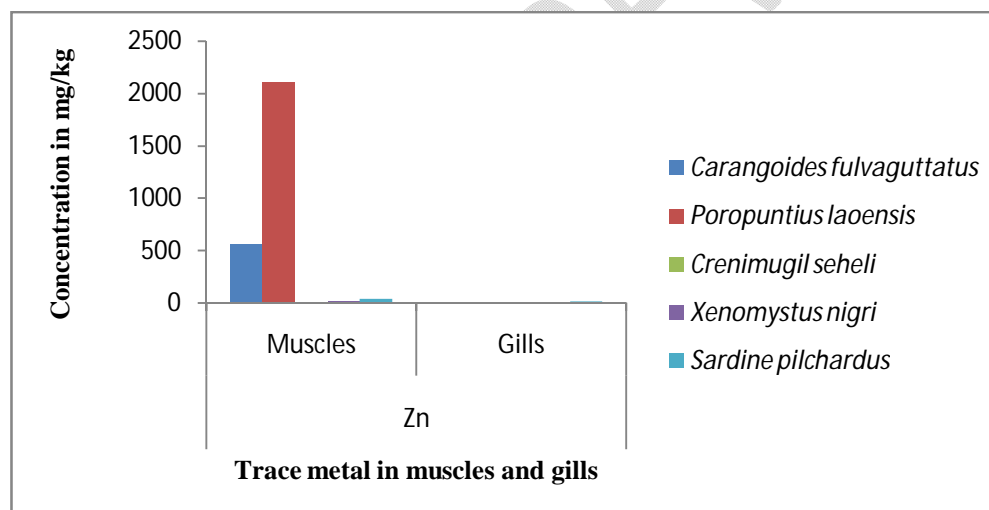


Fig 3: Comparison of Mean Zn Concentrations (mg/kg dry weight) in the Muscles and gills of the different Fish Species

3.2 Levels of trace metals in water samples in mg/L

Based on WHO (2011), the permissible limit of Copper in water is 2.00 mg/L. In this study, the mean level of Cu analysed in the water samples were 9.50 mg/L for (UWB- water collected at the bank of the river) and 10.00 mg/L for (UWA- water collected at the middle of the river) (Table 4). The mean levels of Cu in the water samples analyzed in this study were above the permissible limits based on WHO (2011). Copper concentrations in this study was higher than those reported by [36] in both dry (0.36 mg/L) and wet (0.07 mg/L) season collected from Ogbere River, Ibadan.

However, the Cu value (9.307 mg/L) reported by [37] in Marsa Matrouh, Mediterranean, Egypt River was close to the value obtained in this study.

The mean levels of Mn analysed in the water samples were 10.81 mg/L (UWA) and 46.35 mg/L (UWB) (Table 4). Based on WHO [38], the permissible limit of Mn in water is 0.4 mg/L and the level of Mn in the water samples analyzed in this study were above this permissible limits. The higher Manganese concentrations may be as a result of addition from agricultural runoff, sewage which causes anemia and kidney damage. The values obtained for Mn in this study were higher than those reported by [34] with 1.92 mg/L and [36] in both dry (3.73 mg/L) and wet (4.27 mg/L) season collected from Ogbere River, Ibadan.

In this study, as seen in Table 4, the level of Zinc in the water samples analyzed were below the detection limits. Zinc is insoluble and therefore cannot easily be dissolved in water unless oxidized with heated oxygen. Since the density of Zn is higher in water, there is the possibility of it settling at the bottom of the water body. This may account for the limited quantity of Zn in the water. Various anthropogenic activities in this study area might not result in by-product with Zn being leached into the water body. Kumar [34] reported the value of Zn of (4.65 mg/L) compared to the value obtained in this study.

Lead is a persistent toxic element with no biological role and causes carcinogenic effects in aquatic systems and the human population. In aquatic systems, Pb may come from industrial and smelter discharges, lead containing pesticides, precipitation, the fallout of lead dust, street runoff, and municipal wastewater. Fish can bioaccumulate Pb from water and diet. However, there is evidence that Pb accumulation in fish most probably originated from contaminated water rather than diet. As seen in Table 4, level of Lead in the water samples analyzed in this study were below the detection limits and based on [38], the permissible limit of Lead in water is 0.01 mg/L. This indicates that the water samples collected from Esuk River were not contaminated by lead. [39] reported a higher value (16.712 mg/L) in Marsa Matrouh, Mediterranean, Egypt River compared to the levels obtained in this study.

Based on [38], the permissible limit of Chromium in water is 0.1 mg/L. The water samples analyzed in this study for Cr showed that UWA had mean concentration of 0.95 and UWA 0.07 mg/L (Table 4). The values of Cr obtained in this study were lower than those reported by [34]

with 7.31 mg/L but higher than those reported by [36], who reported the levels of Cr in water collected from Ogbere, Ibadan to be 0.47 mg/L and 0.05 mg/L in dry and wet season respectively. Fig. 3 reveals that trace metal was accumulated more in water collected at the bank of the river (UWB) than in water collected at the middle of the river(UWA) as such UWB was more polluted than UWA.

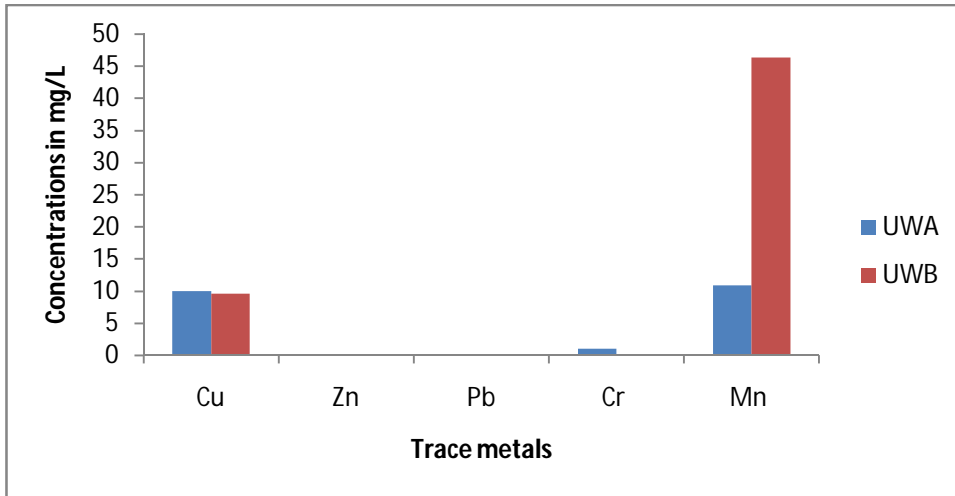


Fig. 4: Comparison of the trace metals concentrations (mg/L) in the water samples collected in this study

Table 4: Mean Concentration of the Trace Metals in the Water (mg/L) and Sediment in mg/kg and WHO Permissible limits

S/N	Sample	Code	Cu	Zn	Pb	Cr	Mn
1	Water	UWA	10.00	0.00	0.00	0.95	10.81
2	Water	UWB	9.50	0.00	0.00	0.07	46.35
3	Sediment	USA	14.70	0.00	0.00	1.65	0.40
4	Sediment	USB	17.40	0.00	0.00	6.30	35.05
	Sediment WHO (2011)		50.00	300.00	50.00	100.00	2000
	Water WHO (2011)		2.00	3.00	0.01	0.1	0.4

UWB- water collected at the bank of the river, UWA- water collected at the middle of the river, USB- sediment collected at the bank of the river, USA- sediment collected at the middle of the river

3.3 Levels of trace metals in sediment samples in mg/kg

The mean level of Cu obtained in this study were 14.70 in the sediment from middle of the river (USA) and 17.40 mg/kg in the sediments collected from the bank of the river (USB). The value

(17.15 mg/kg) reported by [40] were close to the value obtained in this study. Cu concentrations in this study were higher than those reported by [41], but lower than the permissible limit of 50.00 mg/kg based on [38].

Based on [38], the permissible limit of Chromium is 100 mg/kg. In this study, the mean level of Cr analysed in the sediment samples were from 1.65(USA) and 6.30 (USB) mg/kg. The values obtained in this study were lower than those reported by [40] 40.92 mg/kg of Cr.

The mean level of Mn obtained in this study were 0.40 mg/kg (USA) and 35.05 mg/kg (USB) (Table 4). The values (72.54 mg/kg) reported by [34] were higher than the values obtained for Mn in this study. Based on WHO [38], the values obtained in this study were below the permissible limit of 2000 mg/kg.

In this study (table 4), the levels of Zinc and Lead in the sediment samples analyzed were below the detection limits. Based on WHO [38], the permissible limits of Zinc and Lead in sediment are 300 mg/kg and 50 mg/kg respectively. The value of 100.96 mg/kg and 96.85 mg/kg were reported by [40] and [41] respectively compared to the value obtained for Zn in this study. This contradicts the value stated in the preceding sentence for [41].

In conclusion, Fig. 4 reveals that trace metal was accumulated more in sediment collected at the bank of the river (USB) than in sediment collected at the middle of the river(USA) as such USB was more polluted than USA.

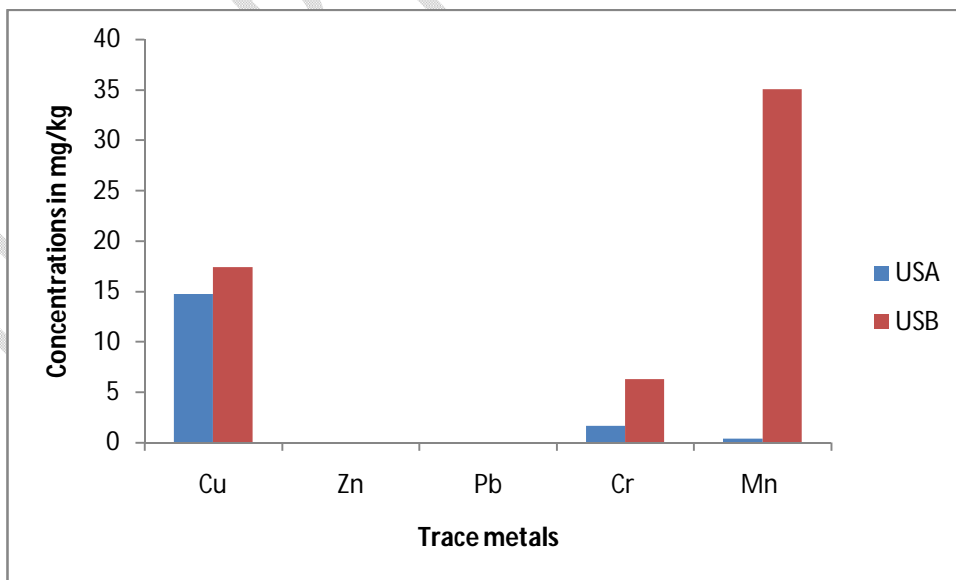


Fig. 5: Comparison of the trace metals concentrations (mg/kg) in the sediment samples collected in this study

3.4 Human Health Risk Assessment

3.4.1 Exposure assessment for fish consumption

The evaluation of human exposure to trace metals in the muscles and gills of the fish species, sediments and water samples were obtained for adults and children. The estimated dietary intake of trace metals in the edible muscles and gills of the fish species, sediments and water samples collected from Esuk are summarized in Table 5. The results reveal that trace metal intake due to consumption of the investigated muscles of the fish species followed the trend $Zn > Mn > Cu > Cr > Pb$ for both adults and children, while the trend for the gills were $Mn > Cu > Cr > Zn > Pb$ for both adults and children. Metal exposure from water and sediments collected from the study area followed the order; $Mn > Cu > Cr > Zn \& Pb$ for both adults and children. Zn, Mn, Cu and Cr showed major contribution through the consumption of edible muscles and gills of the fish species, sediments and water samples for both adults and children; however, Pb was the observed exception. Contribution to trace metals intake through the consumption of the studied fish species muscles were as follows; *Poropuntius laoensis* > *Carangoides fulvaguttatus* > *Sardina pilchardus* > *Crenimugil seheli* > *Xenomystus nigri* for both adults and children respectively. The implication of these results is that consumption of more muscles of *Poropuntius laoensis* would lead to more intakes of trace metals. However, contribution to trace metals intake through the consumption of the gills of the fish species were of the trend *Xenomystus nigri* > *Crenimugil seheli* > *Poropuntius laoensis* > *Sardina pilchardus* > *Carangoides fulvaguttatus* for both adults and children respectively. The implication of these results is that consumption of more gills of *Xenomystus nigri* would lead to more intakes of trace metals. Also contribution to trace metals intake through the water and sediments collected from Esuk were of the trend water/sediments collected from the bank of the river > water/sediments from the middle of the river for both adults and children. These results shows that both the sediments and water collected from the bank of the river (USB) are more polluted than that of the middle of the river (USA); thus the continuous intake of the water by fish or human would leads to adverse health effects.

Table 5: Estimated dietary intake (mg/kg/day) of trace metals in fish species, sediments and water for Children and adults

COD E	Cu		Zn		Pb		Cr		Mn	
	Childre n	Adult n	Childre n	Adult n	Childre n	Adult n	Childre n	Adult n	Childre n	Adult n
CFM	0.0255	0.004 0	1.0602	0.167 4	0.0000	0.000 0	0.0026	0.000 4	0.0861	0.013 6
PLM	0.0217	0.003 4	3.9919	0.630 3	0.0000	0.000 0	0.0000	0.000 0	0.0774	0.012 2
CSM	0.0252	0.004 0	0.0000	0.000 0	0.0000	0.000 0	0.0000	0.000 0	0.0998	0.015 8
XNM	0.0231	0.003 6	0.0376	0.005 9	0.0000	0.000 0	0.0012	0.000 2	0.0083	0.001 3
SPM	0.0241	0.003 8	0.0733	0.011 6	0.0000	0.000 0	0.0205	0.003 2	0.0106	0.001 7
CFG	0.0575	0.009 1	0.0000	0.000 0	0.0000	0.000 0	0.0540	0.008 5	0.0040	0.000 6
PLG	0.0750	0.011 8	0.0000	0.000 0	0.0000	0.000 0	0.0000	0.000 0	0.1442	0.022 8
CSG	0.0466	0.007 4	0.0000	0.000 0	0.0000	0.000 0	0.0044	0.000 7	0.2296	0.036 3
XNG	0.0687	0.010 8	0.0000	0.000 0	0.0000	0.000 0	0.0868	0.013 7	0.4878	0.077 0
SPG	0.0789	0.012 5	0.0200	0.003 2	0.0000	0.000 0	0.0531	0.008 4	0.0010	0.000 2
UWA	0.0190	0.003 0	0.0000	0.000 0	0.0000	0.000 0	0.0018	0.000 3	0.0205	0.003 2
UWB	0.0181	0.002 9	0.0000	0.000 0	0.0000	0.000 0	0.0001	0.000 0	0.0881	0.013 9

USA	0.0279	0.004	0.0000	0.000	0.000	0.000	0.0031	0.0008	0.000
		4		0		0		5	1
USB	0.0331	0.005	0.0000	0.000	0.000	0.000	0.0120	0.0666	0.010
		2		0		0		9	5

Carangoides fulvaguttatus muscles (CFM), *Poropuntius laoensis* muscles (PLM), *Crenimugil seheli* muscles (CSM), *Xenomystus nigri* muscles (XNM), *Sardina pilchardus* muscles (SPM), *Carangoides fulvaguttatus* gills (CFG), *Poropuntius laoensis* gills (PLG), *Crenimugil seheli* gills (CSG), *Xenomystus nigri* gills (XNG), and *Sardina pilchardus* gills (SPG), UWB- water collected at the bank of the river, UWA- water collected at the middle of the river, USB- sediment collected at the bank of the river, USA- sediment collected at the middle of the river

3.4.2 Evaluation of toxic (non-carcinogenic) risks

The hazard quotient (HQ) helps in the evaluation of the magnitude of harm posed to the consumers of fish species contaminated with trace metals. In this study, all the HQ values calculated for adults consuming both the muscles and gills analysed, were all below unity indicating low or no toxic risks are associated with them. An exception was the muscles of *Poropuntius laoensis* that accumulated 2.1010 mg/kg of Zinc (Table 6). The HQ calculated for children consuming the muscles of *Xenomystus nigri*, *Crenimugil seheli*, *Carangoides fulvaguttatus*, *Poropuntius laoensis* and *Sardina pilchardus* were all below unity indicating low or no toxic risks. However, the HQ calculated for children consuming the muscles of the *Carangoides fulvaguttatus*(3.5340) and *Poropuntius laoensis*(13.3063) of Zinc were higher than unity indicating possible risk associated with their consumption (Table 6). The HQ calculated for children consuming the gills of *Xenomystus nigri*, *Poropuntius laoensis*, *Sardina pilchardus*, *Carangoides fulvaguttatus*, *Crenimugil seheli* and Mn of *Crenimugil seheli*, *Poropuntius laoensis* and *Xenomystus nigri* were higher than unity indicating possible risk associated with their consumption. Furthermore, HQ calculated for children consuming the gills of every other studied species analysed were lower than unity indicating low or no toxic risks with their consumption except the hazard quotient for Cu in all the fish species and Mn in some fish species. The HQ value was highest for Zn for the exposed populace and lowest in Pb (Table 6). The HQ of water and sediments for both adults and children were below unity indicating that there are no harmful effects for exposed humans (Table 6).

In this study, it was assumed that the toxic risk due to potentially hazardous chemicals in the same medium was cumulative; therefore, the summation of the HQs gave the overall toxic risk, which is the hazard index. On a general note, as seen in Table 6, all the computed HI values for the muscles, gills, water and sediment were below unity except for the muscles of *Poropuntius laoensis*(2.2808). For children, HI values for *Xenomystus nigri*(muscles), *Sardina pilchardus*(Muscles), and UWA were below

unity indicating that there are no harmful effects to the exposed human population (Table 6) while every other studied species were higher than unity implying harmful effect for them.

Table 6: Hazard Quotient and Hazard Index of trace metals in fish species, sediments and water for Children and Adults

COD E	Cu		Zn		Pb		Cr		Mn		HI	
	Childr en	Adult	Childr en	Adult	Childr en	Adult	Childr en	Adult	Childr en	Adult	Childr en	Adult
CFM	0.6881	0.1086	3.5340	0.5580	0.0000	0.0000	0.0171	0.0003	0.6148	0.0971	4.8540	0.7637
PLM	0.5854	0.0924	13.3063	2.1010	0.0000	0.0000	0.0000	0.0000	0.5530	0.0873	14.4448	2.2808
CSM	0.6804	0.1074	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.7132	0.1126	1.3936	0.2200
XNM	0.6239	0.0985	0.1254	0.0198	0.0000	0.0000	0.0008	0.0001	0.0590	0.0093	0.8092	0.1278
SPM	0.6522	0.1030	0.2445	0.0386	0.0000	0.0000	0.0137	0.0022	0.0760	0.0102	0.9863	0.1557
CFG	1.4405	0.2454	0.0000	0.0000	0.0000	0.0000	0.0360	0.0057	0.0289	0.0046	1.5054	0.2557
PLG	2.0279	0.3202	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	1.0300	0.1626	3.0579	0.4828
CSG	1.2591	0.1988	0.0000	0.0000	0.0000	0.0000	0.0030	0.0005	1.6397	0.2589	2.9018	0.4582
XNG	1.8558	0.2930	0.0000	0.0000	0.0000	0.0000	0.0579	0.0091	3.4854	0.5503	5.3991	0.8525
SPG	2.1326	0.3367	0.0665	0.0105	0.0000	0.0000	0.0354	0.0056	0.0069	0.0011	2.2415	0.3539
UWA	0.5135	0.0811	0.0000	0.0000	0.0000	0.0000	0.0012	0.0002	0.1467	0.0232	0.6614	0.1044

UW	0.07	0.00	0.00	0.00	0.00	0.629	0.09	0.17
0.4878	0.0000	0.0000	0.0000	0.0001	0.00	0	93	1.1170
B	70	00	00	00	00	0	93	64
	0.11	0.00	0.00	0.00	0.005	0.00	0.12	
USA	0.7549	0.0000	0.0000	0.0021	4	09	0.7624	0.04
	92	00	00	00	0.475	0.07	0.21	
USB	0.8935	0.0000	0.0000	0.0080	7	51	1.3772	0.74
	11	00	00	00	7	51	74	

Carangoides fulvaguttatus muscles (CFM), *Poropuntius laoensis* muscles (PLM), *Crenimugil seheli* muscles (CSM), *Xenomystus nigri* muscles (XNM), *Sardina pilchardus* muscles (SPM), *Carangoides fulvaguttatus* gills (CFG), *Poropuntius laoensis* gills (PLG), *Crenimugil seheli* gills (CSG), *Xenomystus nigri* gills (XNG), and *Sardina pilchardus* gills (SPG), UWB- water collected at the bank of the river, UWA- water collected at the middle of the river, USB- sediment collected at the bank of the river, USA- sediment collected at the middle of the river

4. CONCLUSION

In this study, the levels of five trace metals (Cr, Cu, Mn, Zn, and Pb) in the muscles and gills of five different fish species, water and sediments collected from Esuk Idu-Uruan, Akwa Ibom State, Nigeria were determined and the dietary intake as well as the potential non-carcinogenic risk posed by these trace metals were assessed. The results obtained showed that Chromium accumulated more in the gills than in the muscles while Zinc accumulate much in the muscles than in the gills. Zn concentrations in the muscles of the fish species decrease in the following order: *Poropuntius laoensis* > *Carangoides fulvaguttatus* > *Sardina pilchardus* > *Xenomystus nigri* > *Crenimugil seheli*. On a general note, all the computed HI values for adults in the muscles, gills, water and sediment were below unity except for the muscles of *Poropuntius laoensis* that accumulated 2.2808. For children, HI values for *Xenomystus nigri*(muscles), *Sardina pilchardus* (Muscle), and UWA were below unity indicating that they pose no harmful effects for humans while every other studied species were higher than unity implying adverse health risks. In this study, all the HQ values calculated for adults consuming both the muscles and gills analysed, were all below unity indicating that low or no toxic risks are associated with them except for the muscles of *Poropuntius laoensis* that accumulated 2.1010 mg/kg of Zinc. Manganese concentrations in the gills decrease in the following order: *Xenomystus nigri* > *Crenimugil seheli* > *Poropuntius laoensis* > *Carangoides fulvaguttatus* > *Sardina pilchardus*. Manganese concentrations in both muscles and gills of the fish species investigated in this study were higher than the WHO/FAO (1989) limits of 1.00 mg/kg except for the gills of *Sardina pilchardus*.

REFERENCES

1. Ezemonye I, Adebayo O, Enuneku A, Tongo I, Ogbomida E. Potential health risk consequences of heavy metal concentrations in surface water, shrimp (*Macrobrachium macrobrachion*) and fish (*Brycinus longipinnis*) from Benin River, Nigeria. *Toxicol. Rep.*, 2018; 6: 1-9.
2. Briaudeau T, Zorita I, Cuevas N, Franco J, Marigomez I, Izagirre U. Multi-annual survey of health status disturbance in the Bilbao estuary (Bay of Biscay) based on sediment chemistry and juvenile sole (*Solea* spp.) histopathology. *Mar. Pollut. Bull.* 2019; 145: 126 –137. doi: 10.1016/j.marpolbul.05.034
3. Azizi G, Layachi M, Akodad M, Yanez R, Martin I, Baghour M. Seasonal variations of heavy metals content in mussels (*Mytilus gallo provincialis*) from Cala Iris offshore (Northern Morocco). *Res.* 2018;137, 688694. doi:10.1016/j.marpolbul.06.052
4. Rezanian S, Taib S, Mddin F, Dahalan A, Kamyab H. Comprehensive review on phytotechnology: heavy metals removal by diverse aquatic plants species from wastewater,” *Journal of Hazardous Materials*, 2016; 318: 587–599.
5. Nwadinigwe CA, Udo GJ, Nwadinigwe AO. Investigation of heavy metal concentrations in leaves of *Telfairia Occidentalis* Hook F. (fluted pumpkin) in Nigeria. *Pol. J. Environ. Stud.*, 2015; 24(4): 1733-1742.
6. Nwadinigwe CA, Udo GJ, Nwadinigwe AO. Seasonal variations of heavy metals concentrations in sediment samples around major tributaries in Ibeno coastal area, Niger Delta, Nigeria. *International journal of scientific & technology research*, 2014; 3(11): 254-265.
7. Okori BS, Ekanem AN. Physicochemical, Spectroscopic and Bacteriological Analyses of Surface and Ground Water in Epeni Ekor, Yakurr Local Government Area, Cross River State- Nigeria. *Journal of Environmental Treatment Techniques*, 2022; 10(1); 67-75
8. Fu J, Tao X, Yu H, Zhang X. Risk and Toxicity Assessments of Heavy metals in sediments and fishes from the Yangtze River and Taihu Lake, China, *Chemosphere*, 2013; 93(9):1887-1895.
9. Ubong UU, Ekwere IO, Ekanem AN, Ite AE. 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. *J. Mater. Environ. Sci.* 2023; 14(20): 384-394.
10. Ubong UU, Ubong IU, Ubong EU, Etukudo OU. Assessment of Quality of Potable Water Sources in Eket Local Government Area of Akwa Ibom State, Nigeria, *International Journal of Advanced and Innovative Research*, 2015; 4(8): 2278-7844.
11. Yujie Z, Bowu Z, Linfan L, Ziqiang W, Siyuan X, Yue S, Jingye L. Radiation induced reduction: an effective and clean route to synthesize functionalized graphene. *Journal of Materials Chemistry*, 2012; 22(16): 7775.
12. Ali H, Khan E. “What are heavy metals? Long-standing controversy over the scientific use of the term ‘heavy metals’-proposal of a comprehensive definition,” *Toxicological & Environmental Chemistry*, 2018; (100)1: 6–19.
13. Signa G, Mazzola A, Tramati D, Vizzini S. Diet and habitat use influence Hg and Cd transfer to fish and consequent biomagnification in a highly contaminated area: Augusta Bay (Mediterranean Sea). *Environ. Pollu.* 2017; 230: 394–404. doi:10.1016/j.envpol.06.027.
14. Mannzhi P, Odiyo J. Assessment of selected trace metals in fish feeds, pond water and edible muscles of *Oreochromis mossambicus* and the evaluation of human health risk associated with its consumption in Vhembe district of Limpopo Province, South Africa. *Toxicology Reports*, 2021; 8: 705-717. <https://doi.org/10.1016/j.toxrep.03.018>.
15. Esilaba F, Moturi N, Mokua M, Mwanyika T. Human Health Risk Assessment of Trace Metals in the Commonly Consumed Fish Species in Nakuru Town, Kenya. *Environmental health insights.* (2020); 14,1178630220917128. <https://doi.org/10.1177/1178630220917128>.
16. Jakimska A, Konieczka P, Skora K, Namiesnik J. Bioaccumulation of metals in tissues of marine animals, part II: metal concentrations in animal tissues. *Pol. J. Environ Stud.* 2011; 10: 1127–1146.
17. Yousafzai M, Chivers P, Khan R, Ahmad I, Siraj M. Comparison of heavy metals burden in two freshwater fishes *Wallago attu* and *Labeo dyocheilus* with regard to their feeding habits in natural ecosystem. *Pak. J. Zool.* 2010; 42: 537–544.
18. Ihedioha N, Amu A, Ekere R, Okoye B. Determination of level of some trace metals (Pb, Cd and Ni) and their possible health risks from consumption of selected fish and shellfish from Nigerian markets. *International Food Research Journal*, 2016; 23(6): 2557-2563.
19. Kortei N, Clement T. Health risk assessment and levels of toxic metals in fishes (*Oreochromis niloticus* and *Clarias anguillaris*) from Ankobrah and Pra basins: Impact of illegal mining activities on food safety. *Toxicology Reports*. 2020; 7: 360-369. <https://doi.org/10.1016/j.toxrep.02.011>

20. Sun T, Wu F, Wang Q, Ji L, Shan J, Li F. Evaluation on the biomagnification or bio-dilution of trace metals in global marine food webs by meta-analysis. *Environ. Pollut.* 2020; 264: 113856. doi: 10.1016/j.envpol.2019.113856
21. Liu Q, Xu Q, Zeng N, Shi L, Liao B, Du P. Heavy metal concentrations in commercial marine organisms from Xiangshan Bay, China, and the potential health risks. *Mar. Pollut. Bull.* 2019; 141: 215–226. doi: 10.1016/j.marpolbul.02.058
22. Patrick I, Kingsley C, Mercy O, Ahiakwo B, Chukwuebuka E. (2022). Evaluated the metal levels in seafoods from three open market in Bayelsa State, Nigeria. *Chem. Biol. Interact.* 174:183-192.
23. Wilk A, Kalisińska D, Kosik-Bogacka I. "Cadmium, lead and mercury concentrations in pathologically altered human kidneys," *Environmental Geochemistry and Health*, 2017; 39(4): 889–899.
24. Dorsey A, Ingerman L, Swarts S. Toxicological Profile for Copper. Atlanta, GA: Agency for Toxic Substances and Disease Registry. 2014.
25. Ubong IU, Ubong UU, Ubong EU, Ukonta R, Ishmael D. Distribution of particulate matter in Cawthorne channels air basin in Nigeria, *Environment and pollution*, 2015; 4(3):19-26.
26. Wang X, Sato T, Xing B. Health risks of heavy metals to the general public in Tianjin, China via consumption of vegetables and fish. *Sci. Total Environ.* 2005; 350: 28–37.
27. Udosen E, Offiong N, Alade I. Human Health Risk Assessment of Trace Metals due to Dietary Intake of some Edible Fish Species collected from Enyong Creek, Itu–Nigeria, Paper presented at the A Paper Presented at the 37th International Conference of Chemical Society of Nigeria held at Uyo Nigeria. 2014
28. USEPA, (US Environmental Protection Agency) (1989). Risk Assessment Guidance for Superfund. *Human Health Evaluation Manual (Part A), Interim Final. EPA 540/1–89/002 United States Environmental Protection Agency, Washington, DC.*
29. USEPA, (US Environmental Protection Agency) (2010): Risk-Based Concentration Table. Available at <http://www.epa.gov/reg3hwmd/risk/human/index.htm> (Accessed April 15, 2018).
30. Nadal M, Ferre-Huget N, Mart'i-CidR. Exposure to metals through the consumption of fish and seafood by population living near the Ebro River in Catalonia, Spain: health risks. *Hum. Ecol. Risk Assess.* 2008; 14: 780–795.
31. FAO/WHO. National Research Council Recommended Dietary Allowances (10th ed). National Academy Press, Washington, DC. USA. 1989.
32. Alipour H, Banagar R. Health risk assessment of selected heavy metals in some edible fishes from Gorgan Bay, Iran. *Iranian Journal of Fisheries Science* 2016; 17(1): 21-34. doi: 10.22092/IJFS.115582
33. Mensoor M, Said, A. Determination of Heavy Metals in Freshwater fishes of the Tigris River in Baghdad. *Fishes*, 2018; 3(2): 23. doi:10.3390/Fishes3020023
34. Kumar N, Chandan K, Bhushan, S, Singh D, Satish K. Health risk assessment and metal contamination in fish, water and soil sediments in the East Kolkata Wetlands, India, Ramsar site. *Scientific Reports*, 2023; 13:1546. <https://doi.org/10.1038/s41598-023-28801-y>
35. Ardakanil S, Jafari M. Assessment of Heavy Metals (Cu, Pb and Zn) in Different Tissues of Common Carp (*Cyprinus carpio*) Caught from Shirinsu Wetland, Western Iran. *Journal of Chemical Health Risks*, 2014; 4(2), 47–54.
36. Achi C, Omoniyi A, Coker A. Distribution of selected toxic elements in water phases of River Ogbere, Ibadan, Nigeria. *Journal of Environmental protection*, 2021; 12: 429-432. doi: 10.4236/jep.127026.
37. Khaled A, Abdel-Halim A, El-sherif Z, Mohamed, L. Health risk assessment of some heavy metals in water and sediments at Marsa-matrouh, Mediterranean sea, Egypt. *Journal of Environmental protection*, 2017; 8: 74-97. doi: 10.4236/jep.81607.
38. World Health Organization (2011). WHO Guidelines for Drinking Water Quality, 4th ed.; WHO Publications: Geneva, Switzerland; pp. 307–340, ISBN 978 92 4 154815 1.
39. Khaled A, Abdel-Halim A, El-sherif Z, Mohamed L. Health risk assessment of some heavy metals in water and sediments at Marsa-matrouh, Mediterranean sea, Egypt. *Journal of Environmental protection*, 2017; 8: 74-97. doi: 10.4236/jep.81607.
40. Zulkifli S, Mohamat-yusuff F, Arai F, Ismail A, Miyazaki N. An assessment of selected trace element in intertidal surface sediment collected from the Peninsular, Malaysia. *Environmental monitoring and assessment*. 2010; 169(1/4): 457-472.
41. Ahmad K, Shuhaimiothman M. Heavy metal concentrations in sediments and fishes from lake Chini, Pahangmalaysia. *Journal of Biological sciences*. 2010; 10: 93-100.doi:10.3923.

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