

Human Health Implications of Heavy Metal Levels in Three Fish Species from

Lagos Lagoon

*Francis Olumide Oladapo and Helen Abisoye Taiwo

Department of Chemical Sciences, Tai Solarin University of Education, Ijagun, Nigeria

*Corresponding Author: oladapofo@tasued.edu.ng

Accepted: August 25, 2024. Published Online: September 3, 2024**ABSTRACT**

Fish exposure to heavy metals can affect their physiological well-being and economic value. This study examined the levels of selected heavy metals in some fish species sampled from Lagos lagoon and characterized the health risk implications for fish consumers. The fish samples were dissected into different organs, washed, drained of water, weighed and oven-dried to a constant weight. The samples were pulverized and moistened followed by dry-ashing and acid digestion with a mixture of nitric acid, hydrochloric acid and hydrogen peroxide (3:2:1; v/v). The digests were analysed using the atomic absorption spectroscopic (AAS) technique. The results showed that the mean concentrations (in mg/kg) of heavy metals in various organs of the fish samples ranged as follows: Silver catfish (*Chrysichthys nigrodigitatus*), Pb: 12.30 – 14.85, Cu: 9.15 – 11.25, Zn: 4.90 – 6.10, Ni: 3.90 – 4.73, Co: 2.35 – 2.75, Cd: 2.18 – 3.33; tilapia (*Oreochromis niloticus*), Pb: 11.75 – 13.00, Cu: 11.25 – 14.30, Zn: 5.60 – 6.60, Ni: 7.58 – 9.73, Co: 1.83 – 2.25, Cd: 1.50 – 1.70, and catfish (*Clarias batrachus*), Pb: 10.40 – 11.25, Cu: 8.75 – 11.45, Zn: 8.35 – 8.80, Ni: 1.72 – 1.92, Co: 0.44 – 0.50, Cd: 1.30 – 1.45. The heavy metal pollution index occurred in ascending order of *Clarias batrachus* (3.26) < *Chrysichthys nigrodigitatus* (5.27) < *Oreochromis niloticus* (5.57). The Hazard Index (HI) risks that the heavy metals can pose in adults and children consumers are, respectively, 6.72×10^{-4} and 6.71×10^{-4} for *Clarias batrachus*, 7.75×10^{-4} and 7.95×10^{-4} for *Oreochromis niloticus* and 1.04×10^{-3} and 1.01×10^{-3} for *Chrysichthys nigrodigitatus*. The carcinogenic risk (CR) of heavy metals' consumption in fish species investigated in adults and children are projected as follows: Tilapia, adult - 6.84×10^{-7} , children - 7.98×10^{-7} ; catfish, adult - 4.10×10^{-7} , children - 3.78×10^{-7} ; and silver catfish, adult - 6.10×10^{-7} , children - 6.16×10^{-7} . The CR index showed that consumption of these fish species over a lifetime is safe. The entry of carcinogenic metals into the lagoon via effluent discharges should be mitigated.

Keywords: Heavy metal, fish organs, toxicity, hazard quotient, hazard index, carcinogenic risks.

INTRODUCTION

Fishes are aquatic animals that are essential for a healthy and balanced diet. They are rich in amino acids, vitamins, unsaturated fatty acids, eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), long-chain polyunsaturated omega-3 fatty acid (n=3) and trace elements [1, 2]. They are a rich source of essential nutrients, including minerals such as magnesium, selenium, heme iron, zinc, calcium, and phosphorus, as well as vitamins, such as vitamins A, B, B₁₂, niacin, and vitamin D [3]. In the nutritional context, animal proteins are superior to plant proteins because they contain high levels of polyunsaturated fatty acids and all essential amino acids in optimal proportions [3-5]. Fish consumption is associated with a reduction in coronary heart diseases such as arrhythmia, hemodynamics, myocardial infarction and cardiac arrest [6-8].

In spite of the benefits of fish consumption, studies, exposure of fish to environmental pollutants, such as heavy metals [9] dioxins [10], polychlorobiphenyls (PCBs) [10], PBDEs [11], chlorinated pesticides [12,13] and personal care and pharmaceutical products (PCPPs) [14,15], can raise the stress level and alter the physiological, and genetic frameworks of fish. Although some heavy metals, such as Cu and Zn, are beneficial for fish metabolism [16], heavy metals such as cadmium, chromium, mercury, lead, and nickel are non-beneficial and can cause severe toxicity in fish, which can result in acute oxidation stress [9,17]. In effect, oxidation stress will induce a weakened immune system, which can lead to developmental defects and damaged tissue and organs [18, 19].

Literature has cases of reduced fecundity, deformities reproductive organs, diminished gonadosomatic index (GSI), reduced hatching rate, and general underwhelming reproductive performances arising from heavy metal toxicity [20-23]. On the cellular and nuclear levels, deformities associated with blood cells and genetic deformities have been reported [24, 25]. Several developmental anomalies, such as reduced heart performance, elevated heart rate, increased death rate, and malformations in the shape and structure of the vertebral column at various embryonic and larva stages of fish development, have been published [26-29]. In addition, chronic human exposure to heavy metal-contaminated fish can lead to a wide range of health problems, including ill health and mortality. Since most heavy metals are carcinogenic, teratogenic, and mutagenic, serious health problems, such as hepatic and kidney disorders, heart problems, and death, can arise [18,19].

Heavy metals are non-biodegradable in fish and are stored in various fish organs. Studies have shown that the interspecific variation in heavy metals' level of bioaccumulation exists in fish organs subject to their organ type, the metal type, the fish species and the fish size [30]. Eneji *et al.*, [31] reported that the levels of heavy metals in tilapia fish and *Clarias gariepinus* were in descending order of gills < intestine < fish muscles. Fish muscles are commonly considered the most ideal organ to consume (due to reduced toxicity), a few other fish organs, such as the heart, gill, liver, kidney, and intestine, are occasionally consumed. The interest in other fish organs depends on the size and nature of the fish in spite of pollution and toxicity concerns [32]. Some fish species are toxic for consumption as some naturally have biotoxins or are contaminated with chemical substances, such as mercury [33, 34].

Given that consumers inherit the heavy metals that bioaccumulate in fish, frequent exposure may provoke health problems in either immediate or nearest future. Clinically, heavy metal toxicity in fish cannot be directly extrapolated to predict the health of fish consumers. It is not clear if fish toxicity can modulate commensurate health trajectory in human consumers. Studies had been carried out on water, sediment, fish and aquatic invertebrate animal samples in Lagos lagoon, along Makoko axis by Adetutu *et al.* [35]; along the University of Lagos axis by Ajibare and Loto [36]; and along Ologe and Badagry axes by Bassey and Chukwu [37]. Several other works had been conducted along the Lagos lagoon, but due to the volume of domestic, industrial and commercial discharges released into the Lagoon daily, it is necessary to periodically quantify the heavy metals' pollution load indices and, the human health risks' data of these aquatic organisms. Therefore, this work investigated the levels of heavy metals in various organs of three fish species found around Ebute-Metta, one of the highly industrially impacted areas of Lagos lagoon, and to evaluate the likely health risks to humans through consumption of such fish and their organs.

MATERIALS AND METHODS

Description of the Study Area

The Ebute-Metta axis of the Lagos lagoon is situated at a longitude of 3.390° E and a latitude of 6.480° N (Figure 1). The Lagos lagoon extends from Epe to Badagry in Lagos State, Nigeria. People with diverse backgrounds live and ply their trades along the Lagoon. The Lagoon belt is shallow (below 3.2 m were not dredged) receiving effluents from industrial, domestic and commercial sources from within the state and the neighbouring states, such as Ogun, Oyo and

Ondo, thereby impacting on both the water body and the sediment of the Lagoon in that axis. Fish farmers carry out their enterprise on the Lagoon and sell their catch to the people.

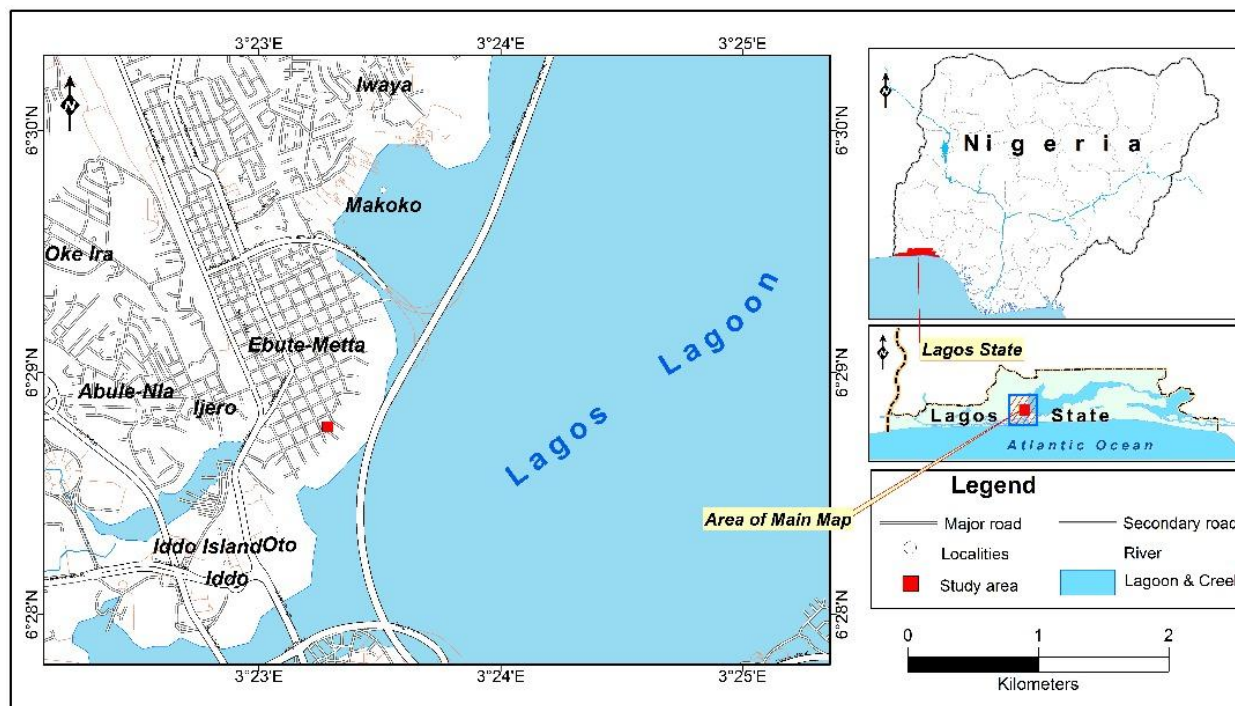


Figure 1: The Map of the Study Area Showing the Ebute-Metta Section of the Lagos Lagoon

Sample Collection and Preparation

Fish species, namely catfish (*Clarias batrachus*), tilapia (*Oreochromis niloticus*) and silver catfish (*Chrysichthys nigrodigitatus*), harvested from the Lagoon during the dry season, were purchased from the fishermen and transported in ice chest to the laboratory. The fish samples were washed with water, descaled, weighed and dissected with rust-free stainless knives. Sterile scissors were used to remove the organs: heart, gill, intestine, kidney, liver, and muscle. They were wrapped in foil paper before drying. The organs were dried in an oven at 105 °C till a constant weight was reached [38]. The dried samples were pulverized with a ceramic mortar and pestle and then stored in the refrigerator at -20 °C before digestion.

Digestion and Analysis of Fish Samples

About 5 g of pulverized fish organ was subjected to dry-ashing at 550 °C for 6 h. It was then cooled down, moistened with water and treated with a mixture of concentrated nitric acid, hydrochloric acid, and hydrogen peroxide (3:2:1; v/v) as used by Olmedo with slight

modifications [39]. The sample solution was digested in an aluminium block set on a hot plate at 90 °C for 45 min. The sample solution was withdrawn from the hot plate and allowed to cool. The digest was washed with water into a funnel laced with Whatman (No. 1) filter paper and 25 mL of distilled water was added to the filtrate to make the final solution. The percentage of acid-insoluble ash content was derived from ashing the residue at 550 °C for 4 h. Then the ash was cooled, moistened with water and acidified with 2 mL conc. HCl. The resulting solution was reduced to dryness. The digest was analyzed for the selected heavy metals using a 700 Atomic Absorption Spectrometer coupled to an air-acetylene burner head and single element hollow-cathode lamp (Perkin Elmer, USA). This procedure was repeated for all the fish organs in this study.

Toxicological Risk Assessment

This was determined for the three fish species. These toxicological risk indices, namely, the metal pollution index (HMPI), total hazard quotient (THQ), hazard quotient (HQ), hazard index (HI), and cancer risk, were evaluated using Integrated Risk Assessment Information System model developed by the United States Environmental Protection Agency [40].

Heavy Metal Pollution Index (HMPI)

The HMPI of the fish species can be calculated using the formula in equation (1)

$$HMPI = \sqrt[n]{M_{Pb} \times M_{Cu} \times M_{Zn} \times M_{Ni} \times M_{Co} \times M_{Cd}} \quad (1)$$

where n = 6 i.e. the number of metals considered in the study. M stands for the concentration of each metal in the fish species being studied.

Potential Heavy Metal Exposure Probability via Fish Organ Consumption

While a few fish organs are hygienically eatable, many others are not. The potential heavy metal exposure level through consumption of fish organs would require evaluation of regularly and infrequently consumed fish organs by humans. Whereas the muscle can be categorized as a frequently consumed fish organ, the others are not. For any fish organ to be considered safe and eatable, it must be mostly fleshy, void of chemical contaminants and having minimal presence of diseases-inducing microbes [41]. In addition, consumption eligibility is equally associated with the nature and size of fish, either very large or extremely small. For the latter, the fish organs are

infinitesimally small so the whole fish can be consumed. Besides, the impact of the fish organs on their toxicity are considered negligible. The projection of such consumable fish proportion can be mathematically estimated using the conditional probability concept. Operating with hundred fish with diverse species and sizes, the hypothetical consumption availability frequency can be drawn. The number of fish available for consumption was 100 and the probability would be 1. The proportion of fish that can be consumed without their internal organs (K) was estimated to be 0.90 corresponding to 90% while those fish which could be context of statistical error, the proportion regarded as non-consumable was placed at 0.05, i.e. consumed with their internal organs (W) was evaluated as 0.10, corresponding to 10%. Within the 5% for both W and K. These represent consumption potential (C) and non-consumption potential (NC) (Table 1).

Table 1: Conditional Probability Concept of Fish Organs Availability for Consumption

| | W | K |
|----|------|------|
| C | 0.05 | 0.85 |
| NC | 0.05 | 0.05 |

The probability of having eatable fish without internal organs (K) with consumption potential (C) can be calculated using the conditional probability expression:

$$\begin{aligned}
 P(K|C) &= \frac{P(K \cap C)}{P(C)} \\
 &= \frac{0.85/1.00}{0.90/1.00} \\
 &= \frac{0.85}{0.90} \\
 &= 0.94
 \end{aligned}$$

$$\% P(K|C) = 94.44\% .$$

The projected analysis indicated about 94.44% of fish population would be eatable whilst 5.56% would not be available for consumption. Since every 17 in 18 fish samples are edible, then the proportion of the fish samples whose internal organs are not edible would be 1/18. It would be appropriate to limit the exposure concentration of any other fish organs measured in this study to 0.001 mg/kg for both adults and children. The exposure concentration for fish muscles has been

pegged at 0.064 mg/kg for adults, as prescribed by FAO/WHO [42] and 0.016 mg/kg for children according to EPA [43].

Total Hazard Quotient

The THQ of the fish organ can be estimated using the formula adopted by Sharma et al [44] expressed in equation (2)

$$PDI = \frac{M \times FC \times EL \times DE \times 10^{-3}}{BW \times LX} \quad (2)$$

Where M is the heavy metal's mean concentration in the organ of the fish (mg/kg), FC stands for the frequency of fish organ consumption {(normal adult = 0.064 kg/day [42], normal children = 0.016 kg/day [43]}, EL is the exposure frequency (350 days/year), and BW is the body weight of the consumer: adult (60 kg) and children (19.6 kg). At the carcinogenic risk level, PDI is estimated by incorporating the exposure duration DE and the meantime or life expectancy, LX, is conservatively pegged at 70 years.

The total presumed daily intake (TPDI) of the fish organs can be estimated by summation of all the PDI for the fish organs.

$$TPDI = \sum_{i=1}^n PDI_i, \text{ where } i = 1, 2, \dots, n \quad (3)$$

Hazard Quotient and Hazard Index

The hazard quotient and hazard index are the two non-carcinogenic health risk indicators pointing to the health status of food items or substances taken as food. They are useful in measuring the health status of food items in parts. The hazard parameters are used to determine the health risk status of heavy metals stored in fish organs. The hazard quotient (HQ) for the fish organs can be estimated with the formula in equation (4)

$$HQ = \frac{PDI}{RfD} \text{ or } \frac{TPDI}{RfD} \quad (4)$$

RfD = Reference dose of the heavy metals; Pb = 0.0035, Cu = 0.042, Zn = 0.30, Ni = 0.020, Co = 3.01, and Cd = 0.001 mg.kg⁻¹.day⁻¹ [45-47].

$$HI = \sum_{i=1}^n HQ_i, \text{ where } i = 1, 2, \dots, n. \quad (5)$$

The hazard index where HI < 1, indicates no health risk, whilst HI > 1 suggests health risk.

Carcinogenic Risk Assessment

The carcinogenic risk status (CR) of heavy metals in fish organs is evaluated using equation (6) derived from equation (4).

$$CR = HQ \times RfD \times CSF \quad (6)$$

The HQ corresponds to the hazard quotient of the heavy metals in fish organs, RfD stands for the reference dose of heavy metals and CSF expresses the ingestion carcinogenic slope factor for the heavy metals investigated and their values are presented as follows: Pb = 8.5×10^{-3} , Ni = 0.84 and Cd = 0.38 [48].

Quality Assurance

The fish samples were thoroughly washed with distilled water and the stainless knives and scissors used for fish preparation were acid-washed and sterilized before use. The ceramic pestle and mortar were cleaned and sterilized before use. All reagents used were analytically pure. The glass apparatus used was soaked in 10% nitric acid for 24 hours, washed with detergent and rinsed with distilled water. The washed glassware was wrapped in foil paper and oven-dried before use. The parameters for validating the analytical methods were evaluated for each metal using solvent blanks and standard solutions at different concentrations. Parameters recommended by the International Union of Pure and Applied Chemistry (IUPAC) [49] and the European Commission (EC) [50], such as recovery, accuracy, precision (repeatability), range of linearity, limit of detection (LOD) and limit of quantification (LOQ), were measured.

The sensitivity of the AAS was tested in replicates using different knock-down concentrations of each metal from the stocks using distilled water containing 2% (0.3M) nitric acid and the recovery studies (%) were performed. The AAS was then applied to the real samples. The knock-down concentrations were prepared from the stock solution to create the calibration curves for all metals with the linearity range $R^2 \geq 0.996$. The linearity range measures the linear regression coefficient (R^2) of the calibration curves. A spiked recovery method was used where a known amount of each assayed metal was added to the predigested sample and then digested. All studies on the recovery of the heavy metals of interest exceeded 92%. The limits of detection (LOD) and limits of quantification (LOQ) are calculated using the following formulas in Equations 7 and 8, respectively:

$$LOD = \frac{3.3 S}{m} \quad (7)$$

And

$$LOQ = \frac{10 S}{m} \quad (8)$$

Where S and m are the standard deviation of the blank solution and m is the slope of the calibration plots.

The recovery studies (%) were calculated using the formula in equation (9)

$$\text{Recovery Studies} = \frac{C_f}{C_i} \times \frac{100}{1} \quad (9)$$

Where C_f and C_i are the concentrations found and introduced into the samples respectively.

Data Analyses

The data obtained from different experimental activities were analyzed using both MS Office 2019 and IBM Statistical Package for Social Science (SPSS statistical software, version 21). The mean and the standard deviation of the data were carried out using the MS Office, whilst the correlation analyses were done using the IBM Statistical Package for Social Science (SPSS) statistical package.

RESULTS AND DISCUSSION

Heavy metals were detected in the heart, gill, intestine, kidney, liver and muscle of catfish, tilapia and silver catfish and the results of the study (mean \pm SD, mg/kg) are presented in Tables 2, 3 and 4 respectively.

Table 2: Concentrations of Selected Heavy Metals in Tilapia Fish Organs (mg/kg)($\bar{x} \pm SD$, n=3)

| | Pb | Cu | Zn | Ni | Co | Cd |
|-----------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Heart | 1.22 \pm 0.29 | 0.10 \pm 0.05 | 1.00 \pm 0.40 | 0.50 \pm 0.02 | 0.22 \pm 0.05 | 0.36 \pm 0.08 |
| Gill | 2.80 \pm 0.38 | 0.83 \pm 0.08 | 1.23 \pm 0.16 | 0.29 \pm 0.04 | 0.24 \pm 0.03 | 0.18 \pm 0.03 |
| Intestine | 3.13 \pm 0.49 | 6.23 \pm 1.54 | 1.05 \pm 0.09 | 3.13 \pm 0.60 | 0.34 \pm 0.13 | 0.21 \pm 0.03 |
| Kidney | 1.72 \pm 0.06 | 0.40 \pm 0.00 | 1.07 \pm 0.10 | 0.63 \pm 0.13 | 0.77 \pm 0.10 | 0.27 \pm 0.03 |
| Liver | 1.93 \pm 0.06 | 4.38 \pm 0.03 | 0.93 \pm 0.06 | 3.52 \pm 0.54 | 0.26 \pm 0.05 | 0.51 \pm 0.09 |
| Muscle | 1.77 \pm 0.60 | 1.03 \pm 0.20 | 0.83 \pm 0.08 | 0.59 \pm 0.02 | 0.19 \pm 0.04 | 0.11 \pm 0.01 |

Table 3: Concentrations of Selected Heavy Metals in Catfish Fish Organs (mg/kg)($\bar{x} \pm SD$, n=3)

| | Pb | Cu | Zn | Ni | Co | Cd |
|-----------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Heart | 2.75 \pm 0.40 | 1.35 \pm 0.18 | 1.82 \pm 0.08 | 0.18 \pm 0.02 | 0.09 \pm 0.01 | 0.31 \pm 0.01 |
| Gill | 1.42 \pm 0.20 | 0.92 \pm 0.18 | 1.70 \pm 0.15 | 0.38 \pm 0.03 | 0.06 \pm 0.01 | 0.04 \pm 0.01 |
| Intestine | 1.67 \pm 0.15 | 1.33 \pm 0.08 | 0.87 \pm 0.08 | 0.15 \pm 0.00 | 0.07 \pm 0.02 | 0.02 \pm 0.01 |
| Kidney | 2.00 \pm 0.05 | 0.45 \pm 0.00 | 1.07 \pm 0.03 | 0.26 \pm 0.05 | 0.03 \pm 0.01 | 0.24 \pm 0.04 |
| Liver | 1.67 \pm 0.10 | 4.53 \pm 0.44 | 1.77 \pm 0.03 | 0.48 \pm 0.09 | 0.04 \pm 0.03 | 0.64 \pm 0.07 |
| Muscle | 1.40 \pm 0.05 | 2.40 \pm 0.09 | 1.32 \pm 0.08 | 0.38 \pm 0.07 | 0.18 \pm 0.03 | 0.11 \pm 0.02 |

Table 4: Concentrations of Selected Heavy Metals in Silver Catfish Fish Organs(mg/kg)($\bar{x} \pm SD$, n=3)

| | Pb | Cu | Zn | Ni | Co | Cd |
|-----------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Heart | 1.87 \pm 0.19 | 0.32 \pm 0.08 | 0.43 \pm 0.08 | 0.47 \pm 0.01 | 0.33 \pm 0.03 | 0.79 \pm 0.61 |
| Gill | 2.95 \pm 0.26 | 2.28 \pm 0.74 | 1.65 \pm 0.41 | 1.14 \pm 0.27 | 0.42 \pm 0.12 | 0.33 \pm 0.03 |
| Intestine | 1.98 \pm 0.03 | 1.42 \pm 0.08 | 0.77 \pm 0.03 | 0.50 \pm 0.03 | 0.43 \pm 0.03 | 0.47 \pm 0.06 |
| Kidney | 3.27 \pm 1.23 | 0.47 \pm 0.06 | 0.67 \pm 0.20 | 0.36 \pm 0.09 | 0.53 \pm 0.05 | 0.49 \pm 0.08 |
| Liver | 1.42 \pm 0.33 | 4.63 \pm 0.69 | 1.12 \pm 0.10 | 1.41 \pm 0.32 | 0.38 \pm 0.03 | 0.21 \pm 0.01 |
| Muscle | 2.05 \pm 0.22 | 0.77 \pm 0.10 | 0.88 \pm 0.15 | 0.48 \pm 0.03 | 0.50 \pm 0.08 | 0.29 \pm 0.01 |

The weights of the three tilapia samples analyzed were 175.20 g, 178.30 g and 180.40 g. Similarly, the weights of the three catfish and silver catfish are 345.10, 351.50, 369.40 g and 55.10, 55.20, 56.40 g respectively.

In the hearts of *Oreochromis niloticus*, the concentration of Pb was the highest (1.22 mg/kg) whilst that of Co was the lowest (0.10 mg/kg). The same trend was observed when the hearts of *Chrysichthys nigrodigitatus* were analyzed. For the three fish species, the concentrations of Pb in the kidney of *Chrysichthys nigrodigitatus* and intestine of *Oreochromis niloticus* were above 3.00 mg/kg followed by that in the gills of *Chrysichthys nigrodigitatus* and *Oreochromis niloticus* respectively. The concentrations of Pb in the kidney, intestine and gill of the fish samples were high and they respectively ranged as 1.72 - 3.27 mg/kg, 1.67-3.13 mg/kg, and 1.42-2.95 mg/kg. The occurrence of Pb in the fish organs is an indication of the prevalence of high-level industrial effluent enrichment along the water body of the study area [51].

The levels of Ni in the gills of *Clarias batrachus* samples were below 0.50 mg/kg but were higher than those of *Oreochromis niloticus*. The concentration of nickel in tilapia's intestine is about six times higher than that of *Clarias batrachus*, but about twenty times more for *Clarias batrachus*. The Co and Cd contents in the fish organs of the three fish species were below 1 mg/kg. The Cu and Zn contents in the fish organs for *Oreochromis niloticus*, *Clarias batrachus* and *Chrysichthys nigrodigitatus* respectively ranged thus: Cu (0.10-6.23 mg/kg), Zn (0.83-1.23

mg/kg); Cu (0.45 – 4.53 mg/kg), Zn (0.87-1.82 mg/kg) and Cu (0.32 – 4.63 mg/kg), Zn (0.43-1.65 mg/kg). The levels of Cu detected in the liver of the three fish samples exceeded 4.00 mg/kg

The statistical analysis of the heavy metals' results revealed that all the six heavy metals were detected in the fish organs at different proportions. Considering that the muscles of fish samples are conventionally consumed, Pearson Correlation analysis was conducted on the selected heavy metals in all three fish samples. The analysis showed a positive correlation between Co and Cd as well as negative correlation between Pb and Cu at $p < 0.05$. Similarly, the Spearman Correlation analysis showed a negative correlation between Cu, Co, and Pb as well as a negative correlation between Zn and Ni at $p < 0.01$.

HMPI of Heavy Metals in Fish Organs

The HMPI for this study was calculated using the formula in equation (1) and the results for tilapia, catfish and silver catfish are 5.57, 3.26 and 5.27 respectively. This implies that the descending order of HMPI in the fish species is tilapia > silver catfish > catfish. This implies that the catfish was the least polluted whilst the tilapia was the most polluted with heavy metals.

THQ and HI of Heavy Metals in Fish Organs

The THQs of heavy metals in adult by fish species are presented in ascending order of Co (7.43×10^{-8}) < Zn (3.11×10^{-6}) < Cu (2.96×10^{-5}) < Ni (3.66×10^{-5}) < Cd (1.37×10^{-4}) < Pb (5.69×10^{-4}) for tilapia; Co (6.27×10^{-8}) < Zn (4.88×10^{-6}) < Ni (2.06×10^{-5}) < Cu (6.17×10^{-5}) < Cd (1.33×10^{-4}) < Pb (4.53×10^{-4}) for catfish; and Co (1.81×10^{-7}) < Zn (3.25×10^{-6}) < Cu (2.22×10^{-5}) < Ni (2.76×10^{-5}) < Cd (3.33×10^{-4}) < Pb (6.52×10^{-4}) for silver catfish (Tables 5, 6 and 7).

Table 5(a): The HI, and THQ of Heavy Metals in Fish Organs of Tilapia for Adult.

| Metal | Pb ($\times 10^{-5}$) | Cu ($\times 10^{-8}$) | Zn ($\times 10^{-8}$) | Ni ($\times 10^{-7}$) | Co ($\times 10^{-9}$) | Cd ($\times 10^{-6}$) | Total ($\times 10^{-5}$) |
|-----------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|----------------------------|
| Heart | 1.28 | 3.81 | 5.33 | 3.99 | 1.17 | 5.75 | 1.90 |
| Gill | 1.43 | 31.6 | 6.55 | 2.32 | 1.27 | 2.88 | 1.78 |
| Intestine | 0.79 | 237.06 | 5.59 | 25.01 | 1.81 | 3.36 | 1.61 |
| Kidney | 0.88 | 15.22 | 5.70 | 5.03 | 4.09 | 4.32 | 1.38 |
| Liver | 0.81 | 166.67 | 4.95 | 28.13 | 1.38 | 8.15 | 2.08 |
| Muscle | 51.73 | 2508.37 | 282.98 | 301.74 | 64.56 | 112.51 | 68.79 |
| Total | 5.69×10^{-4} | 2.96×10^{-5} | 3.11×10^{-6} | 3.66×10^{-5} | 7.43×10^{-8} | 1.37×10^{-4} | 7.75×10^{-4} |

$HI = \sum THQ = 7.75 \times 10^{-4}$ for adult.

Table 5(b): The HI, and THQ of Heavy Metals in Fish Organs of Tilapia for Children.

| Metal | Pb ($\times 10^{-5}$) | Cu ($\times 10^{-7}$) | Zn ($\times 10^{-7}$) | Ni ($\times 10^{-6}$) | Co ($\times 10^{-9}$) | Cd ($\times 10^{-5}$) | Total ($\times 10^{-5}$) |
|-----------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|----------------------------|
| Heart | 3.91 | 1.16 | 1.63 | 1.22 | 3.58 | 1.76 | 5.83 |
| Gill | 4.38 | 9.67 | 2.01 | 0.71 | 3.90 | 0.88 | 5.44 |
| Intestine | 2.40 | 72.57 | 1.71 | 7.66 | 5.53 | 1.03 | 4.94 |
| Kidney | 2.70 | 4.66 | 1.74 | 1.54 | 12.52 | 1.32 | 4.24 |
| Liver | 2.47 | 51.02 | 1.52 | 8.61 | 4.23 | 2.50 | 6.36 |
| Muscle | 39.59 | 191.97 | 21.66 | 23.09 | 49.41 | 8.61 | 52.65 |
| Total | 5.55×10^{-4} | 3.31×10^{-5} | 3.03×10^{-6} | 4.28×10^{-5} | 7.92×10^{-8} | 1.61×10^{-4} | 7.95×10^{-4} |

$HI = \sum THQ = 7.95 \times 10^{-4}$ for children.

Table 6(a): The HI, and THQ of Heavy Metals in Fish Organs of Catfish for Adult

| Metal | Pb ($\times 10^{-5}$) | Cu ($\times 10^{-7}$) | Zn ($\times 10^{-8}$) | Ni ($\times 10^{-7}$) | Co ($\times 10^{-10}$) | Cd ($\times 10^{-7}$) | Total ($\times 10^{-5}$) |
|-----------|-------------------------|-------------------------|-------------------------|-------------------------|--------------------------|-------------------------|----------------------------|
| Heart | 1.26 | 5.14 | 9.70 | 1.44 | 4.78 | 49.54 | 1.83 |
| Gill | 0.65 | 3.50 | 9.06 | 3.04 | 3.19 | 6.39 | 0.79 |
| Intestine | 0.76 | 5.06 | 4.63 | 1.20 | 3.72 | 3.20 | 0.86 |
| Kidney | 0.91 | 1.71 | 5.70 | 2.08 | 1.59 | 38.36 | 1.34 |
| Liver | 0.76 | 17.24 | 9.43 | 3.84 | 2.12 | 102.28 | 2.01 |
| Muscle | 40.91 | 584.48 | 450.05 | 194.34 | 611.66 | 112.51 | 60.41 |
| Total | 4.53×10^{-4} | 6.17×10^{-5} | 4.88×10^{-6} | 2.06×10^{-5} | 6.27×10^{-8} | 1.33×10^{-4} | 6.72×10^{-4} |

$HI = \sum THQs = 6.72 \times 10^{-4}$ for adult

Table 6(b): The HI, and THQ of Heavy Metals in Fish Organs of Catfish for Children

| Metal | Pb ($\times 10^{-5}$) | Cu ($\times 10^{-6}$) | Zn ($\times 10^{-7}$) | Ni ($\times 10^{-7}$) | Co ($\times 10^{-10}$) | Cd ($\times 10^{-5}$) | Total ($\times 10^{-5}$) |
|-----------|-------------------------|-------------------------|-------------------------|-------------------------|--------------------------|-------------------------|----------------------------|
| Heart | 3.84 | 1.57 | 2.97 | 4.40 | 14.63 | 5.12 | 5.59 |
| Gill | 1.98 | 1.07 | 2.77 | 9.30 | 9.75 | 0.20 | 2.41 |
| Intestine | 2.33 | 1.55 | 1.42 | 3.70 | 11.38 | 0.10 | 2.64 |
| Kidney | 2.80 | 0.52 | 1.74 | 6.36 | 4.88 | 1.17 | 4.10 |
| Liver | 2.33 | 5.28 | 2.89 | 11.74 | 6.50 | 3.13 | 6.14 |

| | | | | | | | |
|--------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|
| Muscle | 31.31 | 44.73 | 34.44 | 148.73 | 468.11 | 8.61 | 46.23 |
| Total | 4.46×10^{-4} | 5.47×10^{-5} | 4.62×10^{-6} | 1.84×10^{-5} | 5.15×10^{-8} | 1.47×10^{-4} | 6.71×10^{-4} |

$HI = \sum THQs = 6.71 \times 10^{-4}$ for children.

Table 7(a): The HI, and THQ of Heavy Metals in Fish Organs of Silver Catfish for Adult

| Metal | Pb ($\times 10^{-5}$) | Cu ($\times 10^{-7}$) | Zn ($\times 10^{-8}$) | Ni ($\times 10^{-7}$) | Co ($\times 10^{-9}$) | Cd ($\times 10^{-6}$) | Total ($\times 10^{-5}$) |
|-----------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|----------------------------|
| Heart | 0.85 | 1.22 | 2.29 | 3.76 | 1.75 | 12.63 | 2.17 |
| Gill | 1.35 | 8.68 | 8.79 | 9.11 | 2.23 | 5.27 | 2.06 |
| Intestine | 0.90 | 5.40 | 4.10 | 3.99 | 2.28 | 7.51 | 1.75 |
| Kidney | 1.49 | 1.79 | 3.57 | 2.88 | 2.81 | 7.83 | 2.33 |
| Liver | 0.65 | 17.62 | 5.97 | 0.11 | 2.02 | 3.36 | 1.28 |
| Muscle | 59.91 | 187.52 | 300.03 | 245.48 | 169.91 | 296.62 | 94.22 |
| Total | 6.52×10^{-4} | 2.22×10^{-5} | 3.25×10^{-6} | 2.76×10^{-5} | 1.81×10^{-7} | 3.33×10^{-4} | 1.04×10^{-3} |

$HI = \sum THQs = 1.04 \times 10^{-3}$ for adult.

Table 7(b): The HI, and THQ of Heavy Metals in Fish Organs of Silver Catfish for Children

| Metal | Pb ($\times 10^{-5}$) | Cu ($\times 10^{-6}$) | Zn ($\times 10^{-7}$) | Ni ($\times 10^{-6}$) | Co ($\times 10^{-9}$) | Cd ($\times 10^{-5}$) | Total ($\times 10^{-5}$) |
|-----------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|----------------------------|
| Heart | 2.61 | 0.37 | 0.70 | 1.15 | 5.36 | 3.86 | 6.64 |
| Gill | 4.12 | 2.66 | 2.69 | 2.79 | 6.83 | 1.61 | 6.31 |
| Intestine | 2.77 | 1.65 | 1.26 | 1.22 | 6.99 | 2.30 | 5.37 |
| Kidney | 4.57 | 0.55 | 1.09 | 0.88 | 8.61 | 2.40 | 7.12 |
| Liver | 1.98 | 5.39 | 1.83 | 3.45 | 6.18 | 1.03 | 3.92 |
| Muscle | 45.85 | 14.36 | 22.96 | 18.79 | 130.03 | 22.70 | 72.11 |
| Total | 6.19×10^{-4} | 2.50×10^{-5} | 3.05×10^{-6} | 2.83×10^{-5} | 1.64×10^{-7} | 3.39×10^{-4} | 1.01×10^{-3} |

$HI = \sum THQs = 1.01 \times 10^{-3}$ for children.

The THQ trends of heavy metals were observed for children for all three fish species and were similar to those in adults. The Pb contents in the six fish organs either ranked first or second for the three fish species. These levels of Pb exposure corresponded to the range of 62.69-73.42 % for HI threat in the adults and 61.29 – 69.81 % of HI threat in children. Cd exposure threat comes

next corresponding to a range of 17.68 - 32.02 % in adults and 20.25 – 33.56 % in children. The threat posed by Ni exposure comes third for both tilapia and silver catfish. All these metals are carcinogenic. The HI for consuming heavy metals was highest for silver catfish (Adults: 1.04×10^{-3} ; Children: 1.01×10^{-3}) and lowest for catfish species (Adults: 6.72×10^{-4} ; Children: 6.71×10^{-4}) (Figures 3(a), (b) and (c)). Since the HI of each fish species was less than 1, it does imply that consuming the three fish species cannot elicit non-carcinogenic hazard risk in consumers.

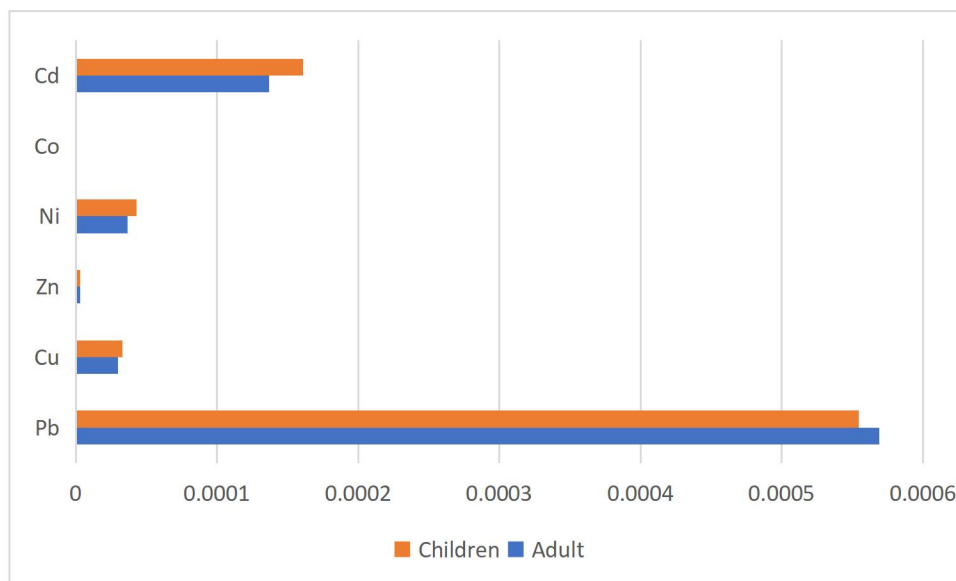


Figure 3(a): The HI of Heavy Metals in Tilapia Fish

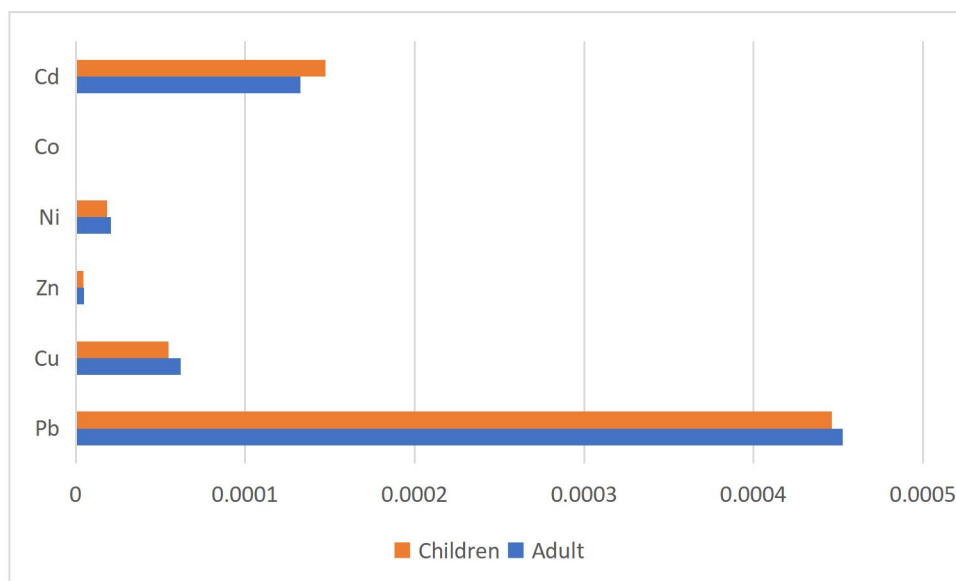


Figure 3(b): The HI of Heavy Metals in Catfish

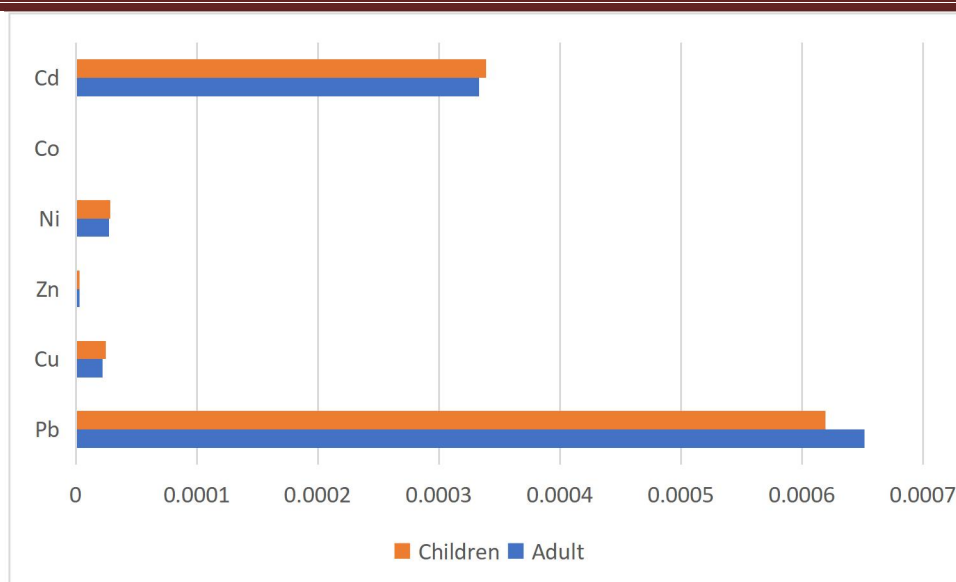


Figure 3(c): The HI of Heavy Metals in Silver Catfish

Pb, Cd, Ni and Cu were the four heavy metals among the studied metals that contributed significantly to the THQs of the fish species investigated. Pb, Cd and Ni are generally considered to be toxic especially at micro-concentrations as they have no biological usefulness in living organisms. The THQs of fish muscles were higher than that of other fish organs (Tables 5, 6 and 7). The hazard index of heavy metals in the fish species is presented in ascending order as follows: catfish < tilapia < silver catfish. According to Figures 4 and 5, the fish muscles of silver catfish bioaccumulated more heavy metals than tilapia and catfish. This implied that the disposition to consume silver catfish for a lifetime may potent toxicity danger.

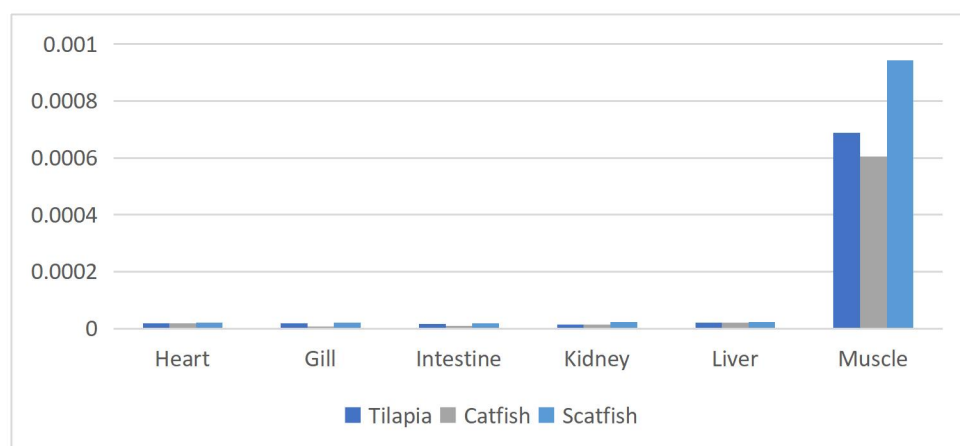


Figure 4: The THQs of Heavy Metals in Fish Organs

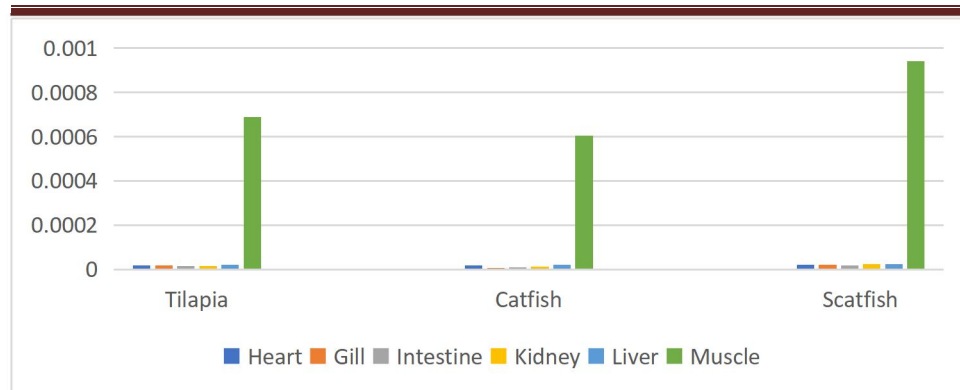


Figure 5: Relative Bioaccumulation of Heavy Metals in the Muscles of the Three Fish Species.

Carcinogenic Risk Assessment (CR) of Heavy Metals in Fish organs

The CR of the heavy metals in the fish organs produced some interesting results. The CRs of Pb, Ni and Cd in tilapia fish are 1.69×10^{-8} , 6.15×10^{-7} and 5.20×10^{-8} respectively for the adults and 1.65×10^{-8} , 7.20×10^{-7} and 6.11×10^{-8} for children. Similarly, the CRs for catfish, following the heavy metal sequence, are 1.34×10^{-8} , 3.46×10^{-7} and 5.03×10^{-8} for the adults and 1.33×10^{-8} , 3.09×10^{-7} and 5.60×10^{-8} for children. The CRs for Pb, Ni and Cd in silver catfish are, respectively, 1.94×10^{-8} , 4.64×10^{-7} and 1.27×10^{-7} for adults and 1.84×10^{-8} , 4.75×10^{-7} and 1.23×10^{-7} for children. The CR values of both adults and children for tilapia, catfish and silver catfish were below the lower threshold for cancer risk (1×10^{-6}) set by the US regulatory authority [52]. The results indicated that the carcinogenic consequences of the heavy metals will be significantly more pronounced in children much more than in adults.

CONCLUSIONS

Heavy metals were detected in the organs of the three fish species investigated. This study has shown that organs of fish species harvested at the impacted areas of Lagoon contained elevated levels of heavy metals. Heavy metals' bioaccumulation varied across the fish organs and the fish species. The health metal pollution index for the three fish samples was within the safe limit. For each fish species, the THQ for each heavy metal was below the risk-inducing level (i.e. $THQ < 1$), while the combined THQ (TTHQ) also fell below 1. These results suggested that fish consumers are not susceptible to any significant health risks. The CR health risk indices for Pb, Cu, Zn, Ni, Co and Cd in each fish species, and their combined CR, were less than the reference threshold for cancer. Hence, the present levels of heavy metals in the three fish species may not induce any health hazard in consumers' lives throughout their lifetime.

REFERENCES

1. Tacon, A.G. & Metian, M. (2013). Fish matters: importance of aquatic foods in human nutrition and global food supply. *Reviews in fisheries Science*, 21(1), 22-38.
2. Richter, C.K., Skulas-Ray, A.C. & Kris-Etherton, P.M. (2016). Recommended intake of fish and fish oils worldwide. In *Fish and fish oil in health and disease prevention* (pp. 27-48). Academic Press.
3. Sheeshka, J. & Murkin, E. (2002). Nutritional aspects of fish compared with other protein sources. *Comments on Toxicology*, 8(4-6), 375-397.
4. Trumbo, P., Schlicker, S., Yates, A.A. & Poos, M. (2002). Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein and amino acids. (Commentary). *Journal of the American Dietetic Association*, 102(11), 1621-1631.
5. Delgado, A.M., Vaz Almeida, M.D., Parisi, S., Delgado, A.M., Parisi, S. & Vaz Almeida, M.D. (2017). Fish, meat and other animal protein sources. *Chemistry of the Mediterranean Diet*, 177-207.
6. Bucher, H. C., Hengstler, P., Schindler, C., & Meier, G. (2002). N-3 polyunsaturated fatty acids in coronary heart disease: a meta-analysis of randomized controlled trials. *The American journal of medicine*, 112(4), 298-304.
7. Chrysoshoou, C., Panagiotakos, D.B., Pitsavos, C., Skoumas, J., Krinos, X., Chloptsios, Y., Nikolaou, V. & Stefanadis, C. (2007). Long-term fish consumption is associated with protection against arrhythmia in healthy persons in a Mediterranean region—the ATTICA study. *The American Journal of Clinical Nutrition*, 85(5), 1385-1391.
8. Kris-Etherton, P.M., Harris, W.S. & Appel, L.J. (2002). Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. *Circulation*, 106(21), 2747-2757.
9. Alam, M., Rohani, M. F., & Hossain, M. S. (2023). Heavy metals accumulation in some important fish species cultured in commercial fish farm of Natore, Bangladesh and possible health risk evaluation. *Emerging Contaminants*, 9(4), 100254.
10. Mikolajczyk, S., Warenik-Bany, M., Maszewski, S., & Pajurek, M. (2020). Dioxins and PCBs—Environment impact on freshwater fish contamination and risk to consumers. *Environmental Pollution*, 263, 114611.

11. La Guardia, M. J., Mainor, T. M., Luellen, D. R., Harvey, E., & Hale, R. C. (2024). Twenty years later: PBDEs in fish from US sites with historically extreme contamination. *Chemosphere*, 351, 141126.
12. Kim, H. H., Lim, Y. W., Yang, J. Y., Shin, D. C., Ham, H. S., Choi, B. S., & Lee, J. Y. (2013). Health risk assessment of exposure to chlorpyrifos and dichlorvos in children at childcare facilities. *Science of the total environment*, 444, 441-450.
13. Reindl, A. R., Falkowska, L., Szumiło, E., & Staniszevska, M. (2013). Residue of chlorinated pesticides in fish caught in the Southern Baltic. *Oceanological and Hydrobiological Studies*, 42, 251-259.
14. Subedi, B., Du, B., Chambliss, C. K., Koschorreck, J., Rüdell, H., Quack, M., ... & Usenko, S. (2012). Occurrence of pharmaceuticals and personal care products in German fish tissue: a national study. *Environmental science & technology*, 46(16), 9047-9054.
15. Yao, L., Zhao, J. L., Liu, Y. S., Zhang, Q. Q., Jiang, Y. X., Liu, S., ... & Ying, G. G. (2018). Personal care products in wild fish in two main Chinese rivers: bioaccumulation potential and human health risks. *Science of the Total Environment*, 621, 1093-1102.
16. Fernandes, C., Fontainhas-Fernandes, A., Cabral, D., & Salgado, M. A. (2008). Heavy metals in water, sediment and tissues of *Liza saliens* from Esmoriz-Paramos lagoon, Portugal. *Environmental monitoring and assessment*, 136, 267-275.
17. Garai, P., Banerjee, P., Mondal, P., & Saha, N. C. (2021). Effect of heavy metals on fishes: Toxicity and bioaccumulation. *J Clin Toxicol. S*, 18
18. Assi, M. A., Hezmee, M. N. M., Sabri, M. Y. M., & Rajion, M. A. (2016). The detrimental effects of lead on human and animal health. *Veterinary world*, 9(6), 660.
19. Shahjahan, M., Taslima, K., Rahman, M. S., Al-Emran, M., Alam, S. I., & Faggio, C. (2022). Effects of heavy metals on fish physiology—a review. *Chemosphere*, 300, 134519.
20. Gupta, G., Srivastava, P. P., Kumar, M., Varghese, T., Chanu, T. I., Gupta, S., ... & Jana, P. (2021). The modulation effects of dietary zinc on reproductive performance and gonadotropins' (FSH and LH) expression in threatened Asian catfish, *Clarias magur* (Hamilton, 1822) broodfish. *Aquaculture Research*, 52(5), 2254-2265.
21. Bhat, R. A., Bakhshalizadeh, S., Guerrero, M. C., Kesbiç, O. S., & Fazio, F. (2023). Toxic effect of heavy metals on ovarian deformities, apoptotic changes, oxidative stress, and steroid hormones in rainbow trout. *Journal of Trace Elements in Medicine and Biology*, 75, 127106.

22. Forouhar Vajargah, M., Mohamadi Yalsuyi, A., Sattari, M., Prokić, M. D., & Faggio, C. (2020). Effects of copper oxide nanoparticles (CuO-NPs) on parturition time, survival rate and reproductive success of guppy fish, *Poecilia reticulata*. *Journal of cluster science*, 31, 499-506.
23. Yan, W., Hamid, N., Deng, S., Jia, P. P., & Pei, D. S. (2020). Individual and combined toxicogenetic effects of microplastics and heavy metals (Cd, Pb, and Zn) perturb gut microbiota homeostasis and gonadal development in marine medaka (*Oryzias melastigma*). *Journal of hazardous materials*, 397, 122795.
24. Ahmed, B., Khan, M. S., & Musarrat, J. (2018). Toxicity assessment of metal oxide nano-pollutants on tomato (*Solanum lycopersicon*): A study on growth dynamics and plant cell death. *Environmental Pollution*, 240, 802-816.
25. Suchana, S. A., Ahmed, M. S., Islam, S. M., Rahman, M. L., Rohani, M. F., Ferdusi, T., ... & Shahjahan, M. (2021). Chromium exposure causes structural aberrations of erythrocytes, gills, liver, kidney, and genetic damage in striped catfish *Pangasianodon hypophthalmus*. *Biological Trace Element Research*, 199, 3869-3885.
26. Huang, W., Cao, L., Shan, X., Xiao, Z., Wang, Q., & Dou, S. (2010). Toxic effects of zinc on the development, growth, and survival of red sea bream *Pagrus major* embryos and larvae. *Archives of environmental contamination and toxicology*, 58, 140-150.
27. Wang, R. F., Zhu, L. M., Zhang, J., An, X. P., Yang, Y. P., Song, M., & Zhang, L. (2020). Developmental toxicity of copper in marine medaka (*Oryzias melastigma*) embryos and larvae. *Chemosphere*, 247, 125923.
28. Witeska, M., Sarnowski, P., Ługowska, K., & Kowal, E. (2014). The effects of cadmium and copper on embryonic and larval development of ide *Leuciscus idus* L. *Fish physiology and biochemistry*, 40, 151-163.
29. Zhang, H., Cao, H., Meng, Y., Jin, G., & Zhu, M. (2012). The toxicity of cadmium (Cd²⁺) towards embryos and pro-larva of soldatov's catfish (*Silurus soldatovi*). *Ecotoxicology and environmental safety*, 80, 258-265.
30. El-Moselhy, K. M., Othman, A. I., Abd El-Azem, H., & El-Metwally, M. E. A. (2014). Bioaccumulation of heavy metals in some tissues of fish in the Red Sea, Egypt. *Egyptian journal of basic and applied sciences*, 1(2), 97-105.
31. Eneji, I. S., Sha'Ato, R., & Annune, P. A. (2011). Bioaccumulation of Heavy Metals in Fish (*Tilapia Zilli* and *Clarias Gariepinus*) Organs From River Benue, North--Central Nigeria. *Pakistan Journal of Analytical & Environmental Chemistry*, 12.

32. Gewurtz, S. B., Bhavsar, S. P., & Fletcher, R. (2011). Influence of fish size and sex on mercury/PCB concentration: importance for fish consumption advisories. *Environment international*, 37(2), 425-434.
33. Alves, R. N., Rambla-Alegre, M., Braga, A. C., Maulvault, A. L., Barbosa, V., Campàs, M., ... & Marques, A. (2019). Bioaccessibility of lipophilic and hydrophilic marine biotoxins in seafood: An in vitro digestion approach. *Food and Chemical Toxicology*, 129, 153-161.
34. Mergler, D., Anderson, H. A., Chan, L. H. M., Mahaffey, K. R., Murray, M., Sakamoto, M., & Stern, A. H. (2007). Methylmercury exposure and health effects in humans: a worldwide concern. *AMBIO: A Journal of the Human Environment*, 36(1), 3-11.
35. Adetutu, A., Adegbola, P. I., & Aborisade, A. B. (2023). Heavy metal concentrations in four fish species from the Lagos lagoon and their human health implications. *Heliyon*, 9(12).
36. Ajibare, A. O., & Loto, O. O. (2023). Risks Assessment of Copper (Cu), Lead (Pb), Mercury (Hg) and Zinc (Zn): A case study of *Tilapia guineensis* in Lagos Lagoon. *Ghana Journal of Agricultural Science*, 58(1), 77-84.
37. Bassey, O. B., & Chukwu, L. O. (2019). Health risk assessment of heavy metals in fish (*Chrysichthys Nigrodigitatus*) from Two Lagoons in Southwestern Nigeria. *J Toxicol Risk Assess*, 5(2), 027.
38. Ashoka, S., Peake, B. M., Bremner, G., Hageman, K. J., & Reid, M. R. (2009). Comparison of digestion methods for ICP-MS determination of trace elements in fish tissues. *Analytica Chimica Acta*, 653(2), 191-199.
39. Olmedo, P., Pla, A., Hernández, A. F., López-Guarnido, O., Rodrigo, L., & Gil, F. (2010). Validation of a method to quantify chromium, cadmium, manganese, nickel and lead in human whole blood, urine, saliva and hair samples by electrothermal atomic absorption spectrometry. *Analytica Chimica Acta*, 659(1-2), 60-67.
40. US EPA (United States Environmental Protection Agency), (2010). Risk-based concentration table. (<http://www.epa.gov/reg3hwmd/risk/human/index>) .
41. Han, F., Huang, X., & Mahunu, G. K. (2017). Exploratory review on safety of edible raw fish per the hazard factors and their detection methods. *Trends in Food Science & Technology*, 59, 37-48.
42. FAO/WHO, (2015). Codex Committee on Food Additives and Contaminants; Adopted in 1995 Revised in 1997, 2006, 2008, 2009 Amended in 2010, 2012, 2013, 2014, 2015, 2016, 2017; World Health Organization: The Hague, The Netherlands, 2017.

43. US EPA (United States Environmental Protection Agency), (2005). United State Environmental Protection Agency's guidelines for carcinogen risk assessment, EPA/630/P-03/001F. Risk Assessment Forum. Washington, DC: US Environmental Protection Agency
44. Sharma, R. K., Agrawal, M., & Marshall, F. M. (2009). Heavy metals in vegetables collected from production and market sites of a tropical urban area of India. *Food and chemical toxicology*, 47(3), 583-591.
45. US EPA (United States Environmental Protection Agency), (2010). Risk-based concentration table. (<http://www.epa.gov/reg3hwmd/risk/human/index>) .
46. US EPA (United States Environmental Protection Agency), (2011a). Recommended Use of BW3/4 as the Default Method in Derivation of the Oral Reference Dose. EPA/100/R11/001. Office of the Science Advisor, USA. [http://www.epa.gov/raf/publications/pdfs/recommended use of bw34.pdf](http://www.epa.gov/raf/publications/pdfs/recommended_use_of_bw34.pdf).
47. US EPA. (United States Environmental Protection Agency), (2011b). Exposure factors handbook 2011 edition (Final); <http://cfpub.epa.gov/ncea/risk/recordisplay.cfm?deid=236252>.
48. Ojaniyi, O. F., Okoye, P. A., & Omokpariola, D. O. (2021). Heavy metals analysis and health risk assessment of three fish species, surface water and sediment samples in Ogbaru Axis of River Niger, Anambra State, Nigeria. *Asian Journal of Applied Chemistry Research*, 9(1), 64-81.
49. López, F. S., Garcia, M. G., Morito, N. S., & Vidal, J. M. (2003). Determination of heavy metals in crayfish by ICP-MS with a microwave-assisted digestion treatment. *Ecotoxicology and environmental safety*, 54(2), 223-228.
50. European Commission, (2002). Commission Decision 2002/657/EC of 12 August 2002 implementing Council Directive 96/23/EC establishes criteria and procedures for the validation of analytical methods to ensure the quality and comparability of analytical results generated by official laboratories.
51. Labonne, M., Othman, D. B., & Luck, J. M. (2001). Pb isotopes in mussels as tracers of metal sources and water movements in a lagoon (Thau Basin, S. France). *Chemical Geology*, 181(1-4), 181-191.
52. US EPA (United States Environmental Protection Agency), (2015). In Quantitative Risk Assessment Calculations 7-9, United States Environmental Protection. <http://www.epa.gov/iris/index.html>.