

Residue Levels and Human Health Risk Assessment of Heavy Metals in Commercial Fish and Shrimp Species from Calabar, Nigeria

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ABSTRACT

Selected heavy metals accumulation and health risk assessment were investigated in the tissues of the widely consumed fish (*Ethmalosa Fimbriata*, *Clarias Gariepenus*, and *Tilapia Guineensis*) and shrimp (*Macrobrachium Felicinum*) species in Calabar. The mean concentrations of Cr, Pb, and Ni were slightly higher than the standard levels and Hg was below detection level for all samples. Zn, Cd and Cu contents of fish and shrimp were below the maximum guideline proposed by the US Food and Drug Administration. The average estimated weekly intake was significantly below the provisional tolerable intake based on the FAO and WHO standards for all

studied metals. The combined Total hazard quotient (THQ) of the studied heavy metals were above 1 that is 7.48 for the fish and 7.0 for the shrimp and was highest for Cr suggesting a possible carcinogenic risk to consumers of this product via Cr. The carcinogenic risk of Pb in both species was within the acceptable range of 10^{-4} - 10^{-6} mg/kg/day. Results of this study reveal an almost safe level of Zn, Cd, Cu, and Hg contents in the fish and shrimp consumed by Crossrivarians but recommend to appropriate authorities to stop the sources and use of Cr, Pb, and Ni.

Keywords: Consumption, Health risk assessment, Heavy metals, Fish, Shrimp, Calabar.

INTRODUCTION

Increasing rates of consumption of Bonga fish (*Ethmalosa Fimbriata*), Catfish (*Clarias Gariepenus*), Tilapia fish (*Tilapia Guineensis*) and shrimps (*Macrobrachium Felicinum*) as popular delicacy in Calabar has raised worries about the safety of health of consumers in the face of perceived rising input of heavy metals in aquatic habitat associated with increasing urbanization. This is as a result of their tendency to absorb toxic pollutants from the surrounding aquatic environment, depending on a variety of factors like; the characteristics of the species under consideration, the exposure period, concentration of the elements, temperature, salinity, pH, seasonal changes etc., (Kwaansa-Ansah et al., 2018). Apart from being part of the local delicacy, these commercially available sea foods are widely consumed because of their high protein, low saturated fat and appreciable omega-3 fatty acids contents that promote good health.

Heavy metals are well known environmental pollutants due to their toxicity, persistence in the environment and bio accumulative nature. Their natural sources include volcanic eruptions and weathering of metal-bearing rocks, while their anthropogenic sources include combustion of fossil fuels, various industrial and agricultural activities etc (Essien et al., 2009). Accumulation of potentially toxic heavy metals in sea foods and biota causes a potential health threat to their consumers including humans. The accumulation of heavy metals in tissues of fish and shrimp may cause actual adverse health effects via their intake and various species of fish and shrimp are normally used as bio indicators to study heavy metals accumulation in aquatic environments, in addition to assessing risks caused by anthropogenic activities (Kwaansa-Ansah et al., 2018).

Government and Communities have been compelled to address the issues of Global changes, which includes growth in human population, temperature, sea level rise and contaminants locally and globally. The formulation of policy and regulations occurs at more local scales and requires information and data to support the regulations (Joanna et al., 2019). This increase in human population has caused more fish and shrimps to be consumed even as more contaminants like heavy metals from urbanization and industrialization could threaten their suitability for human consumption.

In Cross River State, a number of studies have reported Heavy metals in sea foods and Cross River estuary (Essien et al., 2009; Obot et. al., 2016 etc.). However, accrual of heavy metals in muscle tissues of commercially significant seafood and their exposure level to consumers in Calabar metropolis is yet to be reported and the general people and authorities are not mindful of the health implication. Calabar is the capital of Cross River State, Nigeria. It was originally

named Akwa Akpa, in the Efik language and is adjacent to the Calabar and Great Kwa rivers and creeks of the Cross River from its inland delta. Calabar is often described as the tourism capital of Nigeria, especially due to several initiatives implemented during the administration of Donald Duke (1999–2007), which made the city the cleanest and most environmentally friendly city in Nigeria (Achum, 2017). It has an area of 406 square kilometres (157 sq mi) at an elevation of 32 m (105 ft) with a population of 371,022 as at 2006 census and a density of 910/km² (2,400/sq m). The Cross River State Annual Christmas Festival held every year attracts thousands from within and beyond Nigeria. The festival, includes music performance from both local and international artists. Other annual events include the Calabar Carnival, a boat regatta, fashion shows, a Christmas Village, traditional dances and the annual Ekpe Festival (Achum, 2017). Being a tourist destination where fresh fish serve with shrimps is one of the major cuisine delicacies, there is necessity to constantly monitor the accumulation of heavy metals in these sea food species. This work therefore seeks to close this gap in knowledge, by examining the concentration and health risk assessment of selected heavy metals (Cd, Cr, Cu, Ni, Hg, Pb and Zn) accumulation in Bonga fish (*Ethmalosa Fimbriata*), Catfish (*Clarias Gariepenus*), Tilapia fish (*Tilapia Guineensis*) and shrimps (*Macrobrachium Felicinum*) in Calabar Metropolis and evaluate the dietary intake as a basic step to assess consumer's exposure to heavy metals.

MATERIAL AND METHODS

Collection and analysis of samples

A total of eighty-four (84) freshly caught male and female *Ethmalosa Fimbriata* (n=36), *Clarias Gariepenus* (n=24), *Tilapia Guineensis* (n=24) and 2400g of *Macrobrachium Felicinum* were bought from local shops in four (4) different locations, including; Beach, Lagos Street, Mbukpa and Watt Markets between the months of April and September, 2021. The species used in this study was based on their accessibility, popularity among local consumers as well as their consumption rate during the study period. The samples were placed in clean plastic bags (each species in a separate bag), stored on an ice chest to avoid deterioration and transported to the Department of Food Science and Technology, Faculty of Agricultural Science, University of Calabar, Nigeria for taxonomic identification. Total length was measured from the top most part of the mouth to the tip of the caudal fin and standard length were measured from the top most part of the mouth to the tip of hypural bone, using a meter rule. Fresh weights were measured using an electronic weighing balance after removing water and other substances. Samples were washed with distilled water and kept in a refrigerator prior to digestion and chemical analysis. Biological information and trophic level characteristics of the studied fish and shrimp species are shown in Table 1.

Determination of heavy metals

The samples were washed with distilled water and a total of 20 g each of fish and shrimp tissues was removed and dried at 105 °C for 24 h and then transferred to a desiccator until it reached a constant weight. Then, 0.5 g of each sample was powdered and transferred to a beaker containing 5 mL of nitric acid. The mixture was heated up to 140 °C to obtain a clear solution. The solution was then filtered and diluted to 50 mL and used for metal analysis. The concentrations of the selected heavy metals Cd, Cr, Cu, Zn, Hg, Ni, and Pb in the digested solutions were measured using an atomic absorption spectrophotometer (Varian AA-7000). Standard heavy metal solutions (1000 mg/L) were used to plot the calibration curves after

appropriate dilutions. Limit of detection was <0.001 mg/l and levels of heavy metals were expressed as mg/kg wet weight (Miri et al., 2017).

Intake rate limits

Estimated weekly and daily intakes

The estimated daily intake (EDI) was evaluated as optimized by Miri et al., 2017 thus:

$$EDI = \frac{E_F * E_D * F_{IR} * C_F * C_M}{W_{AB} * T_A} * 10^{-3} \dots \dots \dots (1)$$

where ED, EF, CF, W_{AB}, F_{IR}, C_M and T_A are the exposure duration (60 years), exposure frequency (365 days/year), conversion factor (0.208) to convert dry weight of fish/shrimp to wet weight, average body weight for adult (65 kg), ingestion rate (25.2 g/day), heavy metal concentrations in muscle tissues of fish/shrimp and average exposure time (E_F * E_D), respectively (Miri et al., 2017).

The estimated weekly intake (EWI) was evaluated following procedures by USEPA (2011);

$$EWI = \frac{C_M * C_R}{W_{AB}} \dots \dots \dots (2)$$

where C_R represent the fish/shrimp consumption rates (ca. 0.160 kg of fish/shrimp per week) (FAO, 2016).

The percentage of provisional tolerable weekly intake (%PTWI)

The %PTWI was evaluated for each heavy metal using;

$$\% PTW1 = \frac{EWI}{PTWI} \dots \dots \dots (3)$$

The PTWI (mg/kg bw/week) guidelines were employ in this study (Cd=0.007, Cr=23.3, Cu=3.5, Ni=0.365, Pb=0.025 and Zn = 7.0 in mgkg⁻¹ (JECFA, 2003).

Daily intake limit

Daily consumption rate limit (CR_{lim}) of the studied fish and shrimp species was evaluated for carcinogenic effects of contaminants using:

$$CR_{lim} = \frac{ARL * W_{AB}}{CSF * C_M} \dots \dots \dots (4)$$

where ARL and CSF are the maximum acceptable lifetime risk level (10⁻⁵) and cancer slope factor, respectively (Yu et al., 2014). The cancer slope factor value of Pb according to the Integrated Risk Information System is 0.0085 mg/kg day⁻¹ (USEPA, 2011).

The maximum acceptable daily intake of fish and shrimp was also calculated for non-carcinogenic risk of heavy metals contaminant using:

$$CR_{lim} = \frac{RfD * W_{AB}}{C_M} \dots \dots \dots (5)$$

where RfD is the oral reference dose. The RfD values of Cd=0.001, Cr=0.003, Cu=0.04, Ni=0.02, Pb=0.035 and Zn = 0.3, in mg/kg-day⁻¹ respectively (USEPA, 2011). The maximum allowable intake rate of fish and shrimp contaminated with heavy was determined as published in Shakeri et al., 2015:

$$CR_{mm} = \frac{CR_{lim} * T_{ap}}{MS} \dots \dots \dots (6)$$

where MS, T_{ap}, and CR_{mm} are the meal size (0.227 kg fish or shrimps/meals), average time period (30.44 day/month), and maximum allowable intake rate, respectively.

Metal pollution index (MPI)

The MPI was utilized to calculate the accretion of total heavy metals, as shown in Kwaansa-Ansah et al., 2018;

$$MPI = (C_1 * C_2 * C_3 \dots \dots C_n)^{1/n} \dots \dots \dots (7a)$$

Multi-element contamination (MEC)

MEC provides a measure to assess contamination based on the content of heavy metals in studied samples with the PTWI limits Adamu and Nganje (2010). MEC values above 1.0 signify an anthropogenic impact on heavy metal concentration in studied samples. MEC was calculated based on the following formula:

$$MEC = \frac{\frac{C_1}{T_1} + \frac{C_2}{T_2} + \frac{C_3}{T_3} \dots \dots \frac{C_n}{T_n}}{n} \dots \dots \dots (7b)$$

where C—content of heavy metal, T—provisional tolerable levels and n—the number of heavy metals.

Health risks assessments

Target hazard quotient (THQ)

Non-carcinogenic risks were examined based on THQ determination and the hazard index (HI) evaluated by adding all the target hazard quotient values respectively as follows;

$$THQ = \frac{E_F * E_D * F_{IR} * C_F * C_M}{W_{AB} * T_A * RfD} * 10^{-3} \dots \dots \dots (8a)$$

$$HI = \sum THQ \dots \dots \dots (8b)$$

If the THQ or HI is below one, it implies no threat to health, THQ or HI above 1, indicate a higher risk associated with fish/shrimp intake (USEPA, 2011).

Cancer risks (CR)

The CR over a lifetime of Pb exposure was calculated as reported by Kwaansa-Ansah et al., 2018 thus;

$$CR = EDI * CSF \dots \dots \dots (9)$$

Condition factor (K)

The condition factor which reflects the physiological and health states of marine species was evaluated following Froese, 2006 procedures as demonstrated below:

$$K = \frac{W}{L^3} * 100 \dots \dots \dots (10)$$

Where W and L are the fresh weight (g), and length (cm) of fish/shrimp, respectively. A factor of 100 was used to bring the coefficient of condition close to unity. In general, higher K value reveals fatter or thicker species.

Statistical analysis

The statistical analysis was performed using IBM Statistical Package for Social Sciences (SPSS) v. 20 software. A normality test was carried out to evaluate whether the data of each samples were normally distributed. Concentrations of the analyzed Heavy metals were express as mean±standard deviation and the association between fish size (length and weight) and heavy metal concentrations among the fish and shrimp species was assessed using Pearson's correlation analysis. The cluster and principal component analyses were utilized to attain a further understanding of heavy metal distributions by evaluating the similarities or dissimilarities in the different fish and shrimp samples. The principal component analysis was carried out by Ward's Method and Varimax normalized rotation was used to extract components with an eigenvalue greater than one.

RESULTS AND DISCUSSION

Heavy metal concentrations

The concentration of selected heavy metals observed in the tissues of *Ethmalosa Fimbriata*, *Clarias Gariepenus*, *Tilapia Guineensis* and *Macrobrachium Felicinum* are presented in Table 2. Total concentration of heavy metal in mg/kg for the studied species were in the order *Ethmalosa Fimbriata*> *Clarias Gariepenus*> *Macrobrachium Felicinum*> *Tilapia Guineensis* and ranged between 18.38 – 25.90 mg/kg with an overall total of 85.12±9.90 mg/kg. *Ethmalosa Fimbriata* being the only member of its genus and dwells particularly where rivers bring large amount of detritus into the sea has a dissimilar size, age and feeding pattern. This attributes may explain the highest level observed for *Ethmalosa Fimbriata* because this metals have varying accumulations behavior in different species of fish/shrimp and several factors including feeding behavior, swimming pattern, reproductive cycle, as well as sex, size, and age can affect their intake (Canli and Atli, 2003).

Heavy metals recorded in the studied fish and shrimp species followed the order of Cr>Pb>Cu>Ni>Zn>Cd while Hg was below detection levels for all the studied samples (Table 2). This agrees with the finding that Cr, Pb, and Cu are some of the persistent toxic metals known in most environmental matrices (Akinrotimi and Edun, 2015). A two-way ANOVA showed that these metals were not normally distributed ($p < 0.05$; SI1) in the analyzed samples. A low coefficient of variation (CV) was observed for Cr (16.96%), Pb (12.59%) and Cu (12.35%); ($CV \leq 20\%$), moderate for Ni (32.32%); ($21\% < CV \leq 50\%$), high variability for Zn (53.49%); ($50\% < CV \leq 100\%$) and exceeded 100% for Cd (102.31%) Table 2, (Kwaansa-Ansah et al., 2018). These result revealed that the concentration levels of Cr, Pb, and Cu did not vary significantly in the studied fish and shrimp samples while that of Ni indicated a non-homogenous levels. Coefficient of variation (CV) greater than 100% as in the case of Cd is an

indication that the standard deviation exceeded the mean value. The condition factor (K) conveyed a variable health effect with respect to heavy metal contamination. The K value >1 for both fish and shrimp samples (7.02 and 2.28 respectively. Table 3.) revealed that all the studied samples were healthy (Kwaansa-Ansah et al., 2018) and this was higher than the K value in the range of 1.05–1.89 recorded by Chandra and Jhan (2010).

The mean concentration of Cd ranging from 0.03 ± 0.01 mg/kg in *Macrobrachium Felicinum* and *Tilapia Guineensis* to 0.13 ± 0.11 mg/kg in *Clarias Gariepenus*, Zn in the range of 0.46 ± 0.02 mg/kg in *Tilapia Guineensis* to 1.64 ± 0.02 mg/kg in *Ethmalosa Fimbriata* and Cu with a minimum value of 6.25 ± 0.67 mg/kg in *Tilapia Guineensis* and a maximum of 7.73 ± 0.49 mg/kg in *Ethmalosa Fimbriata* were below the level proposed by FAO/WHO (0.50, 100 and 30.0 mg/kg). On the hand, levels of Cr, Ni and Pb ranging between 5.14 ± 0.79 mg/kg in *Tilapia Guineensis* to 7.47 ± 0.02 mg/kg in *Ethmalosa Fimbriata*, 1.12 ± 0.18 mg/kg in *Clarias Gariepenus* to 2.30 ± 0.12 mg/kg in *Ethmalosa Fimbriata* and 5.07 ± 0.28 mg/kg in *Macrobrachium Felicinum* to 6.68 ± 0.05 mg/kg in *Ethmalosa Fimbriata* respectively were higher than the FAO/WHO permissible values of 0.15 mg/kg, 0.6 mg/kg and 2.0 mg/kg respectively (FAO, 2016).

Chromium is naturally present in the environment in trace amounts, but industrial use in rubber and stainless steel manufacturing, chrome plating, dyes for textiles, timber treatment to protect wood from decay fungi, wood-attacking insects and other uses contaminates aquatic systems. Antifouling paints used to prevent growth of marine organism on boats contain Pb and Nickel as important components and they can also be released into the atmosphere by coal-burning power plants and garbage incinerators, Chandra and Jhan (2010). Farombi et al. (2007) have also reported that Cr, Pb, Ni and Cd are the major heavy metals associated with Nigerian crude oils. Thus, the high concentration of Cr, Ni and Pb found in the studied fish and shrimp species from Calabar is suspected because the location of the study is around the local mining, illegal refineries, timber and paint industries areas where wastes produced are not properly disposed. This observation corroborates the assertion that marine biota that is of economic importance as food sources from the sea and coast are also polluted (Farombi et al. 2007). Therefore, research efforts to monitor pollutants, especially metals contained in food sourced from sea and coastal areas should be encouraged.

Despite the high population of Watt market, the highest heavy metal load with regard to sampling sites was reported at Mbukpa market (22.45 ± 2.9 mg/kg; Fig. 1). This probably may be associated with atmospheric precipitation arising from illegal oil bunkering activities in its vicinity.

Heavy metal concentrations measured in the studied fish and shrimp species were compared with earlier studies. The concentration of Pd, Zn, Cd, Ni, and Cu of this study was comparable with those in the Dhaleshwari River, Fosu Lagoon and Hypermarkets (Ahmed et al., 2009; Akoto et al., 2014; Alturiqi and Albedair, 2012) and lower than those in Bodo creek, Cross River Mangrove swamp and Lower Cross River Estuary, Niger Delta, Nigeria. (Abu et al, 2016; Essien et al., 2008, Obot et al., 2016). This comparison discloses that these metals contaminant in fish and shrimp from Calabar may not pose serious health issues compared to those that have been recorded in literature.

Consumption rate limits

The daily consumption of the selected heavy metals was assessed based on their concentrations in the tissues of the investigated fish and shrimp species. The consumption rate limits values are given in Table 3. The concentration of heavy metals recorded in tissues of the studied samples were used to evaluate the health risk posed by the intake of fish and shrimp in Calabar. The estimated daily intake (EDI) was in the trend $\text{Cu} > \text{Cr} > \text{Pb} > \text{Ni} > \text{Zn} > \text{Cd}$ and similar order was also observed for the weekly intake (EWI). The %PTWI revealed: $\text{Pb} > \text{Cd} > \text{Ni} > \text{Cu} > \text{Cr} > \text{Zn}$. The highest EDI values compared with the least PTDI values for all the studied heavy metals indicated that the intake of the studied fish and shrimp species poses no effect on consumers. These EDI values were comparable with earlier studies for several fish species sampled from Chilika lagoon (Parida et al., 2017), Subarnarekha River (Giri and Singh, 2015) and Asafo market, Ghana (Kwaansa-Ansah et al., 2018).

Metal Pollution Index

Pollution indices are widely considered a useful tool for the comprehensive evaluation of the degree of contamination. Moreover, they can have great importance in the assessment of food quality and the forecast of future ecosystem continuity. A rating, reflecting the composite influence of dissolved heavy metals in the studied species was done by calculating the MPI. The trend of total concentrations of heavy metal accretions by MPI in the studied species was in the order: *Ethmalosa Fimbriata* (2.20) > *Clarias Gariepenus* (1.75) > *Macrobrachium Felicinum* (1.28) > *Tilapia Guineensis* (1.21) (Table 2) and support that *Ethmalosa Fimbriata* fish species accumulated the highest heavy metals than the other fish and shrimp species. This agreed well with the pattern of heavy metal concentrations and also indicate that the varying accumulations behavior in different species of fish and shrimp which relied on their feeding habit affected the MPI values more than the weight factor in this case. The MPIs value of this study was more than three order of magnitude higher than those reported for Asafo market, Ghana (0.021 – 0.036) Kwaansa-Ansah et al., 2018 and comparable with the MPIs value of Pikeperch (0.91), Hao et al., 2013.

Health Risk Assessment

The risk assessment results are given in Table 4. The non-carcinogenic daily intake rate effects range between 7.66×10^{-4} kg/day for Cr and 4.99 kg/day for Zn in the analyzed fish and between 8.04×10^{-4} kg/day for Cr and 8.78 kg/day for Zn in the studied shrimp species. Also, the carcinogenic daily consumption rate limit of Pb in the analyzed fish and shrimp species was 3.28×10^{-3} kg/day and 3.77×10^{-3} kg/day respectively. This relatively low values are expected not to cause any adverse effects on human health. The meal size for non-carcinogenic effect of the analyzed fish was in the range 0.10 in Cr to 668.77 meals/month in Zn and between 0.11 in Cr to 1177.88 meals/month in Zn for shrimp. Meals/month of carcinogenic effect of Pb was 0.44 and 0.51 for the fish and shrimp species respectively. THQ was in the trend $\text{Cr} > \text{Pb} > \text{Cu} > \text{Cd} > \text{Ni} > \text{Zn}$ for the fish and $\text{Cr} > \text{Pb} > \text{Cu} > \text{Ni} > \text{Cd} > \text{Zn}$ for the shrimp and were all less than 1 except for Cr which recorded the highest value (8.84 and 6.52) for the fish and shrimps respectively. The combined HI of the studied heavy metals were 7.48 for the fish and 7.0 for the shrimp. These high values which are far greater than one and in addition to the high THQ value of Cr in both fish and shrimp samples revealed high bioaccumulation of Cr as its concentration level on the studied species exceeds the recommended limits. This suggests a possible carcinogenic risk to consumers of this product via Cr consumption. The combined HI of this study was higher than those reported in Sistan region, Iran (26.94×10^{-3}) Miri et al., 2017 and Asafo Market, Ghana

(2.61×10^{-3}) Kwaansa-Ansah et al., 2018 while similar HI value was reported for wild fishes in the Southern Sea of Korea and followed the order; $Cd > Cr > Zn$, Hwang et al. (2017).

The carcinogenic risk factor (CR) of Pb calculated for fish and shrimp consumption in Calabar was 1.60×10^{-5} and 1.39×10^{-5} mg/kg/day respectively. Generally, CR above 10^{-4} are regarded as unacceptable, CR below 10^{-6} as negligible, and ranging from 10^{-4} to 10^{-6} as an acceptable carcinogenic risk factor (USEPA, 2011). Therefore, the CR value of Pb (1.60×10^{-5} and 1.39×10^{-5}) mg/kg/day was in an acceptable range and was lower compared to CR in the range 1.0×10^{-3} to 9.01×10^{-3} mg/kg/day calculated for frequently consumed fish species in Bogra District of Bangladesh (Islam et al., 2016), comparable to CR of 6.75×10^{-5} mg/kg/day in Asafor Market, Ghana (Kwaansa-Ansah et al., 2018) and higher to CR of 1.57×10^{-7} mg/kg/day via fish intake in Sistan region (Miri et al., 2017).

Relationship and source of Heavy metals pollution

The association between studied sea food size and heavy metals concentration was checked by Pearson's correlation analysis for each fish and shrimp species. Most heavy metals had significant correlations ($0.50 < r < 1.0$) with the strongest observed for Pb and Zn ($r = 0.9052$, SI2) with each at $p < 0.05$. The positive correlation between weight and length assent well with earlier findings by Miri et al., 2017 and may be ascribed to their similar spreading bearing, natural and man-made activities. Nonetheless, Cd did not show any significant association with fish and shrimp size for all the studied species except a significant negative correlation between Cd and Cu ($r = -0.1995$; SI2). This might as well be associated to differences in the feeding behavior of each species.

MEC is quite a new index but a widely used tool for generating information about heavy metal origin (Adamu and Nganje 2010). MEC values higher than 1 in this study for both fish and shrimp species (11.83 and 6.12 respectively; Table 3) support strongly the anthropogenic source of Heavy metal contamination.

Multivariate analyses

Principal Component analysis

Principal component analysis (PCA) of heavy metals concentration for *Ethmalosa Fimbriata*, *Clarias Gariepenus*, *Tilapia Guineensis* fishes and *Macrobrachium Felicinum* shrimp species performed using SPSS 20 was expected to identify those elements that co-vary. The PCA results are presented in Fig. 2a where varimax rotation was applied on the first two principal components; PC1 and PC2 accounting for 70.49 and 23.94 % respectively with an overall variance of 94.43%. Zn, Cr and Pb with positive loading and clustering together in PCA 1 indicates the strength of their common relationship as compared with the greater distance observed for Ni. Cu with relative negative loading in PCA 2 reveals the tendency of the fish and shrimp species in accumulating less Cu than others.

Cluster analysis

The measurement regarding similarities and/or differences between size and heavy metals concentration for *Ethmalosa Fimbriata*, *Clarias Gariepenus*, *Tilapia Guineensis* fishes and *Macrobrachium Felicinum* shrimp species by cluster analysis was carried out to obtain information on their association and patterns in data. Result of cluster analysis is shown on the dendogram (Fig 2b) with two main distinguishable clusters. The first cluster comprised of Cd

combining first with the smallest distance to Ni and increased slightly to Zn and relates to a common mixed source of industrial effluents, garbage incinerator and fertilizers application. The second cluster comprised of Cu having the shortest distance which extended slightly to Cr and Pb (persistent toxic metals) and associates with mixed source inputs from industrial effluents and Nigerian crude oils (Farombi et al. 2007).

CONCLUSION

The results revealed that concentration of Cr, Pb, and Ni in fish and shrimp species obtained from markets in Calabar, Cross River State was high and crosses the threshold according to the quality standard set. MPI in the order: *Ethmalosa Fimbriata* > *Clarias Gariepinus* > *Macrobrachium Felicinum* > *Tilapia Guineensis* advises that *Ethmalosa Fimbriata* should be properly processed before consumption and that proper environmental monitoring should be enhanced to check heavy metal hazard. The EDI of the studied heavy metals by fish and shrimp consumption was below the PTWI values which indicate a low risk of non-carcinogenic adverse effects for consumers. More so, the carcinogenic risk of Pb in both fish and shrimp species was within the acceptable range of 10^{-4} - 10^{-6} mg/kg/day. The combine heavy metal concentration and health risk analysis showed that the studied fish and shrimp species could be considered safe to local people and that there may be no potential risks relating to fish and shrimp intake in Calabar. Even though, it is recommended to policy makers and all stakeholders to stop the sources of Cr, Pb, and Ni pollutants, refining, trading and use in all sectors so as not to pollute the environment and increase health risks to the community. The bioaccumulation patterns of metals in this study can be utilized as effective indicators of environmental metal contamination to offer baseline insights on the concentration and health risk of some toxic heavy metals in commercial fish and shrimp species consumed in Calabar and the globe in general.

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Table 1: Biological background information, sampling location and trophic level characteristics of the studied fish and shrimp samples

Biological Information and Trophic level characteristics	Scientific Name	<i>Clarias Gariepenus</i>	<i>Tilapia Guineensis</i>	<i>Ethmalosa Fimbriata</i>	<i>Macrobrachium Felicinum</i>
	English Name	Cat Fish	Tilapia Fish	Bonga Fish	Shrimps
	Local Name	Obubit Inaha	Asat	Ekpai	Obu
	Range of length (cm)	40-60	16-46.2	15-25	3.5-9.5
	Range of Weight (g)	350-550	150-250	100-150	3.70-5.50
	Main Food	Living and dead animal matter, Agro products etc.	Amphibious plants	Plantons	Worms, living and dead organic matter
	Habitat	Fresh water lakes, rivers, swamps, artificial ponds etc.	Shallow streams, ponds, lakes, rivers etc	Fresh and Brackish water, Mangrove creeks, inland rivers etc.	Fresh and Brackish water, Mangrove creeks, inland rivers etc.
	Trophic level	38	2.8	-	2.5
Location and number (quantity) of samples collected	Beach Market	6	6	9	600g
	Lagos Street	6	6	9	600g
	Mbukpa Market	6	6	9	600g
	Watt Market	6	6	9	600g
	Total	24	24	36	2400g

Table 2: Descriptive Analysis of the selected Heavy metals

Seafood Metals (Mg/Kg)	/Heavy Conc.	(Pb)	(Zn)	(Cr)	(Cu)	(Cd)	(Ni)	(Hg)	Total	Range	MPI
Catfish (Clarias Gariepinus)		5.56±0.41	0.84±0.07	6.48±0.29	6.36±0.73	0.13±0.11	1.12±0.18	N	20.48±0.22	0.13-6.48	1.75
Tilapia fish (Tilapia Guineensis)		5.24±0.43	0.46±0.02	5.14±0.79	6.25±0.67	0.03±0.01	1.27±0.08	N	18.38±1.94	0.03-6.25	1.21
Bonga fish (Ethmalosa Fimbriata)		6.68±0.05	1.64±0.02	7.47±0.02	7.73.09±0.49	0.08±0.01	2.3±0.12	N	25.90±0.28	0.08-7.73	2.20
shrimps (Macrobrachium Felicinum)		5.07±0.28	0.56±0.08	6.06±1.01	7.35±0.57	0.03±0.01	1.3±0.04	N	20.36±1.63	0.03-7.35	1.28
Total		22.55±0.71	3.50±0.87	25.15±1.01	19.96±0.86	0.27±0.07	5.99±0.48	N	85.12±9.90	0.27-25.15	-
Range		5.07-6.68	0.46-1.64	5.14-7.47	6.25-7.73	0.03-0.13	1.12-2.3	N	18.38-25.90	-	-
Coefficient of variation (%)		12.59	53.49	16.96	12.35	102.31	32.32	-	-	-	-

Table 3: The PTDI, EDI, and EWI values recorded for the different heavy metals detected in the fish and species

Fish						
Heavy Metal	EDI (mg/kg bw/day)	EWI (mg/kg bw/week)	PTDI	%PTWI	K Value	MEC
Pb	1.8 x 10-3	5.74 x 10-2	3.57	229.5138	7.02	11.83
Zn	3.15 x 10-4	9.62 x 10-3	1000	0.137495		
Cr	2.05 x 10-3	6.26 x 10-2	23.3	0.268832		
Cu	2.19 x 10-3	6.67 x 10-2	500	1.907106		
Cd	2.51 x 10-5	7.96 x 10-4	0.007	11.36996		
Ni	5.03 x 10-4	1.54 x 10-2	0.365	4.210467		
Shrimp						
Heavy Metal	EDI (mg/kg bw/day)	EWI (mg/kg bw/week)	PTDI	%PTWI	K Value	MEC
Pb	1.63 x 10-3	4.99 x 10-2	3.57	199.5815	2.28	6.12
Zn	1.79 x 10-4	5.46 x 10-3	1000	0.078066		
Cr	1.96 x 10-3	5.97 x 10-2	23.3	0.25619		
Cu	2.37 x 10-3	7.23 x 10-2	500	2.066989		
Cd	8.87 x 10-6	2.71 x 10-4	0.007	3.868132		
Ni	4.19 x 10-4	1.28 x 10-2	0.365	3.506849		

Table 4: Estimated CRLim (non-carcinogenic and carcinogenic), CRmm, THQ, CR and HI

Heavy Metal (Fish)	Carcinogenic		Non-carcinogenic		THQ	CR (mg/kg/day)
	CRLim (Kg/day)	CRmm (Meals/month)	CRLim (Kg/day)	CRmm (Meals/month)		
Pb	3.28×10^{-3}	0.44	9.76×10^{-3}	1.31	0.54	1.60×10^{-5}
Zn			4.99	668.77	1.05×10^{-3}	
Cr			7.66×10^{-4}	0.10	6.84	
Cu			0.10	12.86	0.05	
Cd			0.20	26.96	0.03	
Ni			0.21	27.92	0.03	
HI					7.48	
Heavy Metal (Shrimp)	Carcinogenic		Non-carcinogenic		THQ	CR (mg/kg/day)
	CRLim (Kg/day)	CRmm (Meals/month)	CRLim (Kg/day)	CRmm (Meals/month)		
Pb	3.77×10^{-3}	0.51	1.12×10^{-2}	1.51	0.47	1.39×10^{-5}
Zn			8.78	1177.88	5.97×10^{-4}	
Cr			8.04×10^{-4}	0.11	6.52	
Cu			0.09	11.86	0.06	
Cd			0.59	79.24	0.01	
Ni			0.25	33.52	0.02	
HI					7.06	

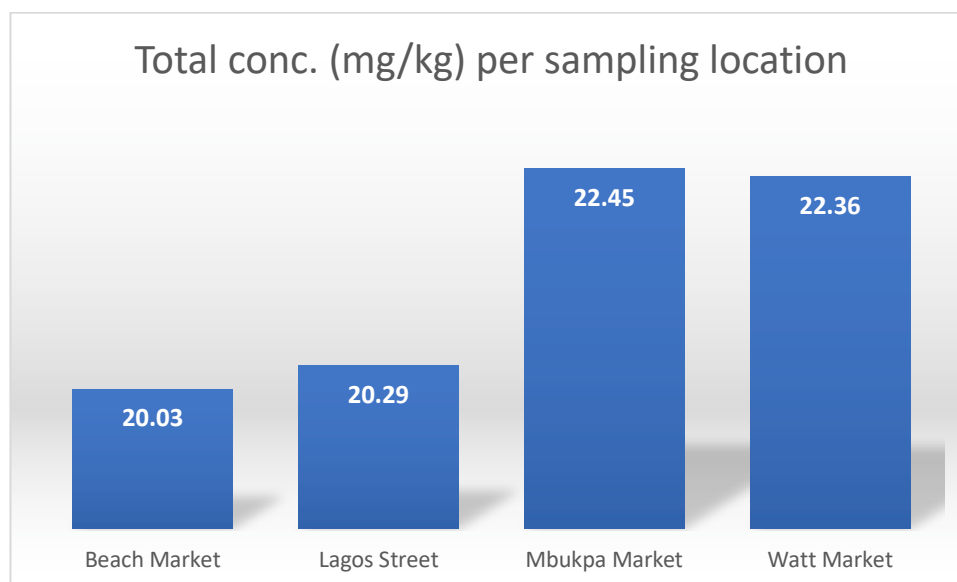


Fig. 1. Heavy Metal concentrations of the four sampling locations in Calabar

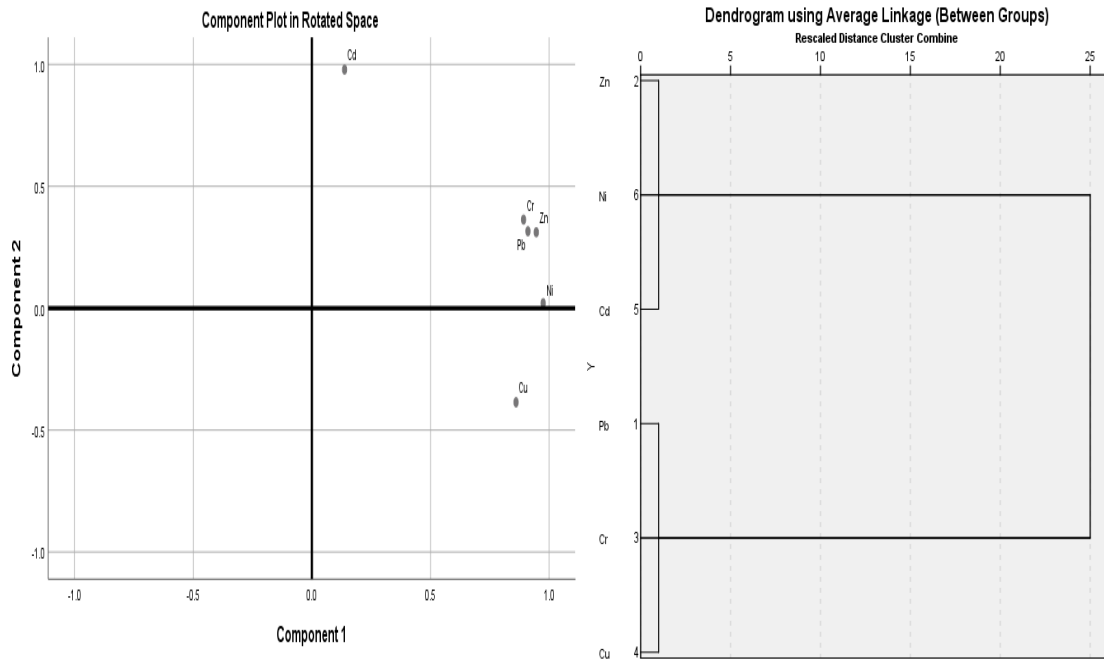


Fig. 2 : (a) Score plot of PCA and (b) Dendrogram of clustering of heavy metals in fish and shrimps species

SI1: Anova: Two-Factor With Replication

SUMMARY	Lead (Pb)	Zinc (Zn)	Chromium (Cr)	Copper (Cr)	Cadmium (Cd)	Nickel (Ni)	Total
<u>Catfish (<i>Clarias Gariepenus</i>)</u>							
Count	4	4	4	4	4	4	24
Sum	22.23	3.37	25.92	25.42	0.53	4.46	81.93
Average	5.5575	0.8425	6.48	6.355	0.1325	1.115	5
Variance	0.22735	0.0060	0.113867	0.706967	0.015692	0.04083	8.0250
<i>Tilapia fish (Tilapia Guineensis)</i>	8	92	0.113867	0.706967	0.015692	3	07
Count	4	4	4	4	4	4	24
Sum	20.97	1.82	20.54	25.01	0.13	5.07	73.54
Average	5.2425	0.455	5.135	6.2525	0.0325	1.2675	67
Variance	0.24189	0.0005	0.827033	0.605292	0.000025	0.00942	6.9024
<i>shrimps (Macrobrachium Felicinum)</i>	2	67	0.827033	0.605292	0.000025	5	17
Count	4	4	4	4	4	4	24
Sum	20.27	2.22	24.25	29.39	0.11	5.2	81.44
Average	5.0675	0.555	6.0625	7.3475	0.0275	1.3	33

	0.10809	0.0094				0.00186	8.8294
Variance	2	33	1.368092	0.432225	9.17E-05	7	93
<i>Bonga fish (Ethmalosa Fimbriata)</i>							
Count	4	4	4	4	4	4	24
Sum	26.73	6.54	29.88	30.92	0.31	9.2	103.58
							4.3158
Average	6.6825	1.635	7.47	7.73	0.0775	2.3	33
	0.00375					0.01786	9.8167
Variance	8	0.0007	0.0004	0.0118	9.17E-05	7	47
<i>Total</i>							
Count	16	16	16	16	16	16	
Sum	90.2	13.95	100.59	110.74	1.08	23.93	
		0.8718				1.49562	
Average	5.6375	75	6.286875	6.92125	0.0675	5	
		0.2320				0.24925	
Variance	0.53738	03	1.212343	0.778892	0.005087	3	
<i>ANOVA</i>							
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>	
	20.7744					2.73180	
Sample	4	3	6.924812	34.99251	4.62E-14	7	
	747.744					2.34182	
Columns	3	5	149.5489	755.7015	1.01E-60	8	
	10.2015					1.80757	
Interaction	4	15	0.680103	3.436702	0.000212	1	
	14.2483						
Within	8	72	0.197894				
	792.968						
Total	7	95					

SI2: Pearson Correlation of the Studied Samples

Heavy Metals	r	N	T-statistics	DF	P-value
Pb with Zn	0.9051615	16	7.9676815	14	1.43549E-06
Pb with Cr	0.7217816	16	3.9020055	14	0.001595445
Pb with Cu	0.6024922	16	2.8245199	14	0.013511874
Pb with Cd	0.2418904	16	0.9327711	14	0.366746797
Pb with Ni	0.811882	16	5.2032761	14	0.000133782
Zn with Cr	0.7359713	16	4.0675047	14	0.001153082
Zn with Cu	0.5078501	16	2.2058286	14	0.044610069
Zn with Cd	0.2898834	16	1.1333062	14	0.276121143
Zn with Ni	0.8787202	16	6.8879414	14	7.46654E-06
Cr with Cu	0.7485358	16	4.2237796	14	0.000850332
Cr with Cd	0.2747822	16	1.0693018	14	0.303024301
Cr with Ni	0.6002304	16	2.8079276	14	0.013960744
Cu with Cd	-0.1994891	16	-0.7617307	14	
Cu with Ni	0.5656931	16	2.5668069	14	0.022375583
Cd with Ni	0.1374725	16	0.5193056	14	0.611659589