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Biomonitoring of heavy metal in selected biomarkers of *Clarias gariepinus* (Burchell, 1822), A comparative study of River Galma, River Kubani and fish farms in Zaria, Nigeria

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ABSTRACT

Levels of contaminants in fish are of particular interest because of the potential risk to humans who consume them. Clarias gariepinus were obtained from River Galma, River Kubanni and some Fish farms in Zaria. The head, liver, gills and muscles were carefully dissected for the determination of heavy metals. Iron, cobalt and manganese were determined using Schemadzu, Atomic Absorption Spectrophotometer (model 6800, Japan) after wet digestion. The results revealed that, Clarias gariepinus liver tissues contained the highest concentration of iron (63%) and cobalt (73%) while the highest concentration of manganese was recorded in gills tissues (36%). Fish muscles appeared to be the least preferred site for the bioaccumulation of the three metals as the lowest concentrations (6%) were detected in this tissue. The order of detection was liver > gills > heads = muscles for iron, liver > gills > heads > muscles for cobalt and gills > heads > liver > muscles for manganese. The mean concentrations of iron and manganese measured in Clarias gariepinus from Rivier Galma and River Kubanni were found to be above the Food and Agricultural Organization (FAO) and US EPA guidelines for consumed food and fish. These findings indicate possible sediment and/or surface water contamination by iron and manganese in River Galma and River Kubanni and that consumption of Clarias gariepinus from the two rivers could pose serious health risk. Implications of findings to public health are fully discussed.

Key words: River Galma, River Kubanni, *Clarias gariepinus*, Heavy metals, bioaccumulation, health risk.

INTRODUCTION

In their interaction with one another and with the environment in which they live and with which they interact, living organisms are continually exposed to naturally occurring and synthetic or manmade chemicals. Some chemicals or their breakdown products (metabolites) lodge in the body of organisms only for a short while before being excreted, arsenic in the human body, for instance, is mostly excreted within 72 hours of exposure. Other chemicals, however, are not readily excreted and can remain for years in the blood, adipose (fat) tissue, semen, muscle, bone, brain tissue, or other organs [1]. The measurement of trace concentration of chemical substances in living organisms is referred to as biomonitoring. Biomonitoring is the science of inferring the ecological condition of an area by examining the organisms that lives there [1]. Biomonitoring therefore may be used to assess the level of chemicals natural or synthetic that is present or has been present in the environment and is best at detecting those chemicals that persist in the environment [1]. Examples of such persistent substances include heavy metals and a number of synthetic organic chemicals, such as PCBs, DDT, PAHs, Dioxins and furans.

Heavy metals are commonly found in natural waters and some are essential to living organisms, yet they may become highly toxic when present at high concentrations. Among environmental pollutants, metals are of particular concern, due to their potential toxic effect and ability to bioaccumulate in aquatic ecosystems. Natural and anthropogenic sources are responsible for their input into the aquatic ecosystem. These metals get distributed in the water body, suspended solids and sediments during the course of their mobility [2]. The rate of bioaccumulation of heavy metals in aquatic organisms depends on the type of organism, the metal concern, chemical form of the metal and the concentration of the metal in the water and sediment. Fishes have been recognized as a good accumulator of organic and inorganic pollutants [3]. Age of fish, lipid content in the tissue and mode of feeding are significant factors that affect the accumulation of heavy metals in fishes [*2*; 4]. Bioaccumulated metals in an organism are often transferred to other organisms (including humans) higher up in the food chain. Heavy metals acquired through the food chain as a result of pollution are potential chemical hazards, threatening consumers [5]. In most cases they become increasingly more concentrated at successively higher trophic levels. Bioaccumulation of heavy metals in tissues of marine organisms has been identified as an indirect measure of the abundance and availability of metals in the marine environment [6]. Monitoring fish tissue contamination serves an important function as an early warning indicator of sediment contamination or related water quality problems and enables appropriate action to be taken in order to protect public health and the environment [6; 7; 8; 9]. The present study aimed at: Monitoring the concentrations of iron cobalt and manganese in different tissues of *Calrias gariepinus* as an indicator of sediment contamination or related water quality problems of River Galma and River Kubanni using fish farms in Zaria as control. Metals concentrations obtained were also compared with Food and Agricultural Organization (FAO)/World Health Organization (WHO) and US EPA guidelines for consumed food and fish. Water quality is a term used to express the suitability of water to sustain various uses or processes. River Galma, River Kubanni receives numerous non-point and point source discharges during their transit through Zaria and serves as a major source of portable water and fish in Zaria. The Zaria dam is on River Galma and supply water to Zaria metropolis while the ABU dam is on River Kubani and supply water to the Ahmadu Bello University community. Fishing is carried out daily on the two Rivers which also serve as a source of water for irrigation as the basins of the two rivers are booming crop farming areas [10; 4].

MATERIALS AND METHODS

Study Location

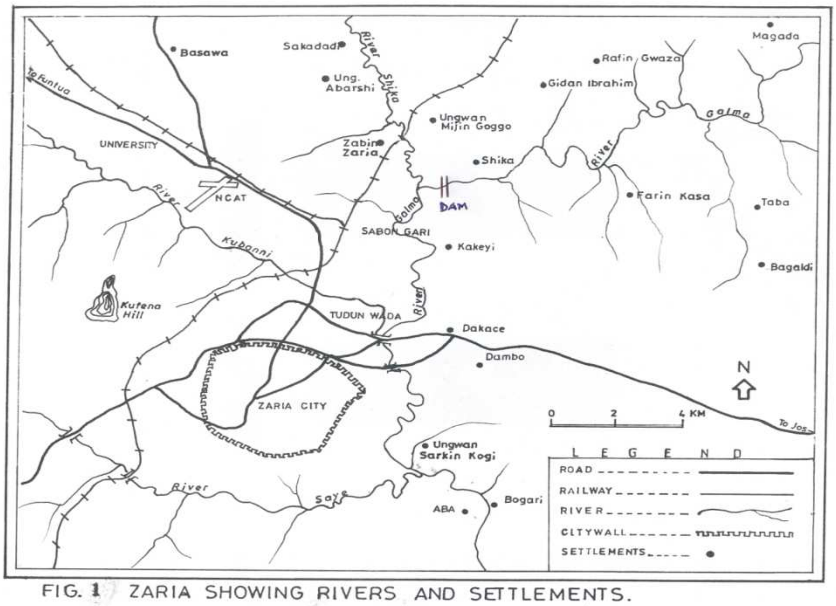

FIG. 1 ZARIA SHOWING RIVERS AND SETTLEMENTS.

Zaria is located at latitude 1103'N and longitude 7040'E, 128km South- East of Kano and 64km North-East of Kaduna City. River Galma is located at the southeastern part of Zaria and its source is the Jos Plateau. The Zaria dam is located on River Galma [10]. Kubanni River originates in the precincts of the Ahmadu Bello University (ABU) Main Campus, Zaria (Northern Nigeria), as a trench in an undulating agricultural land and is fed by a number of tributaries [11]. Kubanni River drains the northwest zone of the city of Zaria and receives effluents mainly from domestic activity and runoff from intense cropping located in the adjoining land. The ABU dam is on the river [11].

Sample collection and preparation

Ten *Clarias gariepinus* each (mean weight 130±3g, Mean length 26.8±2.6cm) were obtained on site from fishermen at the two Rivers (River Galma, River kubani) and five each from two fish farms in Sabon Gari area of Zaria, Nigeria. They were stored in a cooler packed with ice block in order to maintain the freshness and latter transported to the Environmental laboratory of the National Research Institute for chemical technology (NARICT), Zaria, Nigeria for dissection of the organs after being washed thoroughly. 5g (wet weight) of fish tissue was weighed and placed in a beaker, 10ml of freshly prepared concentrated nitric acid / hydrogen per oxide (1:1) was added and covered with a wash glass for initial reaction to subside. The beaker was placed on a water bath at a temperature not exceeding 80°C for two hours to reduce the volume to 3-4ml. The digest was cold filtered into 50mls volumetric flask and made up to the mark with distilled deionized water. Method for sample preparation was adopted from Udiba et al., [12] and APHA, [13].

Metal analysis

Metal analysis was carried out using flame atomic absorption spectrophotometer AA-6800 (Shimadzu, Japan) at National Research Institute for Chemical Technology (NARICT), Zaria-Nigeria. The calibration curves were prepared separately for each of the metals by running different concentrations of standard solutions. The instrument was set to zero by running the respective reagent blanks. Average values of three replicates were taken for each determination and were subjected to statistical analysis. The metals determined includes: Iron, Cobalt, and Manganese.

Analytical Quality Assurance

The result of the analysis was validated by digesting and analyzing Standard Reference Materials, Lichens coded IAEA-336 following the same procedure. The analyzed values and the certified reference values of the elements determined were compared to ascertain the reliability of the analytical method employed. Double distilled and deionized water was used throughout the experimentation. All the reagents used in this work were AnalaR grades from BDH Chemicals (UK).

Statistical Analysis:

All statistical analyses were done by SPSS software 17.0 for windows.

Analysis of Variance Test

Data collected were subjected to statistical tests of significance using Analysis of Variance (ANOVA) with post hoc test analysis to assess significant variation in metal levels of *Clariaas gariepinus* tissues across the three sources (River Galma, River Kubanni and fish farms). A P-value of 0.05 or less was considered statistically significant.

Pearson product moment Correlation Coefficient

Correlation coefficient was used to determine the association between the three metals (iron, cobalt and manganese) under consideration

RESULTS AND DISCUSSION

To evaluate the accuracy and precision of the analytical procedure employed, Standard reference material of lichen coded IAEA-336 was analyzed in like manner to our samples. The analyzed values and the certified reference values of the elements determined were very close, suggesting the reliability of the method (Table 1).

TABLE 1 Results of analysis of reference material (Lichen IAEA -336) compared to the certified reference value (mg/kg)

Element (mg/kg)	Pb	Cd	Cu	Mn	Zn
A Value	5.25	0.140	4.00	55.78	29.18
R value	4.2-5.5	0.1-2.34	3.1- 4.1	56-70	37-33.80

A Value = Analyzed value *R Value = Reference value.*

Table 2: Mean ± S.D of Iron, Cobalt and Manganese in *Clarias garipenus* from River Galma, River Kubanni and fish farms in Zaria, Nigeria

Elements/Sampling Station	River Galma				River Kubanni				Fish Farm			
	Head	Gills	Liver	Muscle	Head	Gills	Liver	Muscle	Head	Gills	Liver	Muscle
Iron	5.05 ±5.50	31.70 ±11.64	84.34 ±82.27	3.44 ±1.09	2.55± 0.45	20.23± 7.15	98.58 ±7.78	3.21 ±3.00	11.2 ±10.86	28.69 ±15.48	19.15 ±5.83	13.62 ±15.57
Cobalt	0.67 ±0.19	1.51 ±0.81	7.92 ±4.33	0.51 ±0.41	0.79± 0.64	0.78 ±0.27	5.34 ±3.85	0.43 ±0.32	0.63 ±0.12	0.58 ±0.59	3.84 ±0.21	0.41 ±0.30
Manganese	3.53 ±1.15	2.69 ±0.64	5.76 ±1.39	0.58 ±0.29	4.36± 6.23	5.81 ±8.90	0.50 ±0.25	0.57 ±0.40	0.12 ±0.11	0.71 ±0.25	0.50 ±0.04	0.25 ±0.11

Table 3: A comparison of Mean of total metal concentrations with maximum limits

Stations	Heavy Metal	Mean of total concentration in fish (mg/kg)	Maximum limits	Reference
Galma	Iron	31.13±4.93	30	FAO (1983)
	Cobalt	2.65±3.71	-	FAO (1983)
	Manganese	3.14±2.10	0.14	EPA, 2004
Kubanni	Iron	31.14±5.31	30	FAO (1983)
	Cobalt	4.07±3.70	-	FAO (1983)
	Manganese	1.07±1.47	0.14	EPA, 2004
Fish farms	Iron	0.58±0.46	30	FAO (1983)
	Cobalt	1.84±1.54	-	FAO (1983)
	Manganese	0.63±0.42	0.14	EPA, 2004

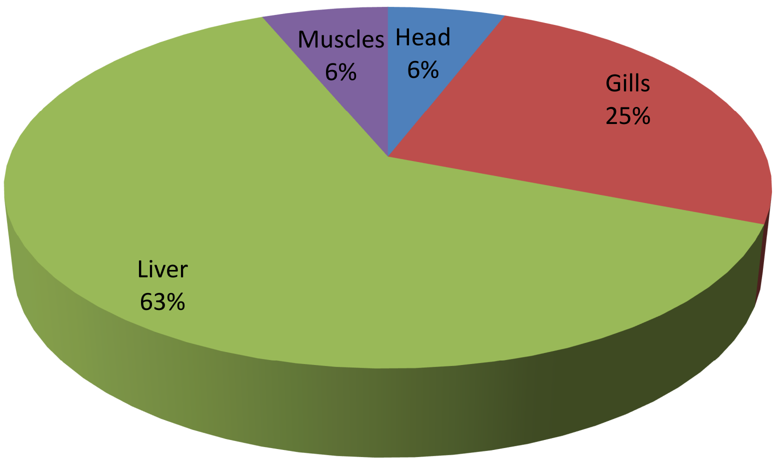

FIG. 2: Percentage concentration of Iron in tissues of Clarias gariepinus tissues

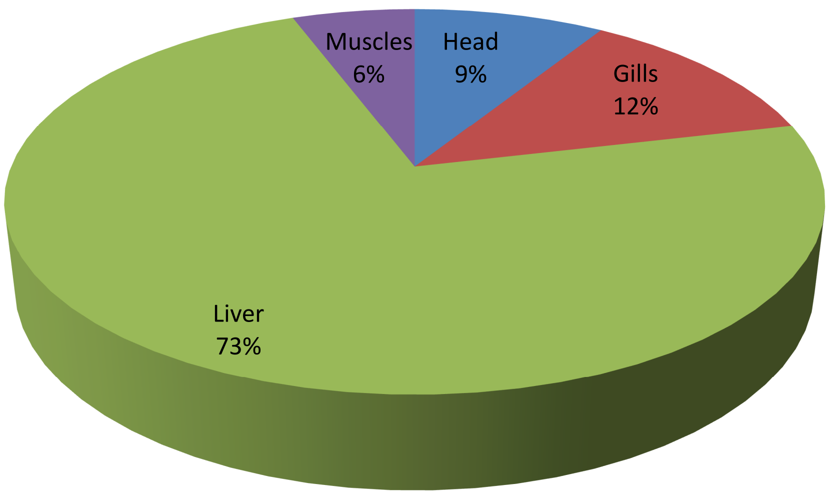

FIG. 3: Percentage concentration of Cobalt in tissues Clarias gariepinus

The Mean concentration and standard deviation of iron, cobalt and manganese of tissues of *Calrias gariepinus* from River Galma, River Kubani and fish farms in Zaria are presented in Table 2. Percentage concentration of Iron, Cobalt and Manganese of tissues of *Calrias gariepinus* from River Galma, River Kubani and fish farms in Zaria are shown in figures 2 – 4 respectively. The distribution of Iron, cobalt and manganese in the head, gills, liver and muscle of *Clarias Gariepinus* across River Galma, River Kubni and fish farms in Zaria are shown in Figures 5, 6, 7 and 8 respectively.

Average concentration of iron in *Clarias gariepinus* was found to be 31.14±4.92 mg/kg for River Galma, 31.14±5.4 mg/kg for River kubanni and 18.16±1.228 mg/kg for Fish farms (Table 3). The order of detection therefore was River Galma = River Kubanni > Fish farms. No statistically significant difference (ANOVA; $P > 0.05$) in overall concentration of iron was observed between *Clarias gariepinus* from river Galma, River Kubanni and Fish Farms. Tissue by tissue analysis revealed that the lowest concentration (0.98 mg/kg) of iron was measured in the heads and the highest concentration (184.64 mg/kg) measured in the gills. Great variation in the level of iron concentration of the tissues was observed, with the degree of iron concentration being present producing the following general sequence: liver > gills > head = muscle. However; only the difference between the iron content of Head and gills, and between gills and muscles were statistically significant at 95% confidence level. Iron content of gills was significantly higher than head and muscles. The percentage concentration of iron in *Clarias gariepinus* tissue was Head 6%, Gills 25%, Liver 63% and Muscle 6% (Figure 2). The trend of iron in tissues of *Clarias gariepinus* per sampling station was gills > liver > head > muscle for River Galma, liver > gills > muscle > head for River Kubanni and gills > liver > muscle > head for Fish Farms (table 2).

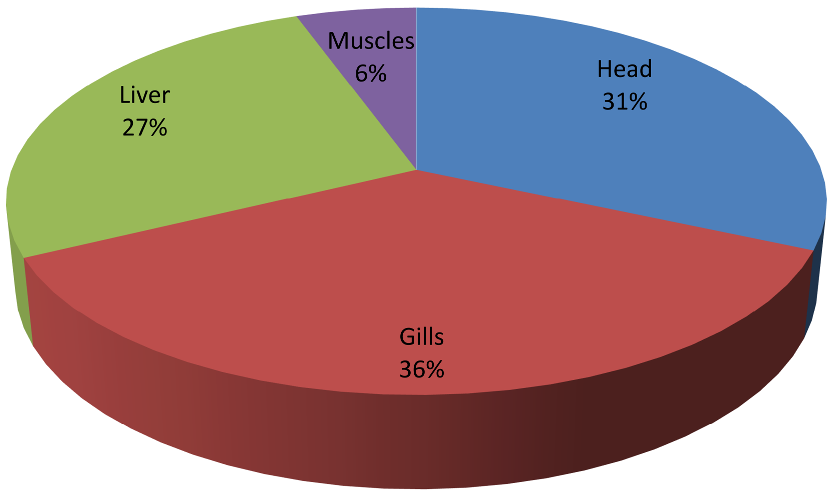

FIG. 4: Percentage concentration of Manganese in tissues Clarias gariepinus

Generally, the levels of iron in the liver and gills irrespective of the of the sample location were higher than the results obtained for head and muscle. This higher level of iron in the liver tissues may indicate that, liver is the target organ for the accumulation of this element. Liver accumulates high concentrations of metals, irrespectively of the uptake route. The liver is considered a good monitor of water pollution with metals since their concentrations accumulated in this organ are often proportional to those present in the environment. The higher level of trace metals in the liver relative to other tissues may be attributed to the high coordination of metallothionein protein with the metals. In addition, the liver is the principal organ responsible for the detoxification, transportation, and storage of toxic substances and it is an active site of pathological effects induced by contamination [11; 4]. For the gills samples, high iron concentration may be due to the fact that freshwater fish gills might be expected to be the primary route for the uptake of water borne pollutants. The gills perform the function of respiration and are directly in contact with water and pollutants that may be present in water. Thus, the concentrations of trace metals in gills reflect the concentration of trace metals in the waters where the fish lives [11]. Finding of this study was found to be consistent with Nnaji et al., [10] who reported that gills are the main sites of heavy metal uptake by fish while the liver and kidney are known to accumulate high amounts of metals. Figure 5- 8 reveals that that iron concentration of the livers in the study followed the order River Kubani > River Galma > Fish Farm, that of the gills followed the order River Galma > Fish farms > River Galma and those of muscles and head followed the order Fish Farms > River Galma > River Kubanni. Recorded data from this study indicates that the mean of total concentrations of iron

(31.13±4.93 mg/kg) measured in *Clarias gariepinus* from Rivier Galma and River Kubanni (31.14±5.31 mg/kg) were above the Food and Agricultural Organization (FAO) guideline of 30 mg/kg [12]. Consuming *Clarias gariepinus* from River Galma and River Kubanni could pose health risk even though the iron contents of fish head and muscles which form a major part of the human diet were below the FAO guidelines. The mean total concentration of iron (0.58 mg/kg) measured in *Clarias gariepinus* from fish farms was below FAO/WHO guideline and therefore poses no significant toxicological risk with respect to iron intoxication. These findings indicate possible sediment and/or surface water contamination by iron in the two rivers. A higher mean iron concentration (58.96mg/kg) was reported for *Clarias Gariepinus from* River Owan, Edo State, Nigeria [14]. Lower mean values of 1.263 mg/kg and 1.340 mg/kg were reported for *Clarias Gariepinus* from River Niger, and River Warri respectively [15; 16]. Iron concentration ranging from 39.60 - 41.07 µg/g was reported for Ikpoba river dam Benin City, Nigeria [17] and 20.0-54.52 mg/kg for fish Species of the River Yamuna (Delhi), India [18]. Liver, kidney and the cardiovascular system are the target organs for iron toxicity

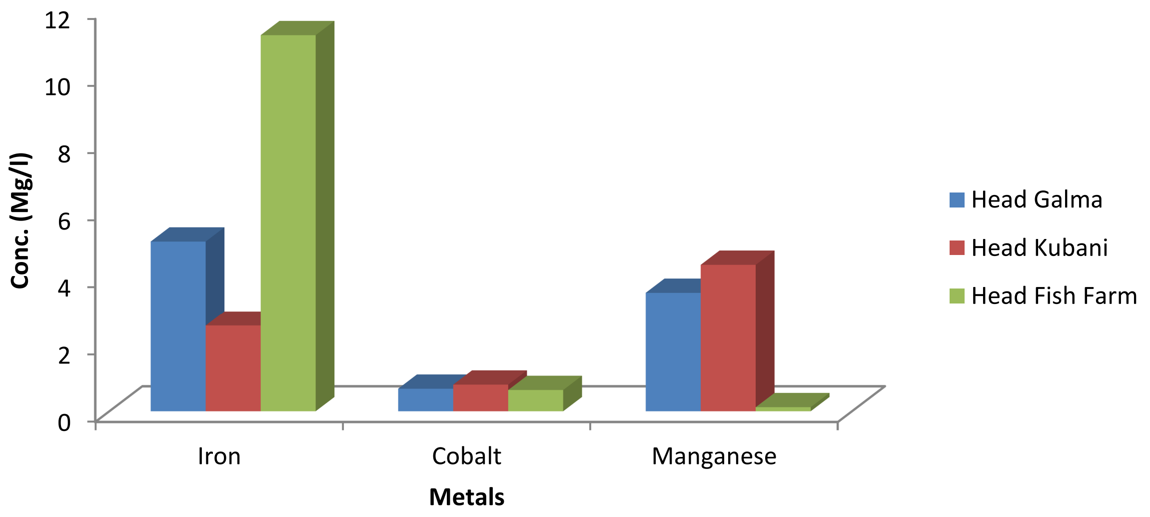

FIG. 5: The dispersion of lead, copper and zinc in the head of Clarias gariepinus across River Galma, River Kubanni and Fish Farms in Zaria

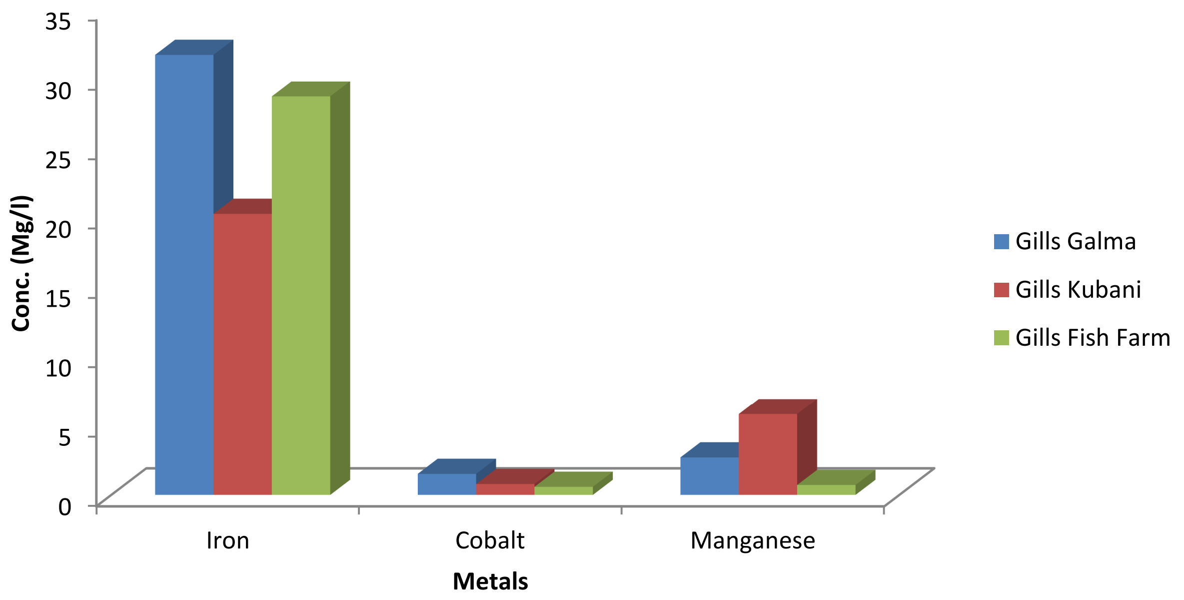

FIG. 6: The dispersion of lead, copper and zinc in the gills of Clarias gariepinus across River Galma, River Kubanni and Fish Farms in Zaria

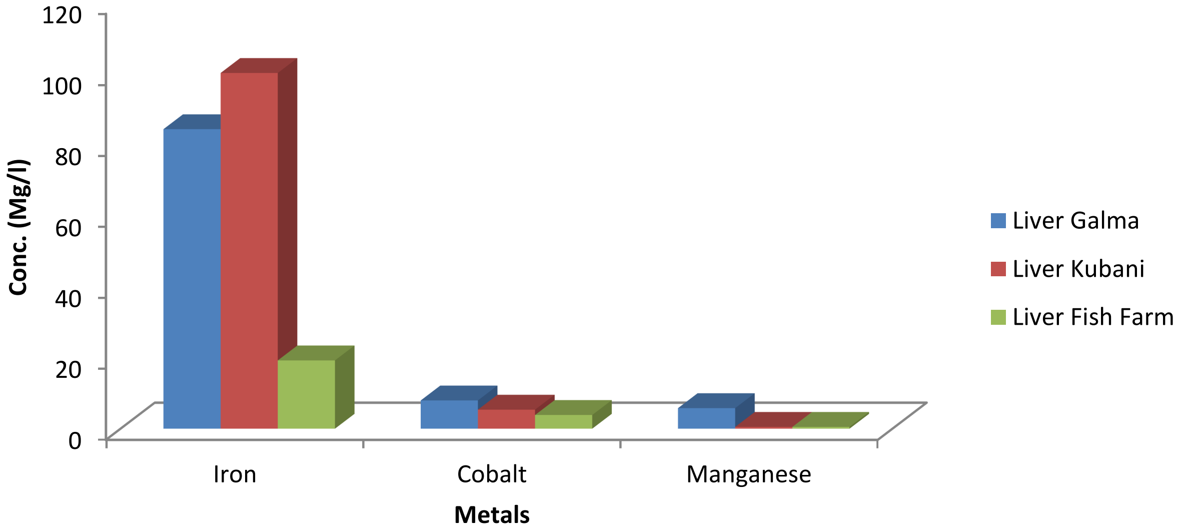

FIG. 7: The dispersion of lead copper and zinc in the liver of Clarias gariepinus across River Galma, River Kubanni and Fish Farms in Zaria

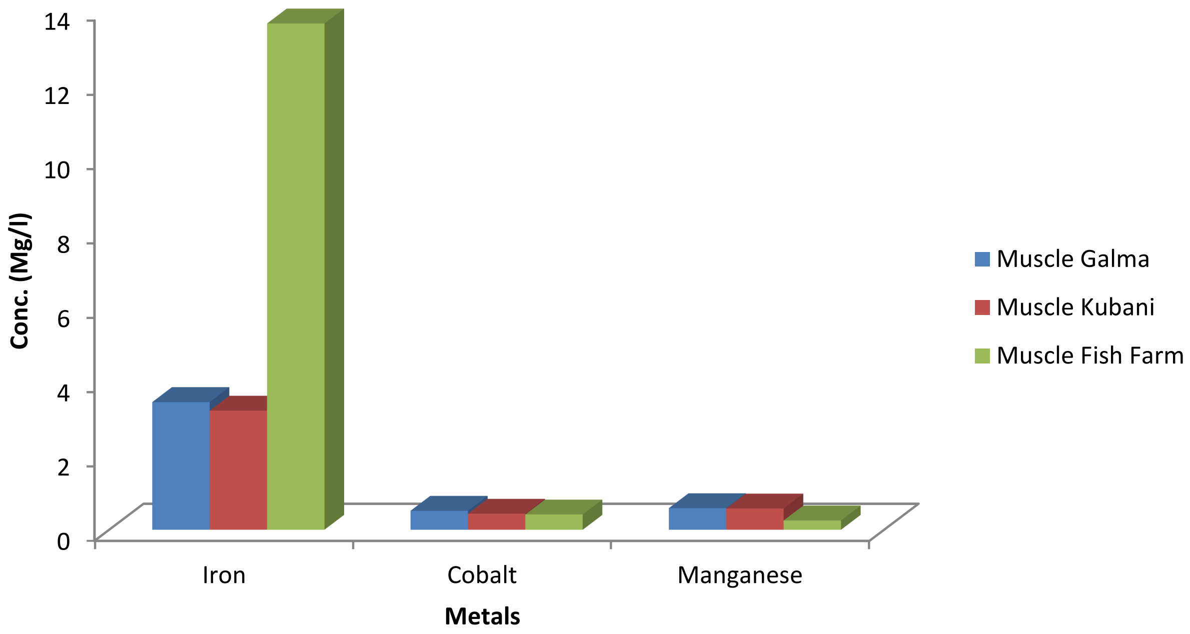

FIG. 8: The dispersion of lead, copper and zinc in the muscle of Clarias gariepinus across River Galma, River Kubanni and Fish Farms in Zaria, Nigeria

Cobalt and its compounds are highly persistent in water and the environment. Cobalt will bioaccumulate or concentrate in the tissues of fish. The average concentration of cobalt was found to be 2.65±3.71 mg/kg, 4.07±3.70 mg/kg and 1.84±1.54 for *Clarias gariepinus* from River Galma, River kubanni and Fish Farms respectively (Table 3). The order of detection therefore was River Kubanni > River Galma > Fish farms. No statistically significant difference (ANOVA; $P > 0.05$) in overall concentration of cobalt was observed between *Clarias gariepinus* from river Galma, River Kubanni and Fish Farms. Result of the present study shows that the lowest concentration (0.07 mg/kg) of cobalt was measured in the head and the highest concentration (12.79 mg/kg) measured in the gills. Gill tissue is very susceptible to changes in concentrations of heavy metals, temperature, pH and other variables in the water environment. For this reason gills are good indicators of water pollution [19]. The percentage concentration of cobalt in *Clarias gariepinus* tissue was Head 9%, Gills 12%, Liver 73% and Muscle 6% (Figure 3). Statistically significant difference was observed between cobalt concentration in liver and head, liver and gills and between liver and muscle with cobalt concentration in liver being significantly higher than the concentration in head, gills and muscles at 95% confidence level. Cobalt concentration in fish tissues followed the order: liver > gills > head > muscles for River Galma and Liver > head > gills and muscles for river Kubanni and Fish farms respectively. Tissue by sample location analysis also revealed that cobalt concentration in the liver, gills and muscles showed the trend River Galma > River Kubanni > Fish farms (Figure 5-8) while cobalt content of fish head followed the order; River Kubanni > River Galma > Fish Farm. The differences in tissue cobalt concentrations across the sampling locations were not statistically significant (ANOVA, P > 0.05). A lower cobalt concentration ranging from 0.06-0.55mg/kg was recorded for Clarias Gariepinus from Ogun coastal waters [20]. In the determination of heavy metals in fish species of the River Yamuna (Delhi), India, Sen et al., [18] reported cobalt levels below detectable limits and Lakshmanan et al., [21] reported cobalt concentrations ranging from 0.006±0.00-0.014±0.00 ppm for Five Commercially Important Fishes of Parangipettai, Southeast Coast of India. Cobalt is an essential element in the human body. It forms the core of vitamin B12 necessary for the formation of all cells, especially red blood cells. Reproductive and developmental effects, bronchial asthma, cardiomyopathy, lungs diseases, eye, nose and throat irritation are some of the effects of cobalt toxicity [22].

Overall mean concentration of manganese was found to be 3.14±2.10 mg/kg, 1.07±1.47 mg/kg and 0.63±0.42 for *Clarias gariepinus* from River Galma, River kubanni and Fish Farms respectively (Table 3). The order of detection therefore was River Galma > River Kubanni > Fish farms. No statistically significant difference (ANOVA; P > 0.05) in overall concentration of manganese was observed between *Clarias gariepinus* from river Galma, River Kubanni and Fish Farms. The lowest concentration (0.13 mg/kg) of manganese was measured in fish muscle and the highest concentration (16.09 mg/kg) measured in the gills. The percentage concentration of manganese in *Clarias gariepinus* tissue was Head 31%, Gills 36%, Liver 27% and Muscle 6% (Figure 4). No Statistically significant (ANVA, P > 0.05) difference was observed in manganese concentration between the tissues studied. However, the concentration in the tissues followed the order: liver > head > gills > muscles for River Galma, gills > head > muscles > Liver for River Kubanni and gills > liver > muscle > head for Fish farms. Comparison of manganese concentration of each tissue across the three sampling locations revealed that the concentration of liver and muscle displayed the order River Galma > River Kubanni > Fish farms while that of gills and head followed the order River Kubanni > River Galma > Fish Farms (Figures 5- 8). The chronic oral dose for Manganese is 0.14mg/kg/day and the guideline value is 0.7 mg/kg [23]. The average manganese concentration recorded in this study for *Clarias Gariepinus* from River Galma and River Kubanni in Zaria were found to be above the EPA limits. These findings may suggest sediment or surface water contamination by the metal manganese in the two rivers. Consumption of fish from the two Rivers therefore poses serious risk with respect to manganese intoxication. Manganese is an essential nutrient that is important for normal processes in the human body, though adverse effect have been reported at higher doses. Exposure to high concentration of manganese is associated with impaired neurological and neuromuscular control, mental and emotional disturbances (muscle stiffness, and lack of coordination). Exposure to very high doses result in impaired male fertility, birth defects, and impaired bone development. Manganese selectively accumulates in the CNS, the bones, liver and kidney [24]. Manganese concentration ranging from 0.38 - 1.34 µg/g was reported for some benthic fishes of Ikpoba river dam Benin City, Nigeria [17]. A range of 1.19-5.14 mg/kg was recorded for in Fish Species of the River Yamuna (Delhi), India [18]. Mean values of 0.292 mg/kg, 0.450 mg/kg and 4.62mg/kg were reported for *Clarias Gariepinus* from River Niger, and River Warri and River Owan, Edo State, Nigeria respectively [15; 16; 14].

Statistical analysis revealed that iron content of *Clarias Gariepinus* tissues correlated positively with cobalt content (r = 0.359) and with manganese content (r = 0.073). Cobalt content was also found to correlate positively with Manganese content (r = 0.185) indicating that increase in iron concentration in *Clarias Gariepinus* tissues is associated with increase in cobalt and manganese concentrations and that increase in cobalt concentration is also associated with increase in manganese concentration, but only the correlation between iron and cobalt was statistically significant at 95% confidence level, suggesting that same source may be responsible for the presence of iron and cobalt at the concentration determined.

CONCLUSION

Calarias gariepinus is a very important source of animal protein in human diet. It forms a significant percentage of captured fish in River Galma and River Kubanni, Zaria and is consumed in great quantities by the local duelers. Iron, cobalt and manganese concentrations were monitored in tissues of *Calarias gariepinus* from the two rivers and selected fish farms in Zaria. Results indicate that liver is the site for maximum accumulation of iron manganese, while muscle is the site for list accumulation. The mean concentrations of iron and manganese measured in *Clarias gariepinus* from Rivier Galma and River Kubanni were above the Food and Agricultural Organization (FAO) and US EPA guidelines for consumed food and fish. Consuming *Clarias gariepinus* from River Galma and River Kubanni could therefore pose serious health risk. These findings suggest possible sediment or surface water contamination by iron and manganese. A study on the heavy metal profile of the two rivers is therefore recommended.

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