



Determination of selected heavy metal and analysis of proximate composition in some fish species from Ogun River, Southwestern Nigeria



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ABSTRACT

The aquatic environment is continuously at the mercy of man's negative impact on it. This study was designed to determine the proximate composition and concentration of some heavy metals in four fish species - *Oreochromis niloticus*, *Malapterurus electricus*, *Parachanna obscura*, *Chrysichthys nigrodigitatus* in Ogun River, Nigeria. The fish species were collected from the Abeokuta axis of the river and analyzed for Manganese (Mn), Lead (Pb), Nickel (Ni), Cadmium (Cd) and Zinc (Zn) using atomic absorption spectrometry. The moisture, crude protein, ash and fat content of the four fish species were analyzed using standard methods. The results showed varying levels of accumulation of the metals among the four fish species. Pb was detected only in *Oreochromis niloticus* while Mn was detected only in *Chrysichthys nigrodigitatus*. Except in a few cases, Pb and Ni in muscles of *Oreochromis niloticus* and *Parachanna obscura* respectively, the liver and gills accumulated more metals than the muscles. The concentrations of the metals in all cases were beyond regulatory limits by International Standards. All the fishes had high moisture content between 75 and 80 %, while fat was the lowest nutrient (0.88–1.89 %) in all fish species. The differences in proximate values were not significant among fish species. The findings in this study may be a reflection of the pollution status of the Ogun River with heavy metals while also portraying the risk associated with the consumption of fish from the river.

1. Introduction

Nigeria, a western African Country has a vast area of freshwater ecosystem, where fishing activities is the main source of livelihood for many. The quest for fish as a food source especially by the local populace is unending, either as a result of its relative affordability and/or because of the nutrients derived from it. Despite the increase in the practice of aquaculture in recent years for adequate fish production due to increasing population, the demand for fish consumption still outweighs aquaculture production. Nigeria produces only about 40 % of the local consumption demand estimated 2.66 million metric tons, importing the balance of 60 % to augment demand [1, 2]. Hence, there is an inevitable need to complement aquaculture with fishing from natural waters which has abundant and diverse fish species. However, fishing from the wild presents its separate challenge as these water bodies are contaminated with chemicals from farmlands, oil spills and heavy metals from industries [3].

The high demand for fish is not only because of its taste but also largely due to its nutritional values which is a function of its proximate composition [4]. Fish protein is easily digestible. Additionally, it is an

important source of both essential and non-essential amino acid [5]. Its amino acid content has a high quantity of cysteine than a large amount of other protein source. Beneficial polyunsaturated fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have been reported [6, 7] to be present in adequate quantities in the tissues of fish. These polyunsaturated fatty acids have been reported to have the ability to both prevent, and also cure some diseases of man including cancers, heart diseases, rheumatoid arthritis and inflammation [8, 9]. The maintenance of acid–base balance is one of the functions of minerals in the body; in addition, they also help in formation of haemoglobin [10]. Furthermore, minerals are involved in catalyzing metabolic reactions, control water balance and helps formation of bones [11].

Heavy metals are important component of pollution in the aquatic ecosystem. This is because of their toxicity, accumulation and ability to bio-magnify along the food chain. The sources of natural aquatic systems contamination of heavy metals are mostly household, manufacturing and man-made activities [12].

Fish can easily accumulate heavy metals into their bodies from any of sediment, water of food by virtue of their position in the aquatic food chain [13, 14]. Some of the heavy metals are beneficial to fish, yet their

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toxicity may overwhelm these health benefits and man by extension can also be affected further the food chain [15]. There is a rich documentation in literature of the adverse effects of heavy metals in man, ranging from liver and kidney damage, cardiovascular diseases and in extreme cases, death [16, 17].

Pollution by heavy metal in aquatic bodies has terrible effects on the environmental balance and the diversity of aquatic species [18]. Aina and Adedipe [19], reported that there has been a steady increase in river pollution in Nigeria. Pollution of Ogun River occur along the course of the river [20]. Domestic sewage, emission from automobiles, mining processes, discharge of effluents from industries, metallurgical activities and agricultural production are some of the sources of anthropogenic heavy metal input into the aquatic environment [21].

Heavy metals bioaccumulate in the food chain, negatively impacting organisms, even leading to death in extreme cases. It is therefore important to analyze the impact of heavy metals in different fish organs. The profiling of the proximate composition of fish is of utmost importance in making sure that fish meet up to standard with the requirements of food regulations and commercial qualifications [22].

Subsistence fishing within the inland fisheries of Nigeria is a provider of employment, especially as the more profitable jobs are not easy to find [23]. In the communities surrounding the Ogun River, fishing is an important source of livelihood. As the majority of families in these areas are low income earners, fish presents an alternative and cheap source of protein compared to other protein sources. A lot of fish species are caught from the Ogun River. Amongst these fish species are *Oreochromis niloticus*,

Malapterurus electricus, *Parachanna obscura* and *Chrysichthys nigrodigitatus*.

The presence of heavy metals is detrimental to fish and by extension, humans and other organism who consume them [24]. Knowledge of the proximate composition of the different fish species is very important, especially if choice is to be made for consumption. The health risks associated with consuming fish that accumulates heavy metals in their bodies far outweighs the nutritional benefits that may be derived from such fish species. Hence, we investigated the proximate composition of four fish species from Ogun River and also analyzed them for some toxic heavy metals with a view to predicting the level of safety associated with their consumption.

2. Materials and methods

2.1. Site of study

The Ogun River was the location for this study. Its coordinates are 8°41'0"N and 3°28'0"E. The river flows from the Shaki area of Oyo State through Abeokuta in Ogun State (where the fish samples were collected) and to Lagos State, Nigeria (Fig. 1). The Ikere Gorge Dam and the Ofiki River both in Iseyin and Shaki respectively, are two of the Ogun River's tributary. The river serves a lot of domestic purposes and a lot of organic wastes from abatoirs also drains into it especially in highly populated areas.

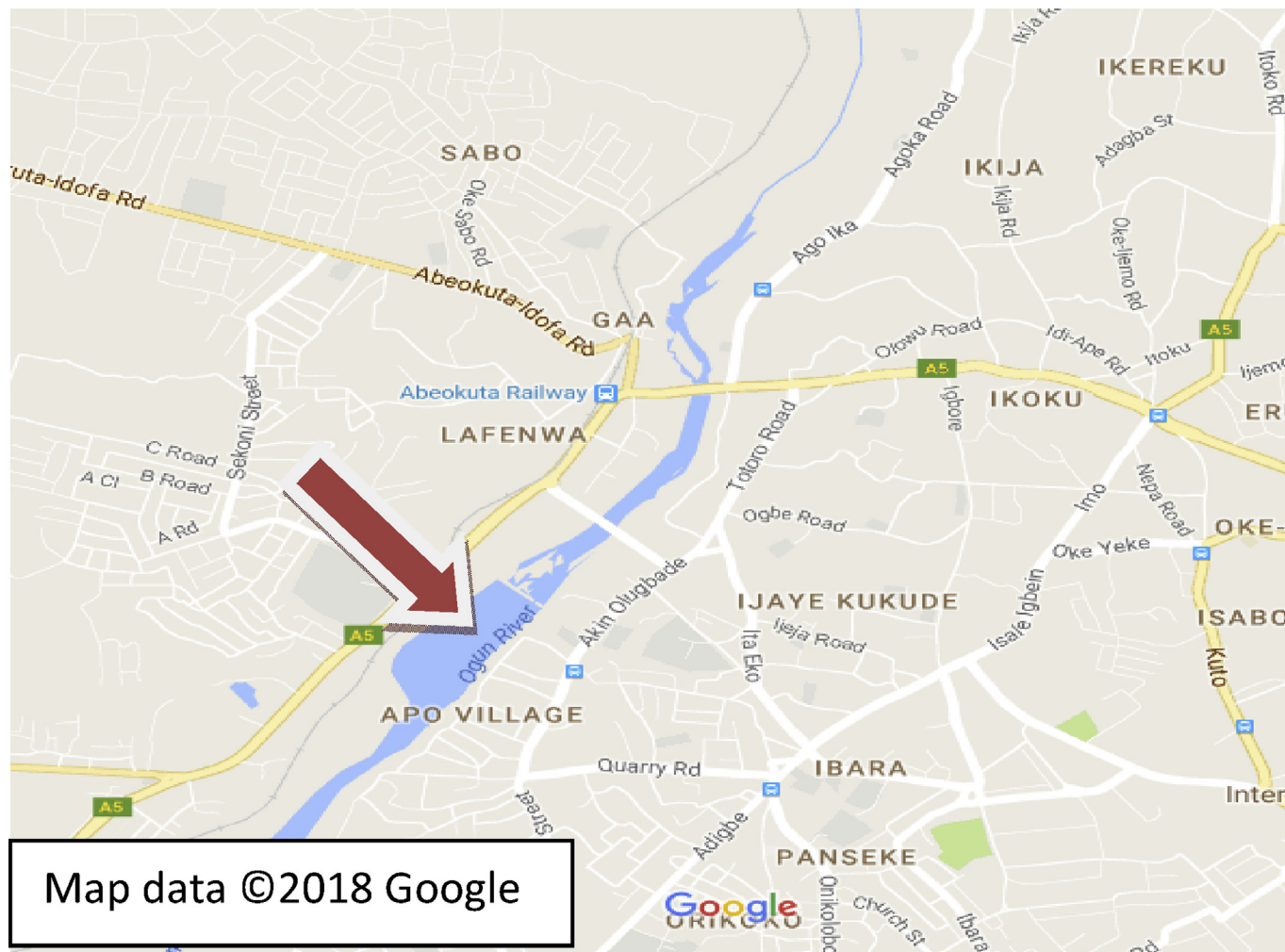


Fig. 1. A map showing the Ogun river.

2.2. Sample collection

Forty (40) fishes, four different species (*Oreochromis niloticus*, *Malapterurus electricus*, *Parachanna obscura* and *Chrysichthys nigrodigitatus*) were used for the study. Fishes with standard lengths of between 25 and 35 cm were randomly collected over a period of six months using appropriate fishing gears with the assistance of local fishermen. They were transported in ice-cold box to the laboratory. The samples were kept at -20 °C until analysis.

2.3. Heavy metal analysis

The frozen fish samples were allowed to defrost, dissected and, the livers, muscles and gills of these fish species were collected for metal analysis. 1g of each of these body parts was dried, made into powder and digested with concentrated nitric acid and hydrochloric acid in the ratio 3:1 at 70 °C for 30 min, while the mixture was allowed to remain on water bath until a colour change was observed. The resulting solution was allowed time to cool and thereafter filtered into a 50 mL flask into which distilled water was added to make up to mark [25]. The following metals, lead, cadmium, nickel, manganese and zinc were analysed using atomic absorption spectrometer (Perkin Elmer Atomic Absorption Spectrometer Pinnacle 900T, Perkin Elmer, U.S.A). Blank was prepared for each sample on which adjustment was made by reference to blank. Accuracy of analytical procedure was ensured using certified reference material (DORM-3). Results were converted to mg kg⁻¹ dry weight of fish. All reagents, HCl and H₂SO₄ used were of analytical grade and 99% purity. Results with recovery percentages of 87.5%, 80.2%, 88.7%, 90.4% and 86.8% were obtained for Cd, Ni and Pb, Zn and Mn respectively.

2.4. Crude protein content analysis

The method used for the Crude protein analysis was according to the Analytical Methods Committee, AOAC [26] which uses the Kjeldahl method to determine nitrogen content, using 6.25 as the conversion factor to get crude protein from total nitrogen. The samples went through the processes of digestion, distillation and titration. 1 g of the samples was weighed into a Kjeldahl flask and selenium catalyst was added. About 25 ml of concentrated tetraoxosulphate (VI) acid was gently added into the tube and the flask was swirled. Digestion procedure was carried out for a minimum of 2 h by heating in a fume cup board until a clear solution, the digest was obtained. The digest was diluted to 100 mL in a volumetric flask and used for the analysis. After digestion, the samples were allowed time cool for about 20 min and then distilled. This was done by preparing 4% boric acid, 35 ml of (NaOH) to the distillation flask. After the first drop of distillate, 4 drops of indicator was added into the conical flask containing 25ml of boric acid which was titrated until a grey colour was visible. The volume of acid used in the titration was recorded. Blank was prepared without the sample using only piece of paper.

2.5. Moisture content analysis

Analytical Methods Committee, AOAC [26] was adopted for determining the level of moisture. A moisture dish was used to dry the samples in an air oven at 101 °C for about 24 h until constant weights were obtained, cooled in a desiccator and re-weighed. The difference between fresh and dry weights was taken as the amount of water present and was converted to percentage.

2.6. Ash content analysis

Ash content was determined following Analytical Methods Committee, AOAC [26]. Samples (pre-dried) from the analysis of moisture content analysis were heated in a muffle furnace at 550 °C for 4 h. The final

weight was subtracted from the initial weight and converted to percentage to give an estimate of the ash content.

2.7. Fat content analysis

To obtain crude fat, 5 g of sample was completely extracted by wrapping in a filter paper in a Soxhlet apparatus. The extractant used was petroleum ether at b.p. 40-60 °C AOAC [26]. This was done each for 4 h.

2.8. Statistical analysis

Mean values for three measurements were taken and subjected to analysis of variance (ANOVA) using GraphPad Prism software to determine whether or not differences were significant. Duncan multiple range test was used to compare means. P-values > 0.05 was considered statistically different.

3. Result

The result for the values of the different metals in the gills, liver and muscles of the four fish species are presented in Table 1. In *Oreochromis niloticus*, the gills had the highest Pb concentration (15.50 ± 30.83 mg kg⁻¹) followed by the muscle though the difference is not significant. The liver had the lowest (8.69 ± 0.00 mg kg⁻¹) concentration of Pb. There was no significant difference in the concentration of Cd in all three tissues. For Mn, not all values were also different significantly. Ni and Zn were not detected (ND) any of the tissues in this fish species (Table 1).

In *Parachanna obscura*, Pb was the only metal that was not detected in any of the tissues. Cd and Ni were found to be highest in the muscle (3.88 ± 0.73 mg kg⁻¹), and lowest in the gill (1.90 ± 0.76 mg kg⁻¹) and liver (15.89 ± 4.26 mg kg⁻¹) respectively. Concentrations of the remaining four metals were not significantly different among the fish tissues (Table 1).

Malapterurus electricus was not positive for Pb and zinc in any of the fish tissues. Cd and Ni were highest in the liver while Mn was highest in the gills. Furthermore the difference in the values recorded for all the metals detected was not significant among the three fish tissues (Table 1).

Table 1 shows that Mn was the only metal detected in *Chrysichthys nigrodigitatus*. However the concentration of Mn in the different tissues was not significantly different. The highest concentration of Mn was observed in the gills (735.84 ± 5.04 mg kg⁻¹) while the lowest concentration was in the liver (715.29 ± 11.48 mg kg⁻¹). The concentrations of all the metals were above regulatory limits that are allowed in food by international standards (Table 2).

The proximate composition for the four fish species are presented in Table 3. *Chrysichthys nigrodigitatus* had the highest crude protein (19.34 ± 5.29%) and moisture content (80.05 ± 1.30%) while *malapterurus electricus* had the lowest values for these same parameters. The highest content of ash was also observed in *Chrysichthys nigrodigitatus*, however ash content was lowest in *Parachanna obscura*. The highest fat content observed was in *Oreochromis niloticus* and this parameter was lowest in *Chrysichthys nigrodigitatus*. All of the proximate parameters were not significantly different among the four fish species.

4. Discussion

Pollution by heavy metals has been reported to ultimately end up in the aquatic ecosystem [29, 30]. To estimate heavy metal pollution in the aquatic ecosystem, fishes are considered to be very useful biomonitors [31]. The concentration of heavy metals as reported in this study further supports earlier claims [32] that the Ogun River is polluted by toxic heavy metals like Pb, Cd and Ni. The long-term health effects of this on the populace depending on the river as a source of drinking water cannot be overemphasized. The river takes its source from Shaki in Oyo State, Nigeria. Here, there are a lot of minning activities taking place. This may be a contributing factor to the pollution state of the Ogun River.

Table 1
Mean concentration and range of heavy metals (mg kg⁻¹), in tissues of fish species.

| <i>Oreochromis niloticus</i> | Metals | Pb | Cd | Ni | Zn | Mn |
|------------------------------------|--------|--------------------------------------|---------------------------------------|---------------------------------------|---------------------------------------|---|
| | Gill | 15.5 ± 3.83 ^a (12.5–20.1) | 1.85 ± 0.60 ^a (1.05–2.81) | ND | ND | 604.32 ± 20.32 ^a (455.41–711.30) |
| | Liver | 8.69 ± 0.00 ^a (7.05–10.1) | 3.50 ± 1.29 ^a (1.88–4.51) | ND | ND | 567.93 ± 22.15 ^a (450.32–621.40) |
| | Muscle | 9.73 ± 1.72 ^a (7.04–10.5) | 1.53 ± 0.58 ^a (1.02–2.45) | ND | ND | 579.56 ± 21.35 ^a (489.10–654.23) |
| <i>Parachanna obscura</i> | Gill | ND | 1.90 ± 0.76 ^a (1.11–2.55) | 17.3 ± 4.73 ^a (12.5–21.3) | 7.69 ± 1.23 ^a (5.85–9.02) | 178.42 ± 27.91 ^a (150.36–210.55) |
| | Liver | ND | 3.88 ± 1.28 ^a (1.90–5.42) | 15.9 ± 4.26 ^a (11.1–18.5) | 11.80 ± 3.74 ^a (8.20–14.5) | 187.39 ± 43.29 ^a (170.23–260.32) |
| | Muscle | ND | 3.48 ± 0.73 ^a (2.11–5.86) | 24.80 ± 5.75 ^a (20.4–31.8) | 6.78 ± 1.94 ^a (4.22–9.06) | 187.39 ± 43.29 ^a (170.23–260.32) |
| <i>Malapterurus electricus</i> | Gill | ND | 6.67 ± 0.57 ^a (3.07–8.14) | 14.7 ± 4.68 ^a (10.5–19.4) | ND | 381.79 ± 24.21 ^a (342.55–406.30) |
| | Liver | ND | 7.88 ± 1.05 ^a (5.81–10.25) | 18.8 ± 5.00 ^a (15.9–20.5) | ND | 366.70 ± 17.68 ^a (315.33–389.02) |
| | Muscle | ND | 6.88 ± 0.98 ^a (3.58–9.66) | 16.74 ± 3.62 ^a (12.7–18.5) | ND | 365.29 ± 26.24 ^a (330.41–390.06) |
| <i>Chrysichthys nigrodigitatus</i> | Gill | ND | ND | ND | ND | 735.84 ± 5.04 ^a (721.02–746.21) |
| | Liver | ND | ND | ND | ND | 715.29 ± 11.48 ^a (700.20–748.65) |
| | Muscle | ND | ND | ND | ND | 727.36 ± 21.04 ^a (698.52–758.11) |
| LOD | | 0.265 | 0.298 | 0.023 | 0.075 | 0.075 |
| LOQ | | 0.875 | 0.983 | 0.077 | 0.250 | 0.250 |

ND – Not Detected; Mean ± S.E, no significant difference in values with same alphabets per row, where n = 10.

Table 2
Permissible limits for heavy metals.

| METALS (mg kg ⁻¹) | LIMIT | |
|-------------------------------|---------------|-----------------------|
| | FAO 2003 [27] | Biney et al 1994 [28] |
| Lead | 0.2 | 0.48 |
| Cadmium | 0.05 | 0.03 |
| Nickel | - | - |
| Zinc | 5 | 4.81 |
| Manganese | 5.5 | 1.10 |

Furthermore, Ogun State is a heavily industrialised state. Discharge from industries could also have been responsible for the high levels of metals being reported in the present study. Run-off, containing these metals, from highways may also have been directly discharged in the river.

When fishes are exposed to pollution from heavy metals, their organs accumulate the metals in varying concentrations [33]. In the present study, it can be observed that no single fish species accumulated all the metals tested for (Table 1). Ni and Zn were not observed in *Oreochromis niloticus*, while Pb was not observed in all the remaining three fish species. Mn was observed only in *Chrysichthys nigrodigitatus*. This disparity may be credited to different rates of metabolism. According to Ademoroti [34], organisms differ in their metabolic rates, amount of food they consume and food requirements. Any of these could have played a role in the differences observed in metal accumulation by the fish species. Furthermore, Adewumi et al. [35] reported activities of microbes in water, interaction with the immediate aquatic environment, rate of feeding, age and feeding habits of fish as other factors that can influence elemental concentrations between and within fish species with the water environment.

The levels of metals reported in this study is higher than in previously reported study [32] of heavy metal accumulation in fish from Ogun River. This may be due to increase in the anthropogenic activities that release these pollutants into the environment. Pb is a non-essential

Table 3
Comparison of the average Proximate Composition and standard error of the four fish species (%).

| Parameters | Fish Species | | | |
|---------------|------------------------------|--------------------------------|---------------------------|------------------------------------|
| | <i>Oreochromis niloticus</i> | <i>Malapterurus electricus</i> | <i>Parachanna obscura</i> | <i>Chrysichthys nigrodigitatus</i> |
| Crude Protein | 17.6 ± 1.49 ^a | 16.8 ± 5.01 ^a | 16.9 ± 0.71 ^a | 19.3 ± 5.29 ^a |
| Moisture | 76.4 ± 0.92 ^a | 75.9 ± 1.54 ^a | 78.6 ± 4.41 ^a | 80.1 ± 1.30 ^a |
| Ash | 3.84 ± 0.53 ^a | 3.95 ± 1.65 ^a | 2.91 ± 0.25 ^a | 7.56 ± 3.58 ^a |
| Fat | 1.89 ± 0.26 ^a | 1.33 ± 0.34 ^a | 1.67 ± 0.30 ^a | 0.88 ± 0.50 ^a |

Mean ± S.E, no significant difference in values with same alphabets per row, where n = 10.

element that constitutes a burden to organisms; it is a great danger to life that even in small quantities is lethal and has no recognized function in biochemical processes [36]. In this study, lead was observed only in *Oreochromis niloticus*, and it was highest in the gills, followed by the muscle and then liver. Primarily, the gill is the route for exchange gases between water and fish [37]. Furthermore, surface of the gills are very large, thus facilitating rapid diffusion of toxic metals [38]. This probably explains why the gills accumulated more lead than the other tissues. Similar higher concentration of lead in fish gills have been previously reported [39, 40]. The low concentration of the lead in the liver as compared with gills and muscle may be attributed to the ability of the liver in detoxifying toxicants. However high levels of Cd was observed in the liver in all the fish species with the exception of *Chrysichthys nigrodigitatus* where Cd was not detected at all. Two reasons may be responsible for this. First, it may be as a result of the ability of Cd to replace the normally metallothioneins associated with essential metals in the liver [41]. Secondly, once Cd accumulates in the liver, it is not easy to excrete [42].

Zn is an essential metal known to play important roles in human metabolic pathways and its shortage can cause appetite loss, retarded growth, skin changes and dysfunction of the immune system [43]. Zn was detected only in *P. obscura* with concentrations in the gill, liver and muscle (6.78, 11.80 and 6.78 mg kg⁻¹ respectively). Jobling [44] credited the high buildup of heavy metals in liver to the metallothionein proteins which are produced in the liver tissues when fishes are exposed to metals. Mn was readily available in all organs of all the fishes. Mn is an essential metal, and low level is necessary for human health [45].

All the metals detected were above regulatory limit (Table 2) in fish food. This may indicate that there is some level of pollution in the water body, most likely as a result of human activities. The presence of these metals in fish will also further impact on the consumers of fish. The negative impacts of these metals have been well documented. In man, the toxicity of lead is known to lead to challenges affecting the muscular, skeletal, nervous, immune, and reproductive systems [46]. Furthermore,

it can significantly affect intelligence quotients and impede the development of children physically [47]. The aged and pregnant women are more sensitive to these effects.

Cd, in acute concentration can cause high blood pressure, renal failure, and can destroy tissues of the testicles and the erythrocytes [48]. Cd also cause demineralization of the bones, impairment of lung function and vulnerability to lung cancer [49]. The high concentration of Zn can lead to death, stunted growth, and reproductive injury [50]. Excess Mn can induce oxidative stress and toxic effects in aquatic organisms [45]. Elevated levels of Ni have also been reported to be toxic [51].

Fish is primarily composed of water (66–81%), protein (16–21%), carbohydrates (<0.5%), lipids (0.2–25%) and ash (1.2–1.5%) [52]. The nutrient composition of the four fish species is presented in Table 3. Yeannes and Almandos [53], opined that differences in the nutrient composition of the fishes could be due to accessibility of these components in water, and/or the ability of the fish to consume and convert these vital nutrients either from food or water. Furthermore, biological variations, seasonal changes and environmental conditions are factors that can influence the biochemical composition of fish [54]. Similar findings were reported by Adewoye and Omotosho [55], Prapasri et al. [56] Ricardo et al. [57] Adewoye et al. [58] and Fawole et al. [59] The observed differences in the proximate compositions in the four fish species were not significant.

The percentage range of the moisture contents of fish species as observed in this study was between 75.98 and 80.05 %. The result shows that moisture is the major constituent of edible fish part. The high moisture content can cause the degradation of polyunsaturated fatty acids, increase the fishes' vulnerability to spoilage by microorganisms, and consequently reducing fish quality for longer periods of preservation [60]. However, high moisture content could also play important roles in metabolic reactions and help to easily solubilize certain elements. High moisture was also reported by Ayanda et al [61] from *Oreochromis niloticus*.

Protein and fat are the most important nutrient present in fish and their composition help to describe the nutritional status of a particular organism [62]. The proximate composition of *Chrysichthys nigrodigitatus*, *Malapterurus electricus*, *Oreochromis niloticus* and *Parachanna obscura* showed high crude protein contents of 19.34%, 16.80%, 17.95% and 16.89%, respectively. The result revealed that all the analyzed species of fish were high-quality sources of protein. The high protein content in the tissue of the fish species in this study may be due to similarity in diet of the fishes that are very rich in protein [63]. The high protein observed in *Chrysichthys nigrodigitatus* makes it a widely cherished fish for food and its aquaculture potential may be investigated to supplement the present species used in aquacultural practice and due to high cost of other protein sources [64].

Ackman [65], placed fish into four categories based on their fat content: lean fish (<2 %), low fat (2–4 %), medium fat (4–8%), and high fat (>8%). Hence the fishes in this study can all be described as lean fat. Fishes use a lot of fat reserves as energy source during spawning activities [66]. This may be a plausible reason for the low fat content in the muscles of the fish species observed in this study. Different types of fatty acids which are nutritionally important for human body were found in the fish meat. Lipids provide energy source during starvation.

The ash content measures of the total mineral content in the tissue [67]. It is also helpful in the development of the body and growth. *Chrysichthys nigrodigitatus* had the highest ash content ($7.56 \pm 3.58\%$) while *Parachanna obscura* had the lowest ash content ($2.91 \pm 0.25\%$). The high value of ash in the fish species is an indication of its high mineral content like magnesium, calcium, potassium, and zinc [68].

5. Conclusion

This study revealed very high concentrations of heavy metals in four fish species from the Ogun River. Hence, it may be safe to say that some of the fish species present in Ogun River are not fit for consumption. In order to prevent health challenges associated with these toxic metals, it is

important to enforce regulations that expose these aquatic ecosystems to pollution.

Declarations

Author contribution statement

Isaac Ayanda: Conceived and designed the experiments; Wrote the paper.

Ukinebo Ekhaton: Performed the experiments; Contributed reagents, materials, analysis tools or data.

Adetutu Bello: Analyzed and interpreted the data.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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