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Heavy Metal Concentration in Fish Species *Clarias gariepinus* (Catfish) and *Oreochromis niloticus* (Nile Tilapia) from Anambra River, Nigeria

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ABSTRACT

Studies have emphasized that the presence of heavy metals in freshwater fish represents a global public health issue. Nigeria, being a developing nation with less emphasis on the quality of seafood consumed by the residents, ranks this study very vital. The policy implication of this study is the advancement of a healthy population in contemporary Nigeria. Hence, this study assessed heavy metal concentration in two fish species, Clarias gariepinus (Catfish) and Oreochromis niloticus (Nile Tilapia), in the Anambra River. The sample included twenty fishes, of which eighteen were collected from the three sampling locations (the fish ports of Anambra River), namely Otu-nsugbe, Otuocha, and Ikemivite) while two control samples were collected from a pond about 200 m away from the river. The levels of heavy metals were determined using Varian AA 240 atomic absorption spectrophotometer (AAS). The results showed that the concentrations of heavy metals (cadmium and arsenic) in the sampled fishes from Anambra River exceeded the joint World Health Organization and Food and Agriculture Organization (FAO/WHO) standard for fish and fish product consumption, while the concentration of chromium, mercury, and lead are within the permissible limit. The study also showed the distribution of the heavy metals in the fish organ varies among fish species. Heavy metals occur higher in *Clarias garepinus* than in *Oreochromis niloticus*, while tissue preference for heavy metal accumulation is in the order of gill > liver > muscle. It was recorded from this study that the heavy metal concentration in the fish from the pond is generally higher than the fish from the river for some metals. The high level of heavy metals in the sampled fish was attributed to heavy metals contamination of the river as a result of various anthropogenic activities such as mining, burning of fossil fuel and emission from the exhaust of boats/vehicles, overuse of fertilizers and pesticides, discharge of effluent, sewage, and hospital waste. This study concluded that long-term consumption of fish from the river may pose health risks to the consumers due to the possible bioaccumulation of heavy metals, especially cadmium and arsenic. It was recommended that continuous monitoring of heavy metal levels in the fish and water, public awareness, and appropriate legislative provisions should be put in place to ensure that harvested fish and fish products may be safe for human consumption.

INTRODUCTION

Studies have shown that the contamination of aquatic ecosystems by a wide range of pollutants has become a matter of concern over the last few decades. Consequently, there is a need to quantify the heavy metal concentration in fish species from the Anambra River, Nigeria. Fish is an aquatic organism that is widely consumed around the world as a major source of dietary protein. It provides humans with low saturated fat and omega-3(n-3) fatty acids, including cardio-protective and reproductive benefits. Fish plays a key role in universal food distribution, and its consumption has increased significantly since 1973 (FAO 2004). It is also estimated that over 200 million people, mostly those living in landless communities, depend on fisheries (via fishing, fish processing, marketing, and transportation of fish and fish products) for their livelihood (Sikoki & Otobotekere 1999). However, these numerous benefits of fish may be offset by the presence of pollutants, especially heavy metals such as (Zn, Cu, Cd, Pb, Hg and Cr). Studies have shown that many of these pollutants originate from various anthropogenic activities such as unsustainable agriculture, industrial activities, and increasing urbanization, which facilitates their entry into rivers, lakes, and oceans (Abou-Arab et al.1996).

This study aims to quantify the level of concentration of heavy metals (cadmium, lead, mercury, chromium and arsenic) in the tissues (muscles, liver, and gills) of two edible freshwater species of fish: African catfish (*Clarias gariepinus*) and Tilapia fish (*Orechromis niloticus*) from Anambra River, Nigeria. Anambra River drains into the River Niger at Onitsha, Nigeria. This study is vital considering the high quantities of fish caught in the river. Heavy metals may contaminate these fish.

Evidence has shown that rapid human population growth, anthropogenic activities, urbanization, industrialization, and unsustainable agricultural practices could increase the concentration of heavy metals in the environment above the acceptable threshold, especially in aquatic ecosystems (Maurya & Malik 2018). Fish are conditioned by their environment, and hence, it is obvious that if the growing and harvesting environment of fish is polluted by chemical or microbiological sources, the fish could also be contaminated through the process of bioaccumulation.

It has been established that the heavy metal load of fish reflects the contamination level of their habitat and, thus, is a potential indicator of pollution sinks. At low concentrations, some heavy metals, such as iron, zinc, copper, and manganese, do not pose health concerns but can become toxic agents at higher concentrations. Heavy metals such as lead (Pb), chromium (Cr), mercury (Hg), arsenic (As), and cadmium (Cd) in fish may become toxic at concentrations exceeding the permissible limits. These heavy metals have a high degree of toxicity and rank among the important metals that are of public health concern, thus classified as either “known” or “probable” human carcinogens (United States Environmental Protection Agency 2012). This is a growing issue, especially in developing parts of the world (such as Nigeria), where attention to safety regulations on food is lacking or inadequate.

Evidence has shown that bio-concentration and bio-magnification are capable of leading to toxic effects of metals in fish, even when environmental exposure level is low (Chovanec et al. 2003, Vanderoost et al. 2003). In the context of an empirical review, Maurya and Malik (2018) identified that heavy metal concentration in fish tissues (muscle, gills, and liver) is increasing steadily in the Ganga River. They also demonstrate that the concentration of Pb, Cd, and Cr are higher than the internationally recommended standard limits of WHO and FAO. In addition, higher concentrations of metals were found in the liver and gills than in muscle. Akpanyungetal (2014) reported on the levels of heavy metals in fish obtained from two fishing sites in Akwa Ibom State, Nigeria, and identified that the levels of Zn and Cu were significantly higher than the maximum recommended levels ($p<0.05$) at both locations and in all the organs. Ali and Khan (2018) reported concentrations of the heavy metals (Cr, Ni, Cd, and Pb and Ni) but observed that Ni accumulation may pose a potential health risk to fish consumers. Research by Gbogbo et al. (2018) reported that lead and cadmium were below detectable levels in all the fish samples, while Cu was not detected in *B. auritus*. The study maintained that the levels of the remaining metals (mg kg^{-1}) were below FAO/WHO maximum permissible limits in fish and occurred in the rank order Se (3.5) > Zn (2.34) > Cu (0.59) > As (0.37) > Hg (0.19) in *C. nigrodigitatus* and Se (2.97) > Zn (2.28) > Hg (0.31) > As (0.21) in *B. auritus*. In a study on the Bioaccumulation of heavy metals (Zn, Pb, Cd, and Cu) in liver, gills, and flesh in benthic and pelagic fish species collected from Lake Geriyo, it was established that the levels of heavy metals varied significantly among fish species and organs (Bawuro et al. 2018). The flesh possessed the lowest concentration of all the metals, while the liver was the target organ for Zn, Cu, and Pb accumulations. However, Cd exhibited a higher concentration in the gills. Fish species showed an inter-specific variation of metal absorption. These differences were discussed for the contribution of potential factors that affected metal uptake, like age, geographical distribution, and species-specific factors. Similarly, Sobhanardakani et al. (2018), Yaradua et al. (2018), and Adeosun et al. (2014) reported that the level of heavy metals all fall below the USEPA (2012) and WHO/FAO (2015) permissible limit for fish, while Akpanyung et al. (2014) maintained that the levels of Zn, Cu, Pb, Cr and As in the fish at Ibaka were above the standards set by WHO/FAO/UNEP and suggested regular monitoring and assessment of fishing sites in the region for heavy metal contamination. It was noted from the review of literature that most of the environmental monitoring studies have focused primarily on contaminant concentrations in the environment rather than in the biota such as fish (Borgmann et al. 2008). To the best of our knowledge, studies reviewed seldom quantify the level of heavy metal concentration in aquatic biota (fish). Hence, the study quantifies the level of heavy metal concentration in fish samples from the Anambra River. The study is unique because it compares the *Clarias gariepinus* and *Tilapia zilli* sampled together at various sampling stations in Anambra River with heavy metal levels in fish from the pond. The study area is characterized by unsustainable agriculture, which is a serious risk factor for pollution.

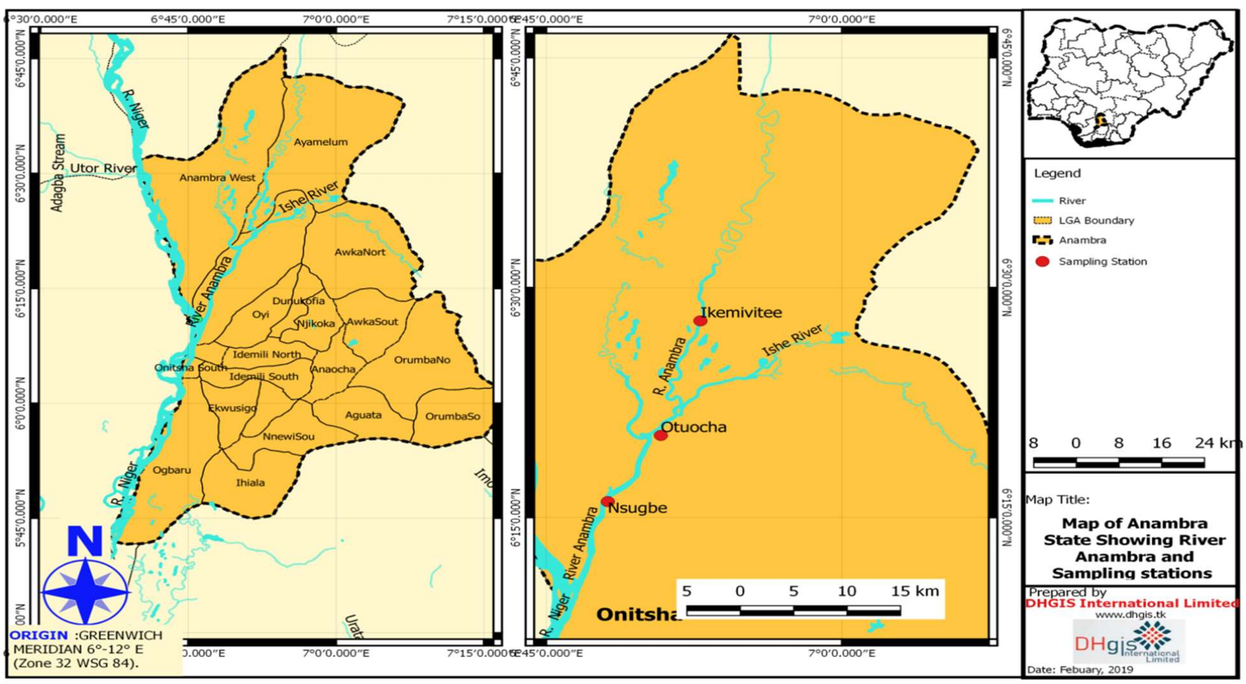
MATERIALS AND METHODS

Study Area

The study was carried out in Anambra River, Anambra State, Nigeria. Anambra River is about 230 km in length and has an extensive basin of about 14,010 km^2. The basin lies between latitude 6°10' and 7°20', longitude 6°35' and 7°40' east of the Niger River into which the Anambra River empties. The Anambra River is the largest tributary of River Niger below Lokoja and is often regarded as a part of the lower Niger lowlands (Awachie & Hare, 1978) in Obiakoret al (2014). The River has a southward course up to the Kogi/ Enugu State boundary; it then meanders through Ogurugu to Nando Ikemivite, Otuocha, and Nsugbe, from where it flows down to form a confluence with the Niger River at Onitsha (Fig. 1).

The sampling areas were located around the major fish landing sites of the main river at the adjacent floodplain of Otu-Nsugbe, Otuocha, and Nando Ikemivite. The three stations, Ikemivite, Otuocha and Otu-Nsugbe, with GPS Latitude 6.3500° and Longitude 6.9167°; Latitude 6.3333° and Longitude 6.8500°, as well as Latitude 6.2667° and Longitude6.8167° respectively, were selected as sampling points. The criteria for selecting these sampling points are based on accessibility, as well as their strategic locations on the various stretches of the Anambra River, and human activities such as fishing, farming, rice mill industry, market, domestic activities, and refuse dumping. The sample collection involved a total of Twenty (20) life-matured fish samples, comprising 18 experimental (nine samples each) of *Clarias gariepinus* and *Oreochromis niloticus* and 2 control samples, made up of one sample of each fish species. The samples were purchased directly from the fishermen at the three sampling locations (Ikemivite, Otuocha, and Otu-Nsugbe fishing ports) of the Anambra River in September 2022. The control samples were bought from a fish pond that uses borehole water about 200m from the river.

Clarias gariepinus and *Oreochromis niloticus* were chosen because they appear to have great economic value and are readily available in terms of populations, suggesting their ecological importance in the area. Similarly, these species were chosen with consideration for the sensitivity of aquaculture organisms in terms of heavy metals contamination and accumulation. The fish samples caught freshly were thoroughly washed and put in sterile polythene bags and then placed in an icebox to maintain the freshness and transported immediately to the laboratory for dissection. Before dissection, the mean weight and mean length of the fish samples were measured with measuring tape and weighing balance, respectively. The mean weight was 1800 g, while the mean length was 30.5 cm. The taxonomical



(Source: DHGIS Limited, 2019)

Fig. 1: Map of Anambra State, Nigeria, showing Anambra River and sampled stations.

Table 1: Mean Concentration of Heavy metal in Fish samples from Anambra River and Fish pond.

Heavy metals (ppm)	Fish	Otuocha		Otu-Nsugbe		Ikem-Ivite		Fish pond (control)		Joint WHO/ FAO standard
Arsenic	tissues	*Clarias garepinus*	*Oreochromis niloticus*	*Clarias garepinus*	*O. niloticus*	*Clarias garepinus*	*O. niloticus*	*Clarias garepinus*	*O. niloticus*	0.26
	Muscle	0.413	0.000	0.105	0.000	0.418	0.000	0.524	0.000	
	Gill	0.000	0.371	0.000	0.000	1.126	0.211	0.693	0.461	
	Liver	0.000	0.000	0.000	0.000	0.131	0.313	0.000	0.000	
Chromium	Muscle	0.000	0.054	0.263	0.057	0.057	0.000	0.106	0.017	1.00
	Gill	0.193	0.028	0.054	0.309	0.061	0.000	0.033	0.077	
	Liver	0.114	0.113	0.021	0.012	0.017	0.014	0.076	0.037	
Mercury	Muscle	0.000	0.146	0.173	0.000	0.000	0.000	0.000	0.000	0.60
	Gill	0.000	0.000	0.324	0.000	0.000	0.000	0.000	0.000	
	Liver	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
Lead	Muscle	1.061	0.253	0.798	0.299	0.737	0.613	0.814	0.458	1.00
	Gill	0.722	0.535	0.365	0.000	1.730	0.956	1.263	0.350	
	Liver	0.218	0.107	0.630	0.212	0.412	0.175	0.222	0.073	
Cadmium	Muscle	0.428	0.307	1.271	0.329	0.484	0.169	0.438	0.390	0.20
	Gill	0.392	0.276	0.468	0.449	0.361	0.304	0.319	0.297	
	Liver	0.286	0.370	0.389	0.333	0.356	0.279	0.306	0.377	

(Source: Authors' Field Work 2022)

identification of the fish species was done according to the field guide to Nigerian freshwater fishes (Olaosebikan & Raji 1998).

The fish samples were separated according to species and location and stored inside the deep freezer at about -100°C and allowed to thaw. Scales were removed and washed with running tap water to remove dirt before being dissected with sterile scissors. The needed fish tissues (gills, liver, and muscles) were transferred into sterile sample bottles, labeled according to species and location, and kept for digestion and analysis of heavy metals. To avoid contamination, all bottles and glassware used in the preparation of the samples were soaked in nitric acid for 15 min and rinsed with deionized water before being used. The prepared sample was left overnight at room temperature.

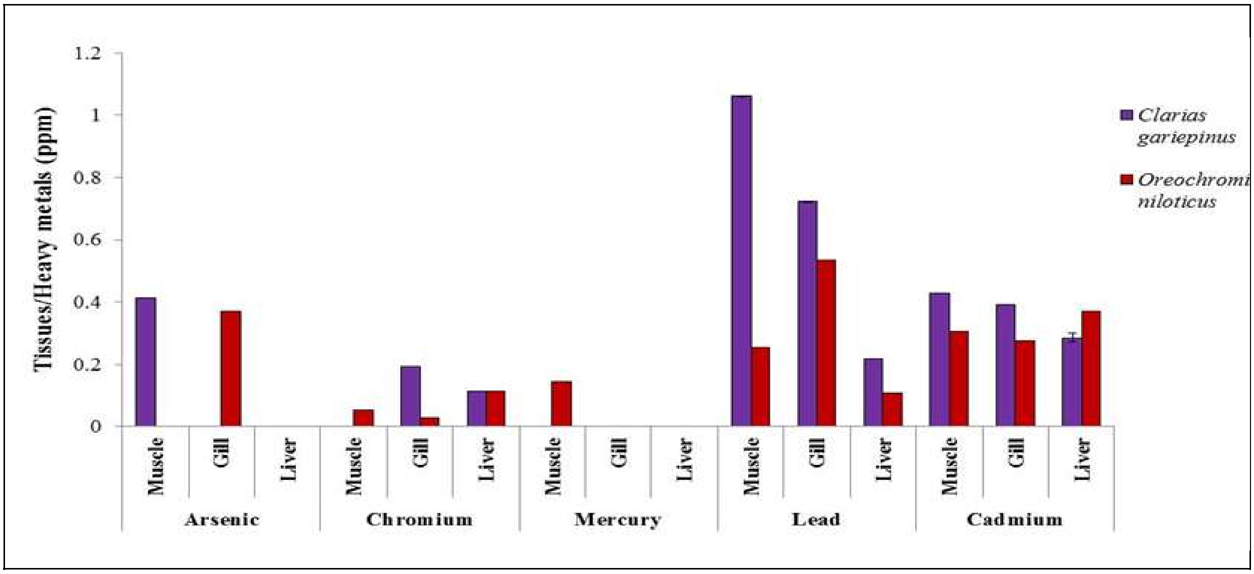
Wet digestion of the sample and determination of heavy metals were conducted to digest the samples. The digested samples were poured into auto-analyzer cups, and the concentration of heavy metals (Cadmium, Mercury, Chromium, Arsenic, and Lead) in each sample was determined using a Varian AA 240 Atomic Absorption Spectrophotometer (AAS) according to the method of APHA 1995 (American Public Health Association). The values of the heavy metal concentrations in the tissues were calculated based on dry weights as this discounted the variability due to inner parts differences in the moisture content of organisms. The accuracy and precision were verified using standard reference materials (MA-A-2/TM) provided by the International Atomic Agency (IAEA) and Zschunke (2013). The absorption wavelengths and detection limits for the heavy metals were noted. The result of the experiment was compared with the Joint WHO/FAO standard for fish consumption. The data collected was analyzed using analysis of variance (ANOVA), and $P < 0.05$ was considered to indicate a statistically significant difference. Means of significant differences were separated using Duncan's Multiple Range Test. Two-way analysis of variance (ANOVA) was used to assess whether the heavy metals concentration in fish varied significantly among the locations and species.

RESULTS AND DISCUSSION

Data in Table 1 shows the level of heavy metal concentration in selected fish samples from the Anambra River. The study quantifies heavy metal concentration in the muscle, gill, and liver of the two species of fish, *Clarias garepinus* (catfish) and *Oreochromis niloticus* (Tilapia fish).

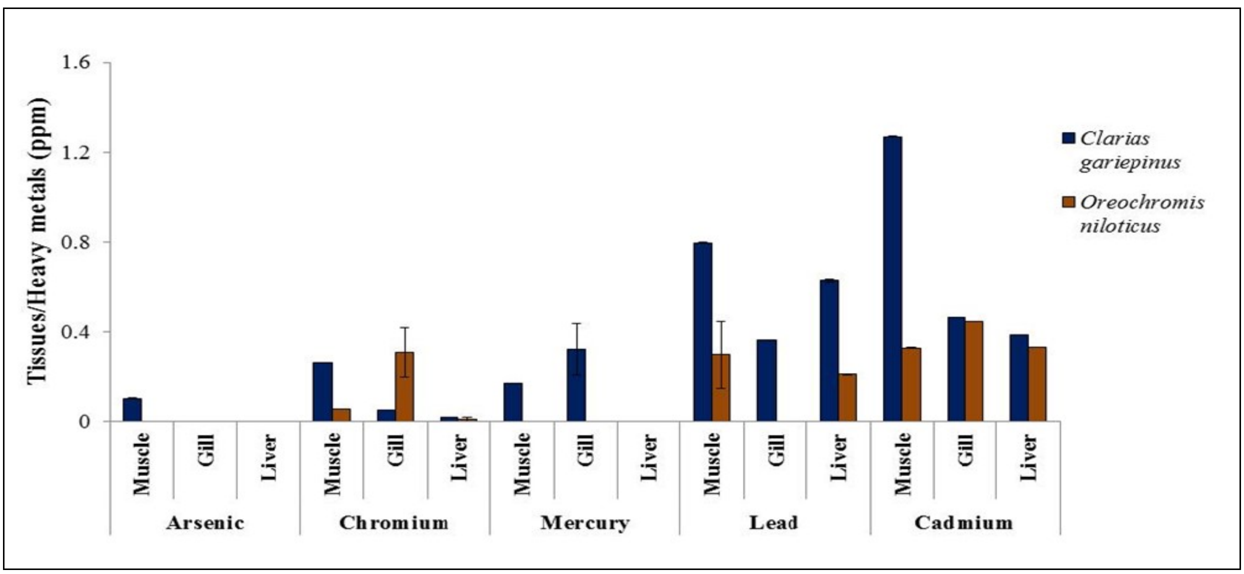
Heavy Metal Concentration in Fish Tissues

The findings of this study showed that the mean concentration of heavy metals in the fish tissues differs significantly amongst the sampled fish species in the Anambra River ($p=0.000$). The variation in the level of heavy was further explained in Table 1. The heavy metal levels in the two species of fish (*Clarias garepinus* (catfish) and *Oreochromis niloticus* (Tilapia fish)) include Arsenic (0.0-0.46ppm), Chromium (0.0-0.309ppm), Mercury (0.0-0.324 ppm), lead (0.0-1.73 ppm) and Cadmium (0.169-0.449 ppm). The fish tissue (gill, liver, and muscle) recorded differences in the rate of heavy metal concentrations. The study also established that heavy metal accumulations were higher in the gill, followed by liver and muscle. In the same vein, the level of heavy metals recorded in *Clarias gariepinus* was higher than in *Oreochromis niloticus*. This finding agrees with Maurya and Malik (2018) and Akpanyunget al. (2014). However, their work was not able to quantify the variation in fish tissue (gill, liver, and muscle). The present study quantifies the disparity in heavy metal accumulation in species-specific factors of fish, with *Clarias gariepinus* recording higher than *Oreochromis niloticus*. The quantity of heavy metal disparity as measured in gill, liver, and muscle are shown in Figs. 2, 3, and 4, respectively.



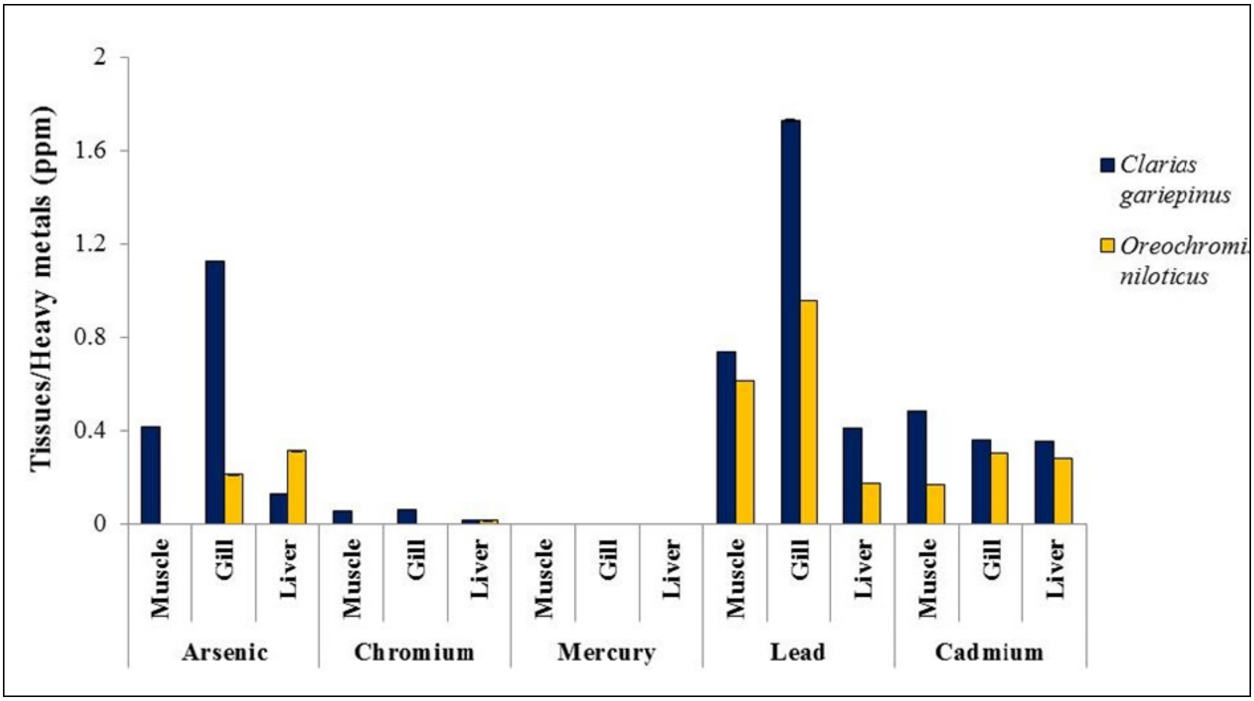
(Source: Result of Researchers' Laboratory Analysis 2022)

Fig. 2: Comparison of heavy metal concentrations in the tissues of *Clarias gariepinus* and *Oreochromis niloticus* at Otuocha in Anambra River, Nigeria.



(Source: Researchers' Laboratory Analysis, 2022)

Fig. 3: Comparison of heavy metal concentrations in the tissues of *Clarias gariepinus* and *Oreochromis niloticus* at Otu-Nsugbe in Anambra River, Nigeria.



(Source: Researchers' Laboratory Analysis 2022)

Fig. 4: Comparison of heavy metal concentrations in the tissues of *Clarias gariepinus* and *Oreochromis niloticus* at Ikem-Ivite in Anambra River, Nigeria.

The disparity in heavy metal accumulation in the fish species studied could be attributed to differences in feeding habits. *Clarias gariepinus is* omnivorous and feeds at all times, while *Oreochromis niloticus* is a carnivore and is typically diurnal. In a similar study carried out by Zhang et al. (2018), they emphasized that metals exhibit various affinities to different organs, and irrespective of the uptake route, gill and liver accumulate high levels of metals and are often used as a good water pollution biomarker.

The differences in metal concentrations can also be attributed to differences in metal uptake, as observed by Hashim et al. (2014) and Adebayo (2017). Other probable reasons include fish size and where fish reside in water. For instance, Adebayo (2017) reported that heavy metal concentrations were higher in omnivorous/herbivore fish such as *Heterotis niloticus* and lower in carnivore fish such as *Clarias anguillaris*. Moreover, Hashim et al. (2014) established that omnivorous fish were detected with elevated concentrations of Cd and Ni, whereas carnivorous fish had the highest concentration of Pb. However, their study was not able to quantify the variation in fish species in different water habitats. Hence, the present study quantifies heavy metal variation in gill, liver, and muscle at different habitats, namely "Otuocha, Otu-Nsugbe, and Ikemivitee" in the Anambra River of Anambra State, Nigeria (Figs. 2 to 4). The concentration of heavy metals recorded in the fish gill, liver, and muscle could also be attributed to the high influx of heavy metals in the Anambra River from anthropogenic activity.

Differences Between Heavy Metal Concentration in the Experimental and Control Samples

The findings of this study showed that there is a significant difference between heavy metal concentration in the experimental and control samples (p=0.010). This finding is further validated by the result in Table 1. The concentration of metals in fish from the pond is generally higher than those from Anambra River except in Chromium and Cadmium, as recorded in *Clarias gariepinus,* and Lead and Chromium, as in *Oreochromis niloticus*. This finding is in line with Zhang et al. (2018), who reported that the concentration proportions of Cd, Cu, and Zn in mixed edible tissues were higher in cultured fish than in wild fish. Akan et al. (2012) identified that sampled wild fish, including *Oreochronmis niloticus,* had low values for the heavy metals tested (Cu, Zn, Co, Mn, Fe, Cr, Cd, Ni, and Pb).

The finding of this study further showed that the concentration proportion of lead and cadmium in both pond and river fish was much higher in gill, followed by liver and muscles in both fish species. Thus, we can deduce that organs like the liver, muscle, and gill were more likely to have higher trace element accumulation abilities in both cultured and wild fish, which differs from other works reviewed.

Similarly, this result agrees with (Ndayisenga & Dusabe 2022), who reported that concentrations of heavy metals values for Fe (0.61 -1.39 ppm) and Mn (0.069-0.186 ppm) for *Tilapia nilotica* and *Clarias gariepinus* reared in fish ponds in 3 districts in Rwanda was above the permissible limit of WHO;. However, the study fails to compare heavy metals in fish ponds and River environments.

These findings further revealed that the heavy metal concentration in the aquatic environment may influence cultured fish more than wild fish, probably because the water used in aquaculture does not flow and may be contaminated either through industrial effluents or possible infiltration, thereby increasing the chances of heavy metal bioaccumulation in the fish tissues. In addition, the feeds and chemicals used in culturing the fish may contain heavy metals.

Differences Between Heavy Metal Concentration in the Fish Tissue and Joint WHO/FAO Standard Limit

The findings of this study also indicate that there is a significant difference between heavy metal concentration in the fish tissue and joint WHO/FAO standard limit (p=0.027). The finding of the study is validated by the result in Table 1. The heavy metal level in the two species of fish (*Clarias gariepinus* (Catfish) and *Oreochromis niloticus* (Tilapia fish)) ranges as follows: Arsenic (0.0-0.46ppm), Chromium (0.0-0.309 ppm), Mercury (0.0-0.324), Lead(0.0-1.73) and Cadmium (0.169-0.449). These set of values differ from joint WHO/FAO standard limit (arsenic with a value of 0.26 ppm, chromium (1.00 ppm), mercury (0.60 ppm), lead (1.00 ppm), and cadmium (0.20 ppm) as presented in Table 1. The concentration of heavy metals (cadmium and arsenic) in fish samples from Anambra River is higher than the joint FAO/WHO standard for fish diet, except Pb. Cr and Hg, which are within the standard. This indicates that consumption of these fish species at present may be a potential health hazard. This may also pose a health risk to regular fish consumers due to possible bioaccumulation as the concentrations of As and Cd exceed the permissible limit. These findings agree with Adebayo (2017), Maurya and Malik (2018), Mensoor and Said (2018), Hashim et al. (2014), Bawuro et al. (2018) Akpanyung et al. (2014), WHO/FAO/IAEA (1996), Murtala et al. (2012), Oumar et al. (2018) who reported heavy metal concentration above the World Health Organization, Food and Agriculture Organization, the European Commission (EC), USEPA, WHO/FAO, UNEP, (FAO, UNEP, FEPA, and Australian National Health and Medical Research Council (ANHMRC) standards. It is important to note that some individuals may consume fish more often, and their dietary intake of these heavy metals would further accumulate, which may lead to elevated health risks. Chronic poisoning with heavy metals (Pb) may lead to anemia and brain damage. Research has shown that long-term low-level Lead exposure in children may lead to diminished intellectual capacity and health risks to pregnant women (WHO 1995). It has also been reported that metals such as iron, copper, cadmium, chromium, lead, mercury, and nickel can produce reactive oxygen species. The result of this is lipid peroxidation, DNA damage, and altered calcium homeostasis. Human exposure to cadmium occurs through contaminated food, water, and cigarette smoking, as well as environmental pollutants with acclaimed toxicity at extremely low concentrations. Cadmium and lead are associated with non-carcinogenic and carcinogenic effects, and continuous exposure to heavy metals has been linked to the development of mental retardation, kidney damage, various cancers, and even death in instances of very high exposure in the human body. Similarly, the toxicity of cadmium affects the bone, liver, renal, reproductive, respiratory systems, and DNA repair mechanism, ultimately precipitating diseases such as hypertension, emphysema, and cancer (Ugwuja et al. 2015)

CONCLUSION

This study showed that the heavy metal concentration in various tissues of fish samples differed significantly. While the levels of Cr, Hg, and Pb are within the standard limit set by the joint FAO/WHO (2015), the concentration of As and Cd exceeds the limit. Heavy metal level in the fish at present is a potential hazard, and long-term consumption of the fish from the area may pose a serious health risk to regular fish consumers due to possible bioaccumulation of the heavy metals, especially cadmium and arsenic. To harvest fish and fish products that are safe for human consumption, there should be continuous monitoring of heavy metal levels to prevent bioaccumulation above permissible levels. The water standards and concentrations of heavy metals in the Anambra River should be monitored routinely to ascertain the heavy metal transfer factor to fish from its environment, as well as the sources to enable sustainable aquatic protection and management practices in the area.

The quality of the fish (both wild catch and aquaculture) for human consumption and the health risks associated with the aquatic products need to be given priority. The toxicity of chemicals like antibiotics, feed additives, water treatment products, and other products used in the aquaculture facility or site need to be adequately evaluated and well documented. Various human activities, such as the location of automobile workshops, dumping of domestic sewage, and other activities that are inimical to the safe use of the river, should be discouraged. Appropriate measures, such as legislative provisions and other tools for effective environmental monitoring, should be mounted and used to protect and enhance the quality of the river.

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