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Assessment of the Impact of Heavy Metals on *Clarias gariepinus* Burchell and *Heterobranchus longifilis* Fish Species of Omambala River Basin of Anambra State Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Heavy metals are natural occurring minerals that are found in nature, as deposits in pure and mostly ore form. They are referred to as transition metals due their variable oxidation state, which as a result have made them to develop special properties among other elements in the periodic

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table. This study aimed to assess the levels of heavy metals (Lead, Chromium, Mercury, Cadmium, Iron, Zinc, Copper and Magnesium) in *Clarias gariepinus* and *Hetrobrancus longifilis* collected from the Omambala River. The fish samples were collected by fishermen at the fish landing site. The heavy metal analysis in the two fish species was studied using Atomic Absorbtion Spectrophotometer (AAS) and the instrument was calibrated with standard solutions. The result of this work showed that the highest compositions of heavy metals were: lead (Pb) content in *C. gariepinus* was 2.69±1.417 mg/l while in *H. longifilis* was 3.14±1.395 mg/l. However, Chromium content in *C. gariepinus* was 3.11±0.826 mg/l and in *H. longifilis* was 4.04±1.073 mg/l. Mercury in *C. gariepinus* was 3.98±1.903 mg/l and *H. longifilis* was 4.77±2.283 mg/l. Cadmium in *C. gariepinus* was 4.90±2.100 mg/l while *H. longifilis* was 4.83±2.019 mg/l. Moreover, Iron in *C. gariepinus* was 3.94±1.414 mg/l and *H. longifilis* was 4.31±2.077 mg/l. Zinc in *C. gariepinus* was 4.02±1.917 mg/l and *H. longifilis* was 5.47±2.607 mg/l. Copper in *C. gariepinus* was 2.62±0.720 mg/l while in *H. longifilis* was 3.01±0.828 mg/l. Magnesium in *C. gariepinus* shows 4.66±2.774 mg/l and *H.longifilis* shows 3.72±1.466 mg/l. The most prevalent heavy metals from the study were Zn, Cd, Hg, Mg, Fe, Cr, Pb, and Cu. It is therefore recommended that further research be made, and awareness created on the high health risk associated with frequent ingestion of these harmful mineral elements found in trace deposits in the Omambala River by the appropriate authority to ascertain their concentrations.

Keywords: Heavy metals; Clarias gariepinus; Heterobranchus longifilis; Omambala River; Anambra state; prevalence.

1. INTRODUCTION

The current global decline in water quality, especially seasonal deposition of heavy metals, is mostly due to increasing human populations, industrialization, agricultural activities and economic development. In freshwater ecosystems, essential and non-essential metals have been shown to accumulate up the trophic chain [1]. Non-essential metals are not known to have any metabolic role, but they can be hazardous to humans, even at extremely low amounts, because of their bioaccumulation in fish and percentage of these heavy metals in fish is relevant for both environmental management and human consumption.

Heavy metals are natural occurring minerals that are found in nature mostly as deposits in pure and mostly ore form. There are referred to as transition metals due to the variable oxidation state which has made them to develop special properties among other element in the periodic table, consequently making them to be used in pigment-dye production, automobile, catalyst in petroleum refining, ornamental designs, agriculture, pharmaceuticals, metal extraction and other technology processes [2,3]. Generally, human activities has significant influence in the distribution and accumulation of heavy metal in our ecosystem which in turn has caused a great deal of negative environmental impact which can be seen in the health, growth and development of plant and animal life (Green and Planchart, 2017). The bioavailability of a specific heavy metal is a factor that depends on the natural and artificial process [2]. Some heavy metals such as Zn, Mn, Co, Cu, Cr, Fe, Mo, Ni, and Se have significant importance in biological function of living things and are essential elements for the survival of the organisms, as it constitute of several key enzymes and playing important roles in various oxidation-reduction reactions [4,5].

Humans require a variety of metals in daily diets. A few metals, such as arsenic (As), chromium (Cr), lead (Pb), and mercury (Hg), are necessary in extremely low amounts, can have harmful effects on the nervous system, kidneys, and other crucially important organs of the body, and this can be life threatening in extreme cases. In general, sources of contamination are contaminated food and beverages and packaging [6,3]. Metals are frequently introduced into food products accidently and unknowingly. If these contaminants are not found, they can pose a significant risk to consumers' health. Arsenic, Cr, Pb, and Hg are metals that occur naturally in chemical compounds, and are of particular concern in food because of their toxicity, especially in the case of long-term (chronic) consumption, when they can accumulate in the body and cause organ failure, especially in vulnerable groups like children [6-9].

Heavy metals are toxic to animals and humans which pose a major risk to people through frequent dietary patterns due to their persistence, extensive biological half-life, and inherent toxicity [10]. The concentration and bioavailability of pollutants in waterbody influence the accumulation of heavy metals in fish. Adsorption and precipitation processes, complexation kinetics, chemical speciation, lipid solubility, and particulate/water partition coefficients are all influenced by physical and chemical variables [2]. Biological factors like species, trophic relationships, and biochemical/physiological adaptability also play an important role [11,12]. In fact, because metals have varying chemical affinities to fish tissues, as well as different absorption, deposition, and excretion rates. Fish are good indicators for the long term monitoring of metal accumulation in the marine environment [13]. In recent decades, adverse effects of unexpected contaminants on marine quality have threatened both food security and human health. The levels of non-essential trace elements in fish are important because fish is an important source of food for the general human population; fish from freshwater bodies receiving industrial effluents have been reported to be unfit for human consumption because of high tissue levels of some heavy metals [14]. The Omambala River is a source of livelihoods for people living along it for many generations. As it has supported fishing, dry season farming agriculture, and provided the local population with drinking water. Most other local population depend on this river as source of protein, which could be in the form of fish, mollusc and crustaceans. Data on the potential health risk of consuming fish especially *Clarias gariepinus* and *Heterobranchus longifilis* sourced from Omambala river are not readily available and this study will help to elucidate that research gap.

2. MATERIALS AND METHODS

2.1 Study Area

This study was conducted on the Anambra River (Igbo: Omambala). It lies between latitudes 6° 00N and 6° 30N and longitudes 6° 45E and 7° 15E.The River is approximately 207.4 km to 210 km in length, rising from the Ankpa hills (305-610 m above sea level) and discharging into the River Niger at Onitsha. The river is in the south central region of Nigeria. The experimental site comprised of five distinct locations and stations established to cover possible impacted and non-impacted area along the river course based on an earlier field reconnaissance tour. The locations **(**EX**)** of the various measured stations are:

E1: Enugu Otu
E2: Ezi Aguleri
E3: Otuocha
E4: Otu Nsugbe
E5: Omambala

2.2 Materials

The major analytical equipment used for this study was the Molecular Plasma-Atomic Emission Spectrometer (MP-AES), which was used to determine heavy metal concentration. Surgical scalpels were also employed in the dissection of marine samples to retrieve its organs of interest. A freezer was used to store the marine samples and its organs. All chemicals and reagents used were of analytical grade and were used without any further purification.

2.3 Field Sampling

One hundred (100) samples each of *Clarias gariepinus* and *Heterobranchus* with a size of 17–21 cm were selected at each sampling site, during the wet (June) and dry (December) seasons by professional fishermen using a multifilament, nylon gill net or trawl from inside the Omambala river in Anambra State, Nigeria. Samples were washed with clean water at the point of collection, separated by species, placed on ice, brought to the laboratory on the same day, and then frozen at −20 °C until dissection, as described by Lake et al. [14].

2.4 Sample Preparation

Fish samples were thawed at room temperature and dissected using stainless steel scalpels. One gram of accurately weighed epaxial muscle on the dorsal surface of the fish, the skin, liver, gill arch, and gill filament from each sample were collected for analysis.

2.5 Digestion and Determination of Heavy Metals

The digestion method by Lake et al., [14] was adopted with some modification. Dissected-selected organs were oven dried, grounded and 1 gramme each transferred to glass beaker. An acid digestion was performed to prepare the sample for heavy metal analysis with 5 mL of nitric acid (65%) and after complete digestion, the samples were cooled to room temperature and diluted to 25 mL with double distilled water. All the digested samples were analysed three times for heavy metals using the Atomic

Emission Spectrophotometer (AES) and the instrument was calibrated with standard solutions.

2.6 Determination of Parameters

The physicochemical parameters measured were temperature, pH, turbidity, conductivity, nitrate, phosphate, BOD, COD, dissolved oxygen, total suspended solids, total dissolved solids, total solids, total alkalinity, total hardness, potassium, sodium, chloride, and calcium. Temperature was determined in situ by using the mercury in a glass thermometer on a centigrade scale. A multi-purpose pH meter model D46 (pH/MV/OC meter) was used to determine the pH of the water samples. The turbidity of the samples was measured in the laboratory using the LABTECH digital Turbidity Meters. The specific conductance of the samples was measured using the battery operated conductivity bridge model MC-1 mark V Electronic switchgear at room temperature. Total dissolved solids, total suspended solids and total solids were measured by gravimetric analysis. Total alkalinity, total hardness, calcium, chloride, dissolved oxygen, chemical oxygen demand, and Biological Oxygen Demand were analysed by the titration method. Potassium and sodium were determined by a flame photometer; while phosphate and nitrate were analysed by UV-visible spectrophotometer.

2.7 Statistical Analysis

In the present study, correlation analysis data were generated separately for the freshwater species *Clarias gariepinus* and *Heterobranchus longifilis*. The correlations of different elements calculated using the different values ($p< 0.05$) for different tissues in two fish samples. All the statistical analyses were done using Minitab 2018 software.

3. RESULTS

3.1 Accumulation of Heavy Metals in *Clarias gariepinus* and *Heterobranchus longifilis*

Table 1 shows degree of accumulation of selected heavy metals in four different organs of *C. gariepinus* from Omambala River. Heavy metal accumulation varied in *Clarias gariepinus* organs. In the organs of *C. gariepinus*, highest accumulations of lead, cadmium, zinc and copper occurred in the gill arch while highest accumulations of chromium, iron and magnesium occurred in the liver. The highest accumulation of mercury was recorded only in the skin. However, lowest accumulations of lead and chromium were found in the gill filament; lowest accumulations of mercury, iron and magnesium were found in the gill arch; lowest accumulations of cadmium and zinc were found in the liver; and lowest accumulation of copper was found in the skin. Except in copper (where $p > 0.05$), the degree of accumulation of the heavy metals was significantly different in the organs of *C. gariepinus* (where $p < 0.05$). This indicates that *C. gariepinus* absorbs these heavy metals using different organs of the body, most especially the gill arch (for lead, cadmium, zinc and copper).

The result of the concentration of heavy metals in the studied organs of *Heterobranchus longifilis* is presented in Table 2. Maximum accumulations of cadmium, iron, zinc and copper occurred in the gill arch; highest accumulations of lead, mercury and magnesium were obtained in the skin; and highest accumulation of chromium was recorded in the liver. However, lowest accumulations of lead, chromium and iron were found in the gill filament; lowest accumulations of mercury and magnesium were found in the gill arch; lowest accumulations of cadmium and copper were found in the skin; and lowest accumulation of zinc was found in the liver. The degree of accumulation of all the heavy metals was significantly different in the tissues of *H. longifilis* ($p < 0.05$). This also indicates that *H. longifilis* absorbs these heavy metals using different tissues of the body, most especially the gill arch (for cadmium, iron, zinc and copper), and the skin (for lead, mercury and magnesium).

Result in Table 3 shows prevalence heavy metals in the organ of *C. gariepinus* and *H. longifilis* from Omambala River. Lead, chromium, mercury, iron, zinc and copper were more prevalence, and the prevalence was significant ($p<0.05$) in the organs of *H. longifilis* than the organs of *C. gariepinus*. On the other hand, cadmium and magnesium were more prevalence in *C. gariepinus* than *H. Longifilis*. The prevalence of magnesium in the fish species were not significantly ($p>0.05$) difference.

Furthermore, the most prevalence heavy metal in the area is as follows: Zinc (5.47±2.607 mg/L) > Cadmium (4.90±2.100 mg/L) > Mercury (4.77±2.283 mg/L) > Magnesium (4.66±2.774 mg/L) > Iron (4.31±2.077 mg/L) > Chromium (4.04±1.073 mg/L) > Lead (3.14±1.395 mg/L) > Copper (3.01±0.828 mg/L). Thus, the type of the heavy metal affected its prevalence in Omambala River in Anambra State.

The order of prevalence of selected heavy metals in four different organs of *C. gariepinus* and *H. longifilis* from Omambala River revealed in Tables 4 and 5 where Zinc is the most prevalence heavy metal because it is found in highest concentration in the skin, gill arch and gill filament of *H. longifilis*, and second highest concentration in the skin, gill arch and gill filament of *C. gariepinus.*

Similar result was reported by Mota et al*.* [15] that the prevalence of these heavy metals may be related with their high availability in the river, since fishes have the tendency to bioaccumulate metals in water through their gills.

Table 1. Degree of accumulation of selected heavy metals in four different organs of *Clarias gariepinus* from Omambala River

Heavy Metals	Organs parts in order of accumulation				p-value
	Clarias gariepinus				
Lead	Gill arch >	Skin >	Liver >	Gill filament	0.042
Chromium	Liver >	Skin >	Gill arch >	Gill filament	0.033
Mercury	Skin >	Liver >	Gill filament >	Gill arch	0.037
Cadmium	Gill arch >	Gill filament >	Skin >	Liver	0.025
Iron	Liver >	Skin >	Gill filament >	Gill arch	0.021
Zinc	Gill arch >	Gill filament >	Skin >	Liver	0.038
Copper	Gill arch >	Gill filament >	Liver >	Skin	0.063
Magnesium	Liver >	Skin >	Gill filament >	Gill arch	0.024

“>” means ‘greater than’

Table 2. Degree of accumulation of selected heavy metals in four different organs of *Heterobranchus longifilis* from Omambala River

Heavy Metals	Organ parts in order of accumulation				p-value
	Heterobranchus longifilis				
Lead	Skin >	Gill arch >	Liver >	Gill filament	0.039
Chromium	Liver >	Gill arch >	Skin >	Gill filament	0.034
Mercury	Skin >	Liver >	Gill filament >	Gill arch	0.028
Cadmium	Gill arch >	Liver >	Gill filament >	Skin	0.043
Iron	Gill arch >	Liver >	Skin >	Gill filament	0.023
Zinc	Gill arch >	Gill filament >	Skin >	Liver	0.021
Copper	Gill arch >	Gill filament >	Liver >	Skin	0.046
Magnesium	Skin >	Gill filament >	Liver >	Gill arch	0.039

“>” means ‘greater than’

Table 3. Prevalence of heavy metals in the organs of *Clarias gariepinus* and *Heterobranchus longifilis* from Omambala River

Heavy Metals (mg/L)	*C. gariepinus*	*H. longifilis*	p-value
Lead	2.69±1.417	3.14±1.395	0.032
Chromium	3.11±0.826	4.04±1.073	0.029
Mercury	3.98±1.903	4.77±2.283	0.035
Cadmium	4.90±2.100	4.83±2.019	0.054
Iron	3.94±1.414	4.31±2.077	0.040
Zinc	4.02±1.917	5.47±2.607	0.021
Copper	2.62±0.720	3.01±0.828	0.041
Magnesium	4.66±2.774	3.72±1.466	0.008

Results are in Mean ± Standard Deviation
(If p>0.05, the difference is not significant)

Table 4. Order of prevalence of selected heavy metals in four different organs of *Clarias gariepinus* from Omambala River

Organs Parts	Heavy metals in order of prevalence							
	Clarias gariepinus							
Skin	Mercury >	Zinc >	Cadmium >	Magnesium =	Iron >	Chromium >	Lead >	Copper
Liver	Magnesium >	Iron >	Mercury >	Zinc >	Chromium >	Cadmium >	Lead >	Copper
Gill Arch	Cadmium >	Zinc >	Mercury >	Chromium >	Lead >	Copper >	Iron >	Magnesium
Gill Filament	Cadmium >	Zinc >	Mercury >	Magnesium >	Iron >	Copper >	Chromium >	Lead

“>” means ‘greater than’
“=” means ‘equal to or the same as the next’

Table 5. Order of prevalence of selected heavy metals in four different organs of *Clarias gariepinus* and *Heterobranchus longifilis* from Omambala River

Organs Parts	Heavy metals in order of prevalence							
	Heterobranchus longifilis							
Skin	Zinc >	Mercury >	Cadmium >	Magnesium >	Chromium >	Iron >	Lead >	Copper
Liver	Mercury >	Zinc >	Cadmium >	Iron >	Chromium >	Magnesium >	Copper >	Lead
Gill Arch	Zinc >	Cadmium >	Iron >	Mercury >	Chromium >	Copper >	Magnesium >	Lead
Gill Filament	Zinc >	Mercury >	Cadmium >	Magnesium >	Iron >	Chromium >	Copper >	Lead

“>” means ‘greater than’
“=” means ‘equal to or the same as the next’

Table 6. Possible source of chemical pollution of heavy metals in three different points at Omambala River.

Heavy metals (mg/L)	Point A	Point B	Point C	p-value
Lead	0.18±0.67	1.19±0.24	0.59±0.20	0.15
Chromium	1.26±0.28	1.32±0.49	0.41±0.63	0.14
Mercury	1.85±0.47	0.54±0.27	1.40±0.23	0.19
Cadmium	0.34±0.11	0.57±0.22	1.01±0.27	0.23
Iron	0.49±0.15	1.92±0.36	1.18±0.15	0.16
Zinc	1.59±0.22	1.37±0.50	1.30±0.86	0.25
Copper	1.38±0.00	1.17±0.23	0.95±0.22	0.22
Magnesium	1.97±0.17	0.45±0.83	1.23±0.78	0.25

Results are in Mean±Standard Deviation
(If $p>0.05$, the difference is not significant)

A total of one hundred fish species of both *Clarias gariepinus* and *Heterobranchus longifilis* were encountered in Anambra River (Table 6). Point A had the highest number of individual species (41) while Point B and Point C had 32 and 27 individuals of fish species respectively. The most abundant fish species in the river was *Clarias gariepinus* with the highest number (25) found in Point A which happened to be the point of greatest pollution in the river. Point A had the highest number of individual fish species (25) belonging to the Family Clarias, followed by Point C (15), while Point B had the least number of individual species (10). For *Heterobranchus longifilis,* the highest number of fish species (22) was recorded in point B, followed by Point A (16). Point C recorded the lowest value (12) of individual fish species of the group.

4. DISCUSSION

In the organs of *C. gariepinus*, the highest accumulations of lead, cadmium, zinc, and copper were found in the gill arch; highest accumulations of chromium, iron, and magnesium were found in the liver; and the highest accumulation of mercury was found in the skin. However, the lowest accumulations of lead and chromium were found in the gill filament; the highest accumulations of mercury, iron, and magnesium were found in the gill arch; the lowest accumulations of cadmium and zinc were found in the liver; and the lowest accumulation of copper was found in the skin. Except in copper (where $p > 0.05$), the degree of accumulation of the heavy metals was significantly different in the organs of *C. gariepinus* (where $p < 0.05$). This is in line with the studies of Yunusa et al. [16] and Israila and Abdullahi [17] which stated that these heavy metals (Zn, Cr, and Fe) were found in the liver of *C. gariepinus* and were less than 0.05. This indicates that *C. gariepinus* absorbs these heavy metals using different tissues of the body, most especially the gill arch (for lead, cadmium, zinc, and copper). The Cu content in the study (Table 1) showed a higher copper concentration was found in the gills than in any other organ of the fish. This supports the findings of Lake et al. [14] which stated that gills had the highest accumulation of Cu in *Cyprinus carpio linnaeus* and *Pelteobagrus fluvidraco.*

Among the organs of *H. longifilis,* the highest accumulations of cadmium, iron, zinc, and copper were found in the gill arch; the highest accumulations of lead, mercury and magnesium were found in the skin; and the highest accumulation of chromium, was found in the liver. However, the lowest accumulations of lead, chromium and iron were found in the gill filament; the lowest accumulations of mercury and magnesium were found in the gill arch; the lowest accumulations of cadmium and copper were found in the skin; and lowest accumulation of zinc was found in the liver. This is consistent with Bawuro et al. [10] in their work (Bioaccumulation of heavy metals in some organs of fish in Lake Geriyo, Adamawa State, Nigeria), which stated that the levels of heavy metals (Zn, Pb, Cd, and Cu) varied significantly among fish species and their organs. The degree of accumulation of all the heavy metals was significantly different in the tissues of *H. longifilis* ($p < 0.05$). This also indicates that *H. longifilis* absorbs these heavy metals using different organs of the body, most especially the gill arch (for cadmium, iron, zinc, and copper), and the skin (for lead, mercury, and magnesium).

Hence, in both species of fish, the gill arches consistently absorbed cadmium, zinc, and copper; the livers consistently absorbed chromium; and the skins consistently absorbed mercury. These variations in the metal accumulation levels may arise from the variations in the reproductive cycle, growth cycle, metabolism, habitat, and feeding strategies of the two fish species.

These accumulations in the gills are expected since the gills are responsible for the uptake of re-suspended and ionised metals from the water column. Higher accumulation of metals in the liver organs is a well-known phenomenon and has been reported in many studies. Metallothioneins found in organs transport metal ions into the liver because liver has a vital role in the synthesis of metal binding proteins and detoxification [18].

The heavy metals lead, chromium, mercury, iron, zinc, and copper from the Omambala River, were more prevalent and significantly higher ($p<0.05$) in the organs of *H. longifilis* than the organs of *C. gariepinus*. On the other hand, cadmium and magnesium were more prevalent and significantly higher ($p<0.05$) in the organs of *C. gariepinus* than the organs of *H. longifilis*.

Furthermore, the most prevalent heavy metals in the area are as follows: Zinc (5.47±2.607 mg/L) > Cadmium (4.90±2.100 mg/L) > Mercury (4.77±2.283 mg/L) > Magnesium (4.66±2.774 mg/L) > Iron (4.31±2.077 mg/L) > Chromium (4.04±1.073 mg/L) > Lead (3.14±1.395 mg/L) >

Copper (3.01±0.828 mg/L). Thus, the type of heavy metal affected its prevalence in the Omambala River in Anambra State. The order of prevalence of selected heavy metals in four different organs of *C. gariepinus* and *H. longifilis* from Omambala River. Zinc is the most prevalent heavy metal because it is found in the highest concentration in the skin, gill arch and gill filament of *H. longifilis*, and second highest concentration in the skin, gill arch, and gill filament of *C. gariepinus.* A similar result was reported by Mota et al. [15] the prevalence of these heavy metals may be related to their high availability in the river since fish have the tendency to bioaccumulate metals in water through their gills. The benefits from the findings of this study are enormous, as they shed light on the heavy metals found in the two fish species from the Omambala River. It has also provided knowledge on the dangers of continuous intake of these fish from the Omambala River.

5. CONCLUSION

The findings of this research revealed that the most prevalent heavy metals in Omambala River were Zn, Cd, Hg, Mg, Fe, Cr, Pb, and Cu. These heavy metals that could be detrimental when detected in large quantity accumulate in the organs of all the fish samples studied. This suggests that consumption of fish from the Omambala river is not completely safe.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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