

# Risk Assessment and Heavy Metals Accumulation in Organs of *Clarias Gariepinus* and *Heterobranchus Longifilis* From Omambala River, Anambra State Nigeria

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## ABSTRACT

Heavy metals are toxic to organisms even at low concentrations. In recent times, there has been the high occurrence of cancer cases locally, traceable to heavy metal contaminations of foods consumed by man and animals. The experimental site for this study was Omambala River which serves as a source point of many fresh fishes as well as its distribution throughout the state both in dry and rainy seasons. One hundred (100) samples each of *Clarias gariepinus* and *Heterobranchus longifilis*, with the size of 17–21 cm were selected from Omambala River. Heavy metal determination was done using the digestion method. All the digested samples were analyzed three times for heavy metals using SP-V21A Vis Spectrophotometer and the instrument was calibrated with standard solutions. For Risk assessment analyses, the target hazard quotients (THQ) index was applied to assess the potential non-cancer risk associated to consumption of the different species of marine organisms sampled. While the total target hazard quotient (TTHQ) was calculated as the arithmetic sum of each THQ value. The lifetime cancer risk (TR) was calculated by multiplying the daily dose by the cancer slope factor (CSF) derived by response-dose curve for toxicant ingestion. The bioaccumulation factor (BAF) was determined by dividing the metal concentration in the tissue of the organism by the metal concentration in the environment or medium. From the correlation analysis, there was a positive correlation between the four different organs of the fish species and the selected heavy metals. The toxicity of magnesium and zinc are higher in *C. gariepinus* while the toxicity of iron is higher in *H. longifilis*. The degree of toxicity is zinc > iron > magnesium > lead > cadmium > copper > mercury > chromium. The water quality index (WQI) for all the heavy metals in four different organs of the two fish species was found to be less than 1 ( $WQI < 1$ ) meaning low pollution; which further means that the consumption of *C. gariepinus* and *H. longifilis* may not pose any health risk to the people of the area. However, there are reports of lifetime cancer risk on daily consumption of Chromium, Cadmium, Lead and Mercury in fish. Chemicals when found in large quantities.

**Keywords:** Heavy metals, *Clarias gariepinus*, *Heterobranchus longifilis*, river, Risk assessment.

## INTRODUCTION

In the recent times, there has been reports on the risks associated with the consumption of heavy metal-contaminated foods across the globe. This has triggered interest in determining heavy metal levels in the marine environment<sup>1</sup>. Attention has also been directed to the determination of heavy metals contamination in public foods especially fish and other aquatic organisms consumed by man. Some examples of heavy metals include lead, zinc, cadmium, copper and manganese. Many of these heavy metals are toxic to organisms at low concentrations. This study has become imperative due to the rise in the case of cancer locally, especially within Anambra State<sup>2</sup>. The role of the Omambala River is a source of food for the exploding populace that depends on it for livelihood and survival. In addition, the economic boom due to industrialization, has constantly released toxic waste into our environment, especially the water body<sup>3,4,5</sup>. Today we have more people who have access to automobile than in previous times which has consequently added to toxic release of pollutants into our environment, which eventually ends up in water bodies<sup>6</sup>. Also other activities such as; quarrying, mining and non-regulated metal works by blacksmiths for which Awka in

Anambra State is famous for and produces abundant heavy metal waste, which are washed down from hinterlands to Omambala River through its connecting tributaries<sup>7,8,9</sup>. At this point, the need to regularly monitor and evaluate our food quality by assessing the minerals and organic content cannot be overemphasised for health risk purposes and ecosystem sustainability. Therefore, this study will be a source of information on the quality of fish resident of Omambara River which are regularly consumed as a source of protein in the environs. The study was limited to risk assessment and bioaccumulation of the heavy metals present in the fish species.

## MATERIALS AND METHODS

### Study Area

This study was conducted in the Anambra River (Igbo: Omambala). This river flows 210 kilometres (130 mi) into the Niger River and is found in Anambra, Nigeria. The river is the most important feeder of the River Niger below Lokoja. The flow of the Omambala River is released into the Atlantic through various outlets forming the 25,000-square-kilometre (9,700 sq mi) Niger Delta region. The socio-economic, socio-cultural and socio-political influence of the Omambala region extends to

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parts of Edo, Delta, Imo, Rivers, Abia, Taraba, Benue, Niger, Nasarawa, Plateau, Akwa-Ibom & Cross-Rivers States of Nigeria<sup>6</sup>.

### Sampling locations and campaign

The experimental site/ station is Omambala River which serves as a source point of many fresh fishes as well as its distribution throughout the state both in dry and raining season.

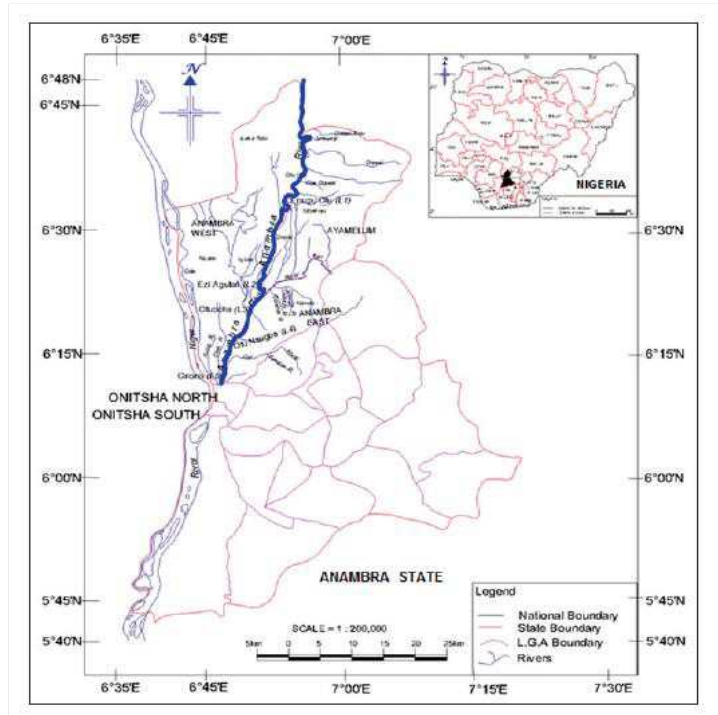


Figure 1: Map of Anambra River Basin. (Source: Obiakor and Okonkwo<sup>6</sup>)

### Field sampling

One hundred (100) samples each of *Clarias gariepinus* and *Heterobranchus longifilis*, with the size of 17–21 cm were selected from Omambala River during the study by professional fishermen using a multifilament, nylon, gill, net or trawl. 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. The water samples were collected with sterile containers, properly labeled, stored in a refrigerator, and taken to the laboratory within 72 hours of collection for analysis of the physicochemical parameters of the River.

### 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.

### Digestion and determination of heavy metals

Heavy metal determination was done using the digestion method. Dissected-selected organs were oven dried, grounded, 1 gram each transferred to glass beaker were performed in an acid digestion 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 analyzed three times for heavy metals using SP-V21A Vis Spectrophotometer and the instrument was calibrated with

standard solutions.

### Risk assessment analyses

#### Determination of target hazard quotient (THQ)

The target hazard quotients (THQ) index was applied to assess the potential non-cancer risk associated to the consumption of the different species of marine organisms sampled. The THQ value was calculated on the base of the metal concentrations recorded in the edible parts of the organisms. THQ values exceeding one unit indicate a potential health risk to the consumers (USEPA, 2001). It was calculated following the United States Environmental Protection Agency (USEPA, 2001).

$$THQ = \frac{EF*ED*FIR*C}{RfD*WAB*TA} * 10^{-3}$$

where: EF and ED represent the exposure frequency (365 days/year) and the average lifetime duration (70 years), respectively; FIR is the fish ingestion rate (36 g day<sup>-1</sup> for person; The Food and Agriculture Organization (FAO, 2005); C is the metal concentration (µg-g<sup>-1</sup>); RfD is the reference oral dose in mg kgbw<sup>-1</sup> d<sup>-1</sup> (1 \* 10<sup>-4</sup> for Hg, 3 \* 10<sup>-4</sup> for As, 1 \* 10<sup>-3</sup> for Cd, 1.5 for Cr, 4 \* 10<sup>-2</sup> for Cu, 2 \* 10<sup>-2</sup> for Ni and Zn, 4 \* 10<sup>-3</sup> for Pb) (USEPA, 2001); WAB is the average body weight for adult consumer (67 kg); TA is the average exposure time (365 days/year \* ED). THQAs was calculated on the base of inorganic As (IAs), which represent the most toxic form, corresponding to ≈2% of the total As.

#### Determination of total target hazard quotient

Since the exposure to two or more pollutants may result in additive effects, the total target hazard quotient (TTHQ) was also calculated as the arithmetic sum of each THQ values :

$$TTHQ = THQ_{(Hg)} + THQ_{(As)} + THQ_{(Cd)} + THQ_{(Cr)} + THQ_{(Cu)} + THQ_{(Ni)} + THQ_{(Pb)} + THQ_{(Zn)} \quad (2)$$

#### Determination of life time cancer risk (TR)

The lifetime cancer risk (TR) was calculated by multiplying the daily dose by the cancer slope factor (CSF) derived by response-dose curve for toxicant ingestion, following the formula:

$$TR = \frac{EF*ED*FIR*C*CSF}{WAB*TA} * 10^{-3}$$

Since CSF of Cr and Hg have not been published by the USEPA, TR was calculated only for As (CSF= 1.5 kg-day/mg), Cd (CSF= 6.3 kg-day/mg) and Pb (CSF= 8.5 \* 10<sup>-3</sup> kg-day/mg)<sup>10</sup>. Acceptable risk levels for carcinogens range from 10<sup>-4</sup> (risk of developing cancer over a human lifetime is 1 in 10,000) to 10<sup>-6</sup> (risk of developing cancer over a human lifetime is 1 in 1,000,000). In this study we consider 10<sup>-5</sup> cancer benchmark.

#### Determination of bioaccumulation factor (BAF)

BAF is calculated by the following equation (Ali et al.<sup>11</sup>).

$$BAF = \frac{C_{organism\ tissue}}{C_{abiotic\ medium}}$$

where C<sub>organism tissue</sub> is the metal concentration in the tissue of the organism and C<sub>abiotic medium</sub> is the metal concentration in the environment or medium.

#### Statistical analysis

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

## RESULTS

The heavy metals detected in the fish samples from this study are Lead, Chromium, Mercury, Cadmium, Iron, Zinc, Copper and Magnesium (Table 1).

**Table 1: List of heavy metals detected in the organs of *Clarias gariepinus* and *Heterobranchus longifilis***

S/N	Heavy metals
1	Lead
2	Chromium
3	Mercury
4	Cadmium
5	Iron
6	Zinc
7	Copper
8	Magnesium

Result in table 2 and 3 shows the degree of concentration/toxicity of selected heavy metals in four different organs of *Clarias gariepinus* and *Heterobranchus longifilis* from Omambala River. From the correlation analysis, there was

positive correlation between the four different organs of the fish species and the selected heavy metals. In *Clarias gariepinus*, the highest concentration of Lead was observed in skin and gill arch with values of  $2.61 \pm 1.162$  and  $2.69 \pm 1.417$  respectively while Chromium was highest in the liver  $3.11 \pm 0.826$ . Cadmium, Zinc and Copper accumulated more in the Gill Arch with values  $4.90 \pm 2.100$ ,  $4.02 \pm 1.917$  and  $2.62 \pm 0.720$  respectively while Iron and Magnesium were detected more in the liver ( $3.94 \pm 1.414$  and  $4.66 \pm 2.774$  respectively). The Liver had the highest concentration of Mercury ( $3.98 \pm 1.903$ ) (Table 2).

For *Heterobranchus longifilis*, the majority of the heavy metals accumulated in the gill arch. Cadmium, Iron, Zinc and Copper with  $4.83 \pm 2.019$ ,  $4.31 \pm 2.077$ ,  $5.47 \pm 2.607$  and  $3.01 \pm 0.828$  values. These were the highest values recorded; however they were not significantly higher than the values obtained from other organs. From the skin, the heavy metals that accumulated more were Lead, Mercury and Magnesium ( $3.14 \pm 1.395$ ,  $4.77 \pm 2.283$  and  $3.72 \pm 1.466$  respectively). Chromium accumulated more in the Liver ( $4.04 \pm 1.073$ ). The differences in the heavy metals accumulation across the organs was not significant.

**Table 2: Degree of concentration/toxicity of selected heavy metals in four different organs of *Clarias gariepinus* from Omambala River**

Heavy Metals	Skin	Liver	Gill Arch	Gill Filament	R-square
<b><i>Clarias gariepinus</i></b>					
Lead	$2.61 \pm 1.162$	$2.46 \pm 1.325$	$2.69 \pm 1.417$	$2.13 \pm 1.259$	0.864
Chromium	$2.81 \pm 1.352$	$3.11 \pm 0.826$	$2.76 \pm 0.760$	$2.52 \pm 0.644$	0.667
Mercury	$3.98 \pm 1.903$	$3.72 \pm 1.735$	$3.12 \pm 1.289$	$3.63 \pm 0.375$	0.691
Cadmium	$3.00 \pm 1.145$	$2.53 \pm 0.552$	$4.90 \pm 2.100$	$3.92 \pm 0.007$	0.849
Iron	$2.90 \pm 0.681$	$3.94 \pm 1.414$	$2.51 \pm 1.662$	$2.75 \pm 0.796$	0.779
Zinc	$3.57 \pm 1.257$	$3.26 \pm 1.730$	$4.02 \pm 1.917$	$3.88 \pm 1.565$	0.966
Copper	$2.34 \pm 1.097$	$2.43 \pm 1.181$	$2.62 \pm 0.720$	$2.59 \pm 1.059$	0.811
Magnesium	$2.90 \pm 1.145$	$4.66 \pm 2.774$	$2.27 \pm 0.941$	$2.77 \pm 1.113$	0.930

Results are in Mean  $\pm$  Standard Deviation

**Table 3: Degree of concentration/toxicity of selected heavy metals in four different organs of *Heterobranchus longifilis* from Omambala River**

Heavy Metals	Skin	Liver	Gill Arch	Gill Filament	R-square
<b><i>Heterobranchus longifilis</i></b>					
Lead	$3.14 \pm 1.395$	$2.46 \pm 1.325$	$2.69 \pm 1.417$	$2.13 \pm 1.259$	0.767
Chromium	$3.65 \pm 1.758$	$4.04 \pm 1.073$	$3.59 \pm 0.988$	$3.28 \pm 0.837$	0.669
Mercury	$4.77 \pm 2.283$	$4.47 \pm 2.082$	$3.75 \pm 1.546$	$4.35 \pm 2.356$	0.734
Cadmium	$4.03 \pm 1.201$	$4.30 \pm 1.375$	$4.83 \pm 2.019$	$4.22 \pm 1.243$	0.828
Iron	$3.47 \pm 1.716$	$4.27 \pm 1.863$	$4.31 \pm 2.077$	$3.44 \pm 0.995$	0.944
Zinc	$4.85 \pm 1.710$	$4.43 \pm 2.353$	$5.47 \pm 2.607$	$5.23 \pm 1.977$	0.704
Copper	$2.69 \pm 1.262$	$2.80 \pm 1.359$	$3.01 \pm 0.828$	$2.98 \pm 1.218$	0.671
Magnesium	$3.72 \pm 1.466$	$2.99 \pm 0.968$	$2.90 \pm 1.204$	$3.55 \pm 1.424$	0.485

Results are in Mean  $\pm$  Standard Deviation.

Result in Tables 4 and 5 show risk assessment of selected heavy metals in four different organs of *Clarias gariepinus* and *Heterobranchus longifilis* from Omambala River. The water quality index (WQI) for all the heavy metals in four different organs of the two fish species was found to be less than 1 ( $WQI < 1$ ) meaning low pollution; which further means that the consumption of *C. gariepinus* and *H. longifilis* may not pose any health risk to the people of the area. However, there are reports of lifetime cancer risk on daily consumption of Chromium, Cadmium, Lead and Mercury in fish (Korkmaz et al., 2019). In another study, Piza et al. (2017) reported that the consumption of fish accumulated with mercury could be dangerous to health.

**Table 4: Risk Assessment of selected heavy metals in four different organs of *Clarias gariepinus* from Omambala River**

Tissue Parts	Heavy metals mg/L							
	Lead	Chromium	Mercury	Cadmium	Iron	Zinc	Copper	Magnesium
<i>Clarias gariepinus</i>								
Skin	3.4 x 10 <sup>-2</sup>	1.2 x 10 <sup>-2</sup>	2.6 x 10 <sup>-3</sup>	3.3 x 10 <sup>-3</sup>	2.8 x 10 <sup>-3</sup>	3.5 x 10 <sup>-3</sup>	4.0 x 10 <sup>-4</sup>	2.3 x 10 <sup>-2</sup>
Liver	2.1 x 10 <sup>-2</sup>	1.4 x 10 <sup>-2</sup>	2.2 x 10 <sup>-3</sup>	1.1 x 10 <sup>-3</sup>	2.0 x 10 <sup>-3</sup>	4.2 x 10 <sup>-3</sup>	4.7 x 10 <sup>-4</sup>	3.5 x 10 <sup>-2</sup>
Gill Arch	5.6 x 10 <sup>-2</sup>	3.3 x 10 <sup>-2</sup>	4.3 x 10 <sup>-2</sup>	3.9 x 10 <sup>-2</sup>	5.2 x 10 <sup>-2</sup>	1.2 x 10 <sup>-3</sup>	2.4 x 10 <sup>-4</sup>	1.0 x 10 <sup>-2</sup>
Gill Filament	5.3 x 10 <sup>-2</sup>	2.7 x 10 <sup>-2</sup>	2.5 x 10 <sup>-2</sup>	2.6 x 10 <sup>-2</sup>	3.9 x 10 <sup>-2</sup>	4.8 x 10 <sup>-3</sup>	4.4 x 10 <sup>-4</sup>	2.5 x 10 <sup>-2</sup>

**Table 5: Risk Assessment of selected heavy metals in four different organs of *Heterobranchus longifilis* from Omambala River**

Organs Parts	Heavy metals mg/L							
	Lead	Chromium	Mercury	Cadmium	Iron	Zinc	Copper	Magnesium
<i>Heterobranchus longifilis</i>								
Skin	3.2 x 10 <sup>-2</sup>	1.6 x 10 <sup>-2</sup>	1.4 x 10 <sup>-3</sup>	3.1 x 10 <sup>-3</sup>	3.2 x 10 <sup>-3</sup>	2.8 x 10 <sup>-3</sup>	4.1 x 10 <sup>-4</sup>	3.3 x 10 <sup>-2</sup>
Liver	2.4 x 10 <sup>-2</sup>	1.8 x 10 <sup>-2</sup>	2.4 x 10 <sup>-3</sup>	1.5 x 10 <sup>-3</sup>	1.8 x 10 <sup>-3</sup>	4.0 x 10 <sup>-3</sup>	3.6 x 10 <sup>-4</sup>	2.3 x 10 <sup>-2</sup>
Gill Arch	2.8 x 10 <sup>-1</sup>	3.5 x 10 <sup>-2</sup>	3.7 x 10 <sup>-2</sup>	3.3 x 10 <sup>-2</sup>	4.7 x 10 <sup>-2</sup>	1.4 x 10 <sup>-3</sup>	1.5 x 10 <sup>-3</sup>	1.2 x 10 <sup>-2</sup>
Gill Filament	4.6 x 10 <sup>-2</sup>	2.3 x 10 <sup>-2</sup>	2.3 x 10 <sup>-2</sup>	3.8 x 10 <sup>-2</sup>	4.2 x 10 <sup>-2</sup>	2.1 x 10 <sup>-3</sup>	2.8 x 10 <sup>-4</sup>	1.0 x 10 <sup>-2</sup>

## DISCUSSION AND CONCLUSION

Degree of concentration/toxicity of selected heavy metals in four different organs of *Clarias gariepinus* and *Heterobranchus longifilis* from Omambala River, the correlation analysis, there was positive correlation between the four different to organs of the fish species and the selected heavy metals. Most of the heavy metals accumulate more in the gill arch and the skin. An increase in the concentrations of the heavy metals in the organs increases the toxicity of the two fish species. The toxicity of Zn is higher in *C. gariepinus*. The quantity of Iron variance between *H. longifilis* and *C. gariepinus*. However, the toxicity is higher in *H. longifilis*. The degree of toxicity is zinc > iron > magnesium > lead > cadmium > copper > mercury > chromium. As reported by Alipour<sup>12</sup> that some metals such as iron, chromium and aluminium may be essential for growth in plants and animals, high concentrations and accumulation in foods may be toxic and very harmful on the reproductive system, the nervous system and the immune system in the human body. Other studies further pointed out that metal toxicity may cause cancer development in the body, cardiovascular diseases, Alzheimer, dementia and Parkinson's disease<sup>13</sup>.

The risk assessment of selected heavy metals in four different organs of *Clarias gariepinus* and *Heterobranchus longifilis* from Omambala River, the water quality index (WQI) for all the heavy metals in four different organs of the two fish species was found to be less than 1 (WQI < 1) meaning low pollution; which further means that the consumption of *C. gariepinus* and *H. longifilis* may not pose any health risk to the people of the area. However, there are reports of lifetime cancer risk on daily consumption of Chromium, Cadmium, Lead and Mercury in fish<sup>14,15,16,17</sup>. In another study, Piza *et al.*<sup>18</sup> reported that the consumption of fish accumulated with mercury could be dangerous to health. Heavy metal accumulation in the gills and organs of fishes have been a subject for discussion for several decades due to the accumulation of these heavy metals in humans as a result of constant indigestion of these chemicals into the human body, these chemicals when found in large quantities can cause adverse health implication or in some cases, cause chronic sickness after a long period of time, thus the need to check the level of these chemicals in rivers and organs of fish that dwell in these water becomes imperative. Heavy metal contamination sometimes occurs naturally but in most cases human activities create a more worrying concern due to human consumption of these fishes as an alternative source of protein for human diary needs.

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