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# Heavy Metal Concentration in Blood and Selected Tissues of Cat Fish From Kwalkwalawa River, Sokoto

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## ABSTRACT
Fish is a cheap source of omega- 3 polyunsaturated fatty acids for humans. However, the presence of toxic heavy metals in water can be detrimental to both fish and humans. The aim of this study was to investigate the levels of heavy metals in the liver, kidney and gills of cat fish obtained from Kwalkwalawa River, Sokoto. Water, sediments, blood and tissue samples of (10) cat fish (*Clarias gariepinus*) species caught from Kwalkwalawa river were analysed quantitatively for the presence of zinc, copper, lead, chromium and cadmium using atomic absorption spectrophotometer. Bioaccumulation of these heavy metals was estimated in these organs. Results showed that lead concentrations in the water was significantly higher ($p < 0.05$) compared to the sediment sample. Also, copper and lead accumulated most in the liver and kidney when compared with that observed for the gills. In conclusion, lead and cadmium levels in the samples were above WHO certified limits hence the concentration of metals in this water should be monitored periodically.
**KEYWORDS**: Heavy metals, Hematology, Bioaccumulation, Toxicity

## INTRODUCTION
For most average Nigerian families, an affordable source of animal protein is fish hence the high rate of fish consumption (Egun and Oboh, 2022). More than three-quarter of the population in Nigeria falls in the low-income category, which further explains the high preference for fish because it is relatively cheap and readily available (Akinbode and Dipeolu, 2012). This makes fish and fish products a top-ranking choice of quality protein within the reach of the poor in most developing countries (Akinbode and Dipeolu, 2012). Other than being an excellent source of protein, fish is also an important source of essential minerals, vitamins and unsaturated fatty acids. The American Heart Association recommends that fish should be consumed at least twice per week in order to meet the daily requirement of Omega-3 fatty acids (Liverpool-Tasie *et al*., 2021). Interestingly the consumption of fish worldwide is on the rise due to increasing awareness of its nutritive and therapeutic value (Rimm *et al.,* 2018; Supartini *et al*., 2018). Despite this great nutritional value of fish, it can be detrimental to human health if its quality is compromised. Water pollution by heavy metals does not only impair fish productivity and reproductive capabilities but greatly affect man that depends on it as a major source of protein (Fonge *et al*., 2011; Amqam *et al*., 2020). Over the years, scientist have been carrying out various researches to elucidate the harmful effect of heavy metals on living systems as well as its negative impacts (Castro-Gondalez and Mendez, 2008; Al-busaidi *et al*., 2011). This includes stressing the living organisms which causes renal failure, liver damage, cardiovascular diseases and increased mortality (Al-busaidi *et al*., 2011; Madiha *et al*., 2022).

Due to their bioaccumulation and chronic toxicity, it is worthy to note that all heavy metals have the potential to cause harm to most organisms at excessive levels of exposure (Adedeji and Okocha, 2011; Madiha *et al*., 2022). Heavy metals are classified as metallic elements which have relatively high atomic weight and are toxic at low concentration (Butu and Igiusi, 2013). They exist naturally in the environment or are anthropogenically produced and accumulate to certain amount high enough to cause harm to aquatic life (Jiang *et al*., 2020). Many researchers have investigated the concentrations of heavy metals in different rivers and sediment in Nigeria (Butu and Iguisi *et al*., 2013; Bawuro *et al*., 2018; Olayinka-Olagunju *et al*., 2021). Studies have been conducted on heavy metals in relation to Kwalkawlawa river in Sokoto state. Sani *et al*. (2015) reported concentrations above tolerable limits of Fe, Pb and Cd in three fish species obtained from river Kwalkwalawa. Similarly, other works have reported high Zn, Cu, Cd, Cr and Pb levels compared to World Health Organization limits in three different species from Kwalkwalawa river Sokoto (Dabai *et al*., 2013; Raji *et al*., 2016; Ejike and liman, 2017). Many of these studies, however, were focused on the concentrations of heavy metals in the fish, water and sediments. Nevertheless, the bioconcentration and histopathological effects of these metals in the fish tissue is also important. Organ/tissue bioaccumulation and/or bio-concentration of heavy metals are important markers for toxicity monitoring and could serve as indicators for contaminants or pollutants to which humans are at risk of exposure (Chaves *et al*., 2021). Therefore, periodic monitoring of contaminants in fish and water bodies is imperative (Meche *et al*., 2010; Yilmaz *et al.*, 2007; Zhao *et al.*, 2012). In an attempt to bridge this gap, the present study was designed to compare environmental or habitat levels of heavy metals with bioaccumulated levels in the liver, gills and kidney of cat fish caught from Kwalkwalawa river.

## MATERIALS AND METHODS
### Site Description
Cat fish was collected from Kwalkwalawa river Sokoto, (latitude 13° 06’ 28” and longitude 05° 12’ 46”). The climate of Kwalkwalawa is typically hot, or semi-arid tropical with an annual precipitation of 600 mm between the months of June to September. The remaining 8 months of the year is usually very dry resulting in an increase in activities around the river bank. Farmers and

other villagers cultivate land and wash clothes at the river bank which could serve as a source of heavy metals due to application of pesticides/fertilizer and detergents. The area is characterized by mean annual maximum temperature of 41 °C, with monthly means varying from 40.0 °C in May to 33.0 °C in December (Misau *et al*., 2015; Akilu and Oladeji, 2017).

**Field Sampling and Fish Dissection**
Live cat fish (n = 10) with an average weight of 630 g were captured from the Kwalkwalawa river, Dundaye, Sokoto. They were then transported on iceto the Veterinary Pharmacology and Toxicology laboratory, Usmanu Danfodiyo University, Sokoto for dissection and sample collection.

**Heavy Metal Determination**
Concentration of the heavy metals Cu, Cr, Cd, Pband Zn in samples of river water and sediments, as well as blood and selected tissues of captured catfish were determined using Atomic Absorption Spectrometry method (Shimadzu-UK, "AAS3600") at the Energy Research Centre Usmanu Danfodiyo University, Sokoto. Samples were first pre-digested following procedures described below:

**Water Sample**
Water samples (state number of samples collected) from Kwalkwalawa river, Dundaye, Sokoto were collected in one litre sterile plastic containers and taken to the laboratory. Five millilitres of concentrated hydrochloric acid was added to 250 mL of sample and evaporated to 25 mL at 90 $^{o}$C for 5 minutes. The concentrate was further transferred to a 50 mL flask then diluted to mark with distilled water and used for analysis (Eaton *et al.,* 1998).

**Sediments**
Kwalkwalawa River was collected into pre-cleaned polythene bag and mixed gently. Samples were air dried for three days and sieved with 200 mm mesh screen. Exactly 5 g of prepared sediment was placed in a 150 mL conical flask then 50 mL of 0.1 M HCl was added and the flask was agitated on an orbital shaker for 30 min at 200 rev/min. The content was filtered into another 50 mL flask and filled up to mark with 0.1 M HCl for the determination of Cu, Cr, Cd, Pb and Zn.

**Blood and Tissue Samples**
Five (5) mL of blood was collected from each cat fish via caudal venepuncture using a 5 mL syringe and dispensed into a 100 mL flask and 10 mL $HNO_3$ was added. The blood sample was digested by placing the mixture on a hot plate at 80 $^{o}$C for 120 minutes. After allowing to cool, the solution was then filtered and digestion was completed by the addition of 10 mL of $H_2O_2$.

Harvested tissue samples (liver, kidney and gills) were placed in an oven (80℃) for 24 hours to achieve a stable weight. Each sample was then grinded with a mortar and pestle. To 5 g of each dried tissue sample, 3 mL of nitric acid (65%) and 1 mL of analytical grade hydrogen peroxide (35%) were added. The tube was then placed in a microwave set at 200 ℃ for 25 minutes. After cooling to room temperature, the content of the digestion tube was transferred into a volumetric flask and 50 mL of deionized water was added to it and finally filtered with Whatman No. 1 filter paper (Du Preez and Steyn,1992).

**Haematological Assessment**
With the aid of a 23 guage needle, 2 mL of blood was drawn from the posterior caudal vein of each cat fish anesthesized in clove oil solution for six minutes as described by Argungu *et al*. (2015). The blood samples were then transferred into labelled sterile EDTA sample bottles for assessment of haematological parameters. The packed cell volume (PCV) was determined by haematocrit method while hemoglobin (Hb), total white blood cell (WBC), red blood cell count (RBC) and differential cell counts were determined using the method of Dacie and Lewis (1975). The mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular haemoglobin (MCH) were calculated as described by Dacie and Lewis (1975). Haematological analysis was done at the Chemical Pathology Laboratory of the Department of Veterinary Pathology, Usmanu Danfodiyo University Sokoto.

**Enzyme Assay**
For enzyme assay, 2 mL of blood was collected from each cat fish and centrifuged at 3000 rpm for 10 minutes to obtain serum which was used to assay for activities of alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and creatinine kinase (KC). The enzyme analyses were done using Randox commercial kits (Randox Laboratories Ltd., United Kingdom).

**Evaluation of Bioconcentration Factor**
The biocencentration factor (BCF)of catfish exposure to heavy metals was evaluated following methods described by Olayinka-Olagunju *et al*. (2021) using the relationship:

$$\text{BCF}= \frac{\text{Mean metal concentration in sample } (\text{mg kg} - 1)}{\text{Mean metal concentration in Kwakwalawa river } (\text{mg L} - 1)}$$

**Statistical Analysis**
Data obtained from this experiment were expressed as mean ± SD. Statistical analysis was performed with the aid of Graph Pad Prism (version 8.0). The mean values of heavy metals in water and sediment sample were compared using T test and heavy metals in the blood and selected tissues were analysed using one-way analysis of variance test with Tukey's post hoc test for comparison. $P < 0.05$ was considered significant.

**RESULTS**

**Heavy Metal Concentrations in Water and Sediment from Kwalkwalawa River, Sokoto**

Metal concentrations determined in water and sediment samples are shown in Table 1. The results of heavy metal analysis showed that metals were detected in varying concentrations in water and sediment samples of Kwalkwalawa River, Sokoto. Chromium, cadmium and lead levels in water sample were above the WHO and DWAF limits for heavy metals in water. The heavy metals in the sediment sample were within the recommended limits (Table 1). Copper and Zinc levels in the sediment samples were significantly higher (P < 0.05) compared to that of the water sample. Lead concentration in the water sample was significantly higher (P < 0.05) in the water sample compared to the sediment sample.

**Table 1:** Heavy Metal concentrations in water (mg/L) and sediments (mg/kg) sample collected from Kwalkwalawa River, Sokoto.

| Metal | Water Sample (mg/L) | Sediment Sample (mg/kg) |
|---|---|---|
| **Cu** | 0.113± 0.010[a] | 0.155± 0.001[b] |
| ***Cr** | 0.120± 0.005[a] | 0.150 ± 0.080[a] |
| ***Cd** | 0.030± 0.001[a] | 0.034 ± 0.002[a] |
| ***Pb** | 0.600± 0.059[a] | 0.560± 0.054[b] |
| **Zn** | 0.161 ± 0.013[a] | 0.190± 0.020[b] |

Data are presented as mean ± SD of ten determinations, [a,b]Values with different superscripts are significantly different at $p < 0.05$. *Heavy metal levels above WHO (2017) and DWAF (DWAF, 1996) recommended limits.

**Serum and Tissue Heavy Metal Concentrations of *Clarias gariepinus* Obtained from Kwalkwalawa River, Sokoto**

The results in Table 2 showed that the fish obtained from Kwalkwalawa River Sokoto, exhibited wide range of variations in heavy metal levels. Chromium concentration was significantly higher (P < 0.05) in the serum compared to that in the liver and kidney. The cadmium concentration in the kidney was significantly lower compared to that of the blood and gills. Furthermore, Lead concentration in the blood and gills was significantly higher ($p < 0.05$) than that in the liver and kidney. There was no significant difference ($p > 0.05$) in the lead concentrations of the liver and kidney. Cu and zinc levels in tissues were not significantly different from that in serum. The concentration of cadmium and lead in serum was above the WHO recommended levels. Cadmium was also above the WHO recommended limits in the liver, kidney and gills. In the gills, lead concentration was above WHO recommended levels

**Table 2:** Heavy metals in the serum and tissues of *Clarias gariepinus* from Kwalkwalawa River, Sokoto

| Metal (mg/kg) | Concentration in Blood (mg/L) | Liver (mg/kg) | Kidney (mg/kg) | Gills (mg/kg) |
|---|---|---|---|---|
| **Cu** | 0.054 ± 0.009 | 0.06 ± 0.001 | 0.050 ± 0.001 | 0.02 ± 0.001 |
| **Cr** | 0.103 ± 0.001[a] | 0.07 ± 0.02[b] | 0.040 ± 0.001[c] | 0.070 ± 0.002[a] |
| **Cd** | *0.013 ± 0.0001[a] | *0.008 ± 0.0002[a,b] | *0.006 ± 0.001[b] | *0.030 ± 0.0001[c] |
| **Pb** | *1.107 ± 0.01[a] | 0.30 ± 0.007[b] | 0.500 ± 0.0 20[b] | *0.900 ± 0.008[a] |
| **Zn** | 0.070 ± 0.008 | 0.04 ± 0.004 | 0.060 ± 0.001 | 0.054 ± 0.004 |

*****Heavy metals levels above WHO (2017) recommended limits for blood and tissues. Data are presented as mean ± SD of ten determinations, [a,b]Values with different superscripts are significantly different at $p < 0.05$

**Hematological Changes Associated with Heavy Metals of Cat Fish Obtained from Kwalkwalawa River, Sokoto**

Table 3 shows the erythrocyte indices of cat fish from Kwalkwalawa River, Sokoto. The RBC, Hb, and PCV were found to be 1.80 X $10^6$ µL, 6.23 g/dl, 25%, below the normal reference value, respectively. Also, the total WBC and Monocytes were found to be 14.33 × $10^3$ µL and 1.56, respectively and were below the normal reference values. Basophils were not detected

**Enzyme Activities in serum of cat fish Obtained from Kwalkwalawa River, Sokoto**

The serum biochemical result reveal that AST, ALT and ALP were found to be 104 U/L, 59.1 U/L and 81.9 U/L, respectively and higher than the normal reference value in *Clarias gariepinus.* Creatinine kinase was 39.4 U/L compared to the 45.40 U/L (Table 4).

Table 5 presents the bio concentration of heavy metals in Liver, Kidney and Gills of *Clarias gariepinus* from Kwalkwalawa River, Sokoto. The results show that the gills have higher affinity for bioconcentration of chromium, cadmium, lead and zinc than the liver and kidneys. However, the concentration of copper is highin liver and kidney (BCF 0.425 and 0.433, respectively) compared to that of gills (BCF 0.212).

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**DISCUSSION**

It was observed from this study that the mean concentrations of chromium, cadmium and lead in the Kwalkwalawa river, was more than allowable limits (Mollo *et al*., 2022). The findings from the present study are similar to a study of Olayinka-Olagunju *et al*. (2021) where chromium and lead levels were greater than what was recommended by WHO. Chromium and lead concentrations above permissible limit are deleterious to fish and human health. Long term exposure to heavy metals has been reported to cause severe damage to fish tissue both at gross, histopathological and immunological examinations (Islam *et al*., 2019).

**Table 3:** Alteration in blood indices associated with heavy metal pollution in cat fish from Kwalkwalawa River, Sokoto

| Parameter | Mean ± SD | Reference Values* |
|---|---|---|
| **WBC ($10^3$/µL)** | 14.33 ± 3.93 | 18.66-25.61 |
| **RBC ($10^6$/µL)** | 1.80 ± 0.850 | 3.05-8.64 |
| **Hb (g/dL)** | 6.23 ± 3.510 | 10.2-18.64 |
| **PCV (%)** | 25.00 ± 11.40 | 32.6-45.7 |
| **MCV (fl)** | 147.10 ± 12.80 | 136 - 148 |
| **MCH (pg)** | 36.70 ± 03.70 | 30.2 - 46.7 |
| **MCHC (g/dL)** | 25.00 ± 1.80 | 38.2 - 46.7 |
| **Neutrophil (%)** | 38.44 ± 11.91 | 27.6 – 40.14 |
| **Lymphocytes (%)** | 53.89 ± 11.91 | 51.4 - 70.2 |
| **Monocytes (%)** | 1.56 ± 0.53 | 1.86 - 4.01 |
| **Basophil (%)** | ND | ND |

* Akinrotimi *et al.*, 2011

**Table 4**: Alteration in serum enzyme activities associated with heavy metal in *Clarias gariepinus* from Kwalkwalawa River, Sokoto.

| Parameter | Mean ± S. D | Reference Values* |
|---|---|---|
| **AST (U/L)** | 104.00 ± 24.9 | 50.52 ± 0.44 |
| **ALT (U/L)** | 59.10 ± 8.66 | 39.58 ± 0.17 |
| **ALP (U/L)** | 81.80 ± 8.10 | 8.65 ± 0.66 |
| **Creatinine Kinase (U/L)** | 39.40 ± 2.64 | 45.40 ± 4.77 |

*Reference values adopted from Okorie-Kanu and Unakalamba (2015).

**Table 5:** The BCF of copper, chromium, lead and zinc in liver, kidney and gills of cat fish from Kwalkwalawa River, Sokoto

| Metal | Liver | Kidney | Gills |
|---|---|---|---|
| Cu | 0.425 | 0.433 | 0.212 |
| Cr | 0.433 | 0.225 | 0.550 |
| Cd | 0.233 | 0.200 | 0.966 |
| Pb | 0.317 | 0.645 | 1.422 |
| Zn | 0.168 | 0.288 | 0.338 |

The other metals assessed in this study i.e., copper and zinc were within the WHO permissible limit in both water and sediment samples. The increased Pb and Cd levels in water sample may be due to human activities which take place at river banks with run-offs containing these heavy metals (Jabeen *et al*., 2018). Furthermore, the high Pb and Cd levels could also be as a result of the use of fertilizers and pesticides as well as washing of clothes with soaps and detergents by both farmers and villagers at the Kwalkwalawa river bank. Cadmium followed by lead has been reported to be the most toxic of all heavy metals with zinc having the lowest toxicity (Huseen and Mohammed, 2019). Exposure to very minute concentrations of heavy metals results in adverse changes in the different fish tissues (Tabrez *et al*., 2021). Heavy metals in water are deleterious to fish, then periodic monitoring of heavy metals in water and aquatic life is very important. Unfortunately, year in year out, the activities around the river banks coupled with run-offs from flooding makes heavy metals to be ranked as the major pollutant of water bodies (Jabeen *et al*., 2018). The heavy metal accumulation (cadmium and lead) is above WHO/FAO and DWAF permissible limits, for both domestic and agricultural use, hence it can be said that fish from the Kwalkwalawa river may constitute human health hazards. Analysis of enzymes from the blood is an important part of toxicity testing. Heavy metals are known to cause changes in enzyme activities, which directly or indirectly affect the overall health of the organism. The activities of serum ALP, ALT, AST and creatinine kinase of cat fish used for this study may be attributed to heavy metal bioaccumulation in cat fish (Khan *et al*., 2020). Serum liver enzymes like ALT, AST and ALP are enzymes used to assess liver damage in fish (Khan *et al*., 2020). Following this damage, these enzymes leak into the circulation hence their elevated values. Prolonged exposure to heavy metals even at low concentrations could cause leakage of these enzymes (Tabrez *et al*., 2021). It has been reported that heavy metal accumulation results in high ALT, AST and ALP concentrations in *Mystus species* and *Oreochromis niloticus* in rivers with high heavy metal pollution (Khan *et al*., 2020; Tabrez *et al*., 2021). The values of the serum tranaminases in this study for cat fish could be attributed to stress on the liver from heavy metal intoxication (Khan *et al*., 2020). Although, there are other stressors like parasite burden which was not explored in this study. Elevated creatinine enzymes in fish are indicators of kidney damage which may be as a result heavy metal bioaccumulation (Tabrez *et al*., 2021). Information from the analysis of a blood sample is very important for the detection of changes following exposure to any toxicant (Ghadhave *et al*., 2014). The mean PCV of cat fish (*Clarias gariepinus*) from Kwalkwalawa river was 25.00± 11.4 % which falls below the normal range of PCV for cat fish (Akinrotimi *et al*., 2011). However, the mean PCV of cat fish from the present study was at the lower limit which may be attributed to effect of heavy metal concentration in the fish. The RBC and Hb of cat fish from Kwalkwalawa river in this study were lower than the reference values for cat fish as reported by (Adeyemo *et al*., 2014; Okorie-Kanu and Unakalamba, 2015). This agrees with the findings of Khan *et al*. (2020) and Tabrez *et al*. (2021) where heavy metals accumulation in fish caused significant decreases in Hb, PCV and RBC.

White blood cell (WBC) counts are useful information to assess stress in fish. Basophils and Band neutrophils were not detected in the blood samples of cat fish in this study which is in agreement with a study by Tabrez *et al*. (2021). The WBC was below the reference value for cat fish (28.33±1.64 $10^3/\mu L$) reported by Akinrotimi *et al*., (2011). It has been established that exposure to heavy metals like cadmium decreases WBC viability and consequently cell death (Witeska and Wakulska, 2007).

The concentration of chromium, copper and zinc in the liver, kidney and gills was within permissible limits in this study for fishes and water and this may be attributed to increase amount and high flow of water, which allowed for movement of heavy metals at the time of collection of the fish samples at middle of the rainy season. This result is similar to the observation of Olayinka-Olagunju *et al*. (2021) where there was low concentration of heavy metals in fish collected between April to October, 2019 in south west Nigeria.

Cd and Pb were above permissible limits implying that they bio accumulate in fish. These metals are highly toxic and can be and stored in the fish from the water and food chain (Weber *et al*., 2013). Bio concentration factor (BCF) is the heavy metal concentration in fish compared to water where it is found (Bawuro *et al*., 2018)). It is expressed mathematically as the ratio of the mean heavy metal concentration in a selected fish tissue (mg $kg^{-1}$) wet weight to the heavy metal concentration in water ($mgL^{-1}$) (Olayinka-Olagunju *et al*., 2021). Factors which determine BCF include: the level of heavy metal pollution in the water, type of heavy metal and the degree of metabolism of the heavy metal in selected fish tissue (Uysal *et al*., 2009).

Based on the BCF result from the present study, gills have higher affinity for the bio concentration of chromium, cadmium, lead, and zinc than kidney and liver. This may be because gills are a medium of heavy metal ion exchange which enables it interact with heavy metals in the river due to their large surface area (Bawuro *et al*., 2018). These therefore suggest that metals bio accumulated in gills are basically concentrated from water. Liver and kidney are the dominant organs for the bioconcentration of copper in catfish from the present study and this agrees with the findings of Subotic *et al*. (2013).

## CONCLUSION

Observations from this study revealed that bioconcentrarion of heavy metals in cat fish varies between tissues, Gills has had higher affinity for chromium, cadmium, lead and zinc than liver and kidneys with the exception of copper. The presence of heavy metals above the recommended limits in water for agricultural and domestic use may not necessarily result in outright mortality of the fish but may cause haematological and biochemical changes. The presence of this metals constitutes environmental health hazard for humans who consume the fish.


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