Clarias gariepinus (African Catfish) Liver and Gills Susceptible to Heavy Metal Toxicity in Fish Farms Harvested in Benin Metropolis, Nigeria

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Abstract

Heavy metal toxicity is increasing in fish organs, particularly in the gills and livers due to their susceptibility over time. The gill is the respiratory organ of the fish, while, the liver conducts metabolic activities including detoxification. Heavy metals (cadmium, Chromium, zinc, lead and copper) enter water bodies through natural and human activities and bioaccumulate in these organs after absorption. *Clarias gariepinus (*African catfish) has been widely consumed in Nigeria with no clear cut on bioaccumulated heavy metal's impact on consumers' health. This study, therefore, examines heavy metal toxicity in the liver and gill and histopathological changes in *C. gariepinus.* The levels of Cu, Cr, Cd, Pb, & Zn in homogenized fish organs were measured by atomic absorption spectrophotometry, while histopathology was examined after processing tissues histologically via microscopy. Data were analyzed using ANOVA and independent samples t-test. Results showed that the concentration of Cd and Pb were slightly higher in gills and liver but in a tolerable range compared to Cu, Zn and Cr, respectively. Though Cu recorded the highest concentrations in fish tissue (33.48 and 28.78mg/kg) but was below the expected daily nutritional requirement (120mg/kg) for humans and posed no toxicological risk. Liver section - L1 showed regular branching cords of hepatocytes whereas L2 and L3 showed the presence of necrosis of the hepatocytes, and L4 revealed destruction of the hepatocytes. On the other hand, sections of the gills revealed normal fusion of lamellae (G1), total destruction of the gills (G2), and mild and total desquamation of the gills (G3) and (G4) respectively. Under the prevailing circumstances, we conclude that the number of heavy metals in fish's liver and gill in this study is minimal to cause harm to consumers. However, further research may be needed molecularly to complement this and possible molecular concerns.

Keywords: Heavy metal, Bioaccumulation, Copper, Cadmium, Fish Organs

Introduction

Though essential to maintaining biochemical and physiological functions in living organisms, metals become noxious when the threshold concentrations are exceeded (Odigie and Adejumo, 2018). Its toxicity depends on the absorbed dose, the route and the duration of exposure, which can lead to various disorders and result in excessive damage due to oxidative stress induced by free radicals (Warraich *et al*., 2020). Heavy metal toxicity has proven to be a significant threat with several associated health risks (Engwa *et al*., 2019). These metals enter the surroundings by natural means and through human activities, and after consumption in higher concentration, may pose health dangers in the system (Rahman et al., 2016: Odigie and Adejumo 2018). On the other hand, fish and its products are consumed as food across the globe with other sea foods. It provides the world's prime source of high-quality proteins; 14-16% of animal protein is consumed worldwide (Sall *et al*., 2020). Consumption of contaminated fish may predispose to many diseases and cancer (Valerio et al., 2016). However, fish gill and liver remain the most susceptible organs to toxic metals because gill serves as respiratory organ in direct contact with water, having an unhindered relationship with metals interacting with the ecosystem (Legorburu *et al.*, 2015). The liver is the main metabolic organ where detoxification occurs and is thus susceptible to damage by metallic toxicants. Therefore, inspecting fish and their ponds is a practical step to ensure that fish and their products do not harm unsuspecting consumers (Mejiberg *et al*., 2016). From the foregoing, there is limited information in support of the safety of consumable fish and its bye products in this locality as far as we know. For example, African catfish (*C. gariepinus)* is often consumed in Benin metropolis with no clear cut on possible health implications to consumers. It is with the understanding that this study may bring about robust policy on fish farming and guide towards wholesome fish produce; hence, we examined the liver and gills of *C. gariepinus* susceptible to heavy metal intoxication from varying fish farms in Benin metropolis. We also investigated the level of heavy metals toxicity in liver and gill of the fish and compared it to standards established by WHO, FAO and related agencies.

Materials and Methods

Harvesting, Collection and Preparation of Sample

Adult catfish was obtained from various fish farms in Ovia north east local government area, Egor local government area and Oredo local government area, all in Edo state and weighed using a digital electronic balance (Gilbertini, Italy; sensitivity = 0.001g). The fish was incised longitudinally and the internal organ carefully removed in search for the liver. The operculum in the head was opened and the gills harvested. The liver and gills were rinsed carefully in normal saline and placed in 10% formalin preservative. This procedure was repeated for all fish obtained from varying fish farms.

Sample Processing

All collected samples were placed in a labeled container to which 10% buffered neutral formalin has been added for at least 6 hours and 72 hours to enable accurate results from histochemical techniques.

Grossing and Histology

The liver and gills were carefully observed for physical features like colour and appearance; length and breadth were measured and recorded. A part of the organ was excised, placed in a tissue cassette, and labeled appropriately. Tissue processing was done by taking tissues through: dehydration, clearing and infiltration. Afterward, tissues were orientated in a tissue mould and embedded with molten paraffin wax. Tissue blocks were then trimmed at zero microns and sectioned at 3μm using a rotary microtome. Sections were stained with haematoxylin and eosin techniques to demonstrate general tissue structure. Slides were mounted and observed through the microscope with ×10 and ×40 objectives.

Tissue Homogenization

Tissue was cut into small pieces with a knife and Teflon cutting board. A blending container was packed with dry ice i.e the area beneath the blade in particular. The pieces of tissue were placed on top of the ice in the blending container and more dry ice added as tissues were allowed to freeze for 30 seconds. The blending container was covered with Teflon linen and the tissue-dry ice mixture was blended at a high speed until a homogenized consistency is obtained. The tissue homogenate was transferred into a glass jar and stored below 1000C overnight. The lid of the glass jar was loosely attached to allow CO_2 sublimation. The homogenate container lid was then tightened the following day and stored in a freezer below 1000C.

Determination of Heavy Metals Concentration

A working and intermediate standard solution containing 1000 mg L-1 was prepared. An appropriate wavelength and slit width was selected accordingly. Analysis was done for heavy and toxic metals in the homogenates by applying calibration curves after the parameters (lamp alignment, wavelength and slit width adjustment and burner alignment) were optimized for maximum signal intensity and sensitivity of the instrument. Finally, the reading was recorded and the concentrations were determined.

Statistical Analysis

Statistical analysis was done by ANOVA and independent samples t-test (P value < 0.05 was considered statistically significant) using SPSS (version 20) statistical software.

Results

Histopathology (Plate 1) revealed that the liver tissue section; L1 showed normal branching cords of hepatocytes, whereas Slide L2 and L3 showed the presence of necrosis of the hepatocytes, while Slide L4 showed vast destruction of the hepatocytes. On the other hand, sections of the gills showed: normal fusion of lamellae (G1), total destruction of the gills (G2), mild and total desquamation of the gills (G3) and (G4) respectively. Meanwhile, Figure 1 showed a graphical representation of heavy metal concentration in gills and liver of fishes obtained from varying locations in Benin City. Comparison of the measured variables (in mg/kg) between the liver and gills and their respective controls in fishes suggested that the concentrations showed a statistically significant difference ($p \leq 0.05$); Cu was significantly higher in the liver than in the gills statistically, Zn was significantly lower in the liver than in the gills, Cr was significantly lower in the liver than in gills, Cd was significantly higher in the liver than in the gills. At the same time, Pb was significantly higher in the liver and the gills (Table 1).

Table 1: Comparison of measured variables of the concentration of heavy metals between the gills and liver of fish

HEAVY METALS	Organ		P Value
	Liver	Gills	
Cu	33.48 ±0.93^a	28.78 ±1.34^b	0.006
Zn	6.70 ±0.24^a	15.55 ±0.44^b	0.000
Cr	2.39 ±0.07^a	3.08 ±0.09^b	0.000
Cd	0.24 ±0.01^a	0.10 ±0.03^b	0.000
Pb	0.43 ±0.02^a	0.26 ±0.01^b	0.000

Note: Values in the same row and sub-table not sharing the same subscript are significantly different at p< .05 in the two-sided equality test for column means (Values are Mean ± SEM; P≤0.05).

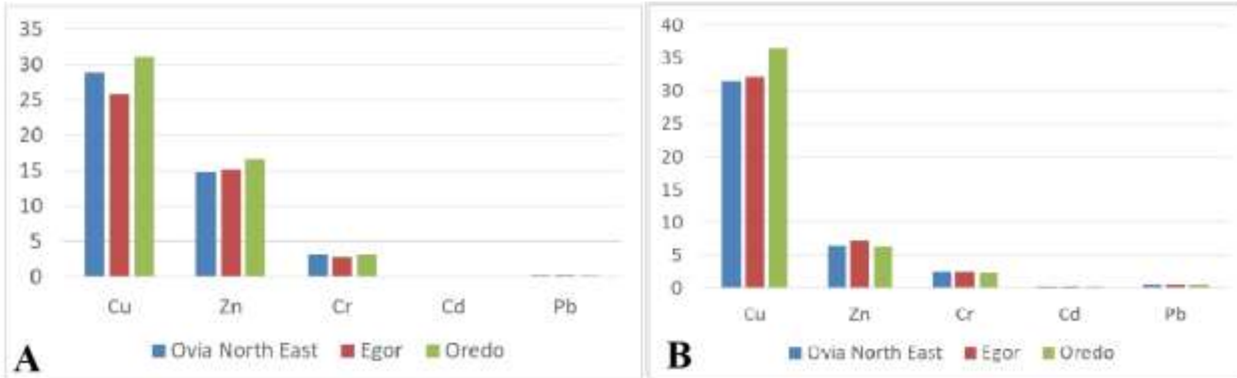

Figure 1: showed a graphical representation of heavy metal concentration in gills and liver of fishes obtained from varying locations in Benin metropolis, Nigeria.

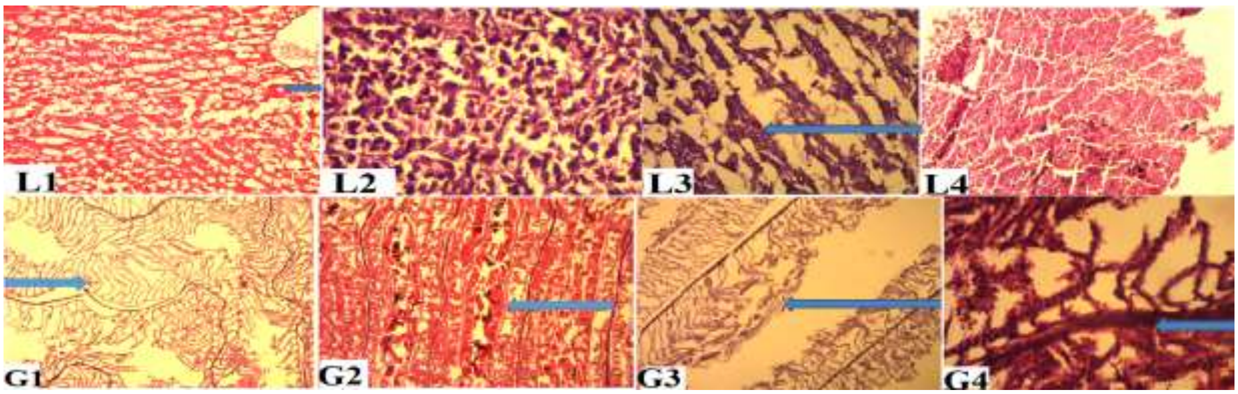

Plate 1: showed pathological appearances in liver and gills of fishes. L1 - normal branching cords of hepatocytes; L2 and L3 - necrosis of the hepatocytes; L4 - destruction of the hepatocytes; G1 -normal fusion of lamellae; G2 - destruction of the gills; G3 and G4 - mild and total desquamation of the gills (H&E at x400 magnifications).

Discussion

Fish are considered biomonitors of aquatic ecosystems for estimating heavy metals pollution. Different organs of fish are known to accumulate varied concentrations of heavy metals when exposed (Mehana *et al.,* 2020). In this study, the concentration of copper in the liver and gills were in decreasing order of liver>gills. The low concentration of Cu observed in the fish species indicated that food is the primary pathway for the uptake of copper, and the fish feeds contain lesser amounts of copper. The mean concentration of Cu recorded in this study is 33.48mg/kg for liver and 28.78mg/kg, which is much lower than the maximum allowable limit of 120 mg/kg in diet. It is also observed that Oredo local government area fish specimens had higher concentrations of Cu (36.56mg/kg) and (31.11mg/kg) in gills and liver compared to Ovia northeast and Egor local government area, respectively. Most of the fish ponds in Oredo as gathered from the fish farmers still use more formulated feeds containing more copper than commercial feeds used in Ovia north east and Egor (Irungu *et al.,* 2018). In the present study, Zinc (Zn) is the only metal readily available in the organs of all examined fish species. The highest concentration of zinc (17.89mg/kg) is recorded in gills (G4) from Oredo. Low concentration of Zn in the liver of the fish species investigated has been linked to lower levels of binding proteins (metallothioneins) in the liver (Moselhy *et al.,* 2014). The mean concentration of Zn, ranging from 6.70 mg/kg in the liver to 15.55 mg/ kg in the gills of fish, was lower compared to the maximum allowable limit of 50 mg/kg in dietary meal. In addition, Chromium (Cr) concentration is significant ($p<0.05$) in the liver and gills of the fish species in the present study. The distribution pattern is in the decreasing order of (gill > liver). In other words, gill is the organ which accumulates the highest concentration of Cr compared to the liver. The highest mean value of Cr (3.86 mg/kg) is recorded in the fish gill from Ovia North east local government area. The concentrations of Cr recorded in the present study are lower than the maximum allowable limit of 8 mg/kg in food. Cadmium (Cd) concentrations in fish organs showed a significant difference ($p<0.05$). The distribution pattern is also in decreasing order of (liver > gills). The highest (0.287 mg/kg) and lowest (0.038 mg/kg) level of Cd is found in the liver and gills respectively. Cadmium concentration in the liver of the examined fishes showed little disparity amongst the three local government areas. The concentration of Cd in the gills showed comparable values in all of the fish organs examined. This is in agreement with the work of Rahimzadreh *et al.,* in which it was reported that Cd is stored in the body in various tissues, including bone and exoskeleton (Rahimzadreh *et al.,* 2017). The acute toxicity of Cd to aquatic organisms is variable, even between closely related species, and is related to the free ionic concentration of the metals. Cadmium interacts with calcium metabolism in fish to cause abnormally low calcium levels (hypocalcaemia), probably by inhibiting calcium uptake from the water (Islam et al., 2010). However, high calcium concentration in water protects fish from Cd uptake by competing at the uptake site. The maximum amount of Cd in fish for human consumption specified by the WHO is 0.05 mg/kg. Therefore, the mean concentration of Cd (0.10mg/kg for gills and 0.24 mg/kg for liver) in this study is higher than the maximum allowable limits by the world health organization. In addition, Lead (Pb) in the present study showed a higher liver concentration than in gills. The highest concentration (0.542 mg/kg) is recorded in the liver (Plate 1: Slide L3) from Oredo local government area. The concentration of Pb in the liver ranged between 0.239 mg/kg to 0.512 mg/kg, with an average value of 0.43mg/kg. Gills showed a similar concentration range of between 0.143 mg/kg to 0.290 mg/kg with an average value of 0.26mg/kg. Lead is a neurotoxin that causes behavioural deficits in vertebrates and can cause a decrease in survival, growth rate, learning and metabolisms (Wani et al., 2015). However, juvenile fish is more susceptible to lead than adults or eggs (Mohammed, 2013). The mean concentration of Pb recorded in fish species is higher than the permissible limit of 0.2 mg/kg in fish.

On the other hand, pathological changes in the liver sections L3 and L4) showed necrosis of hepatocytes, a pathologic response of the fish liver to toxins. Recall that fish liver has the function of eliminating toxins. From the present results, liver sections have a high concentration of lead which has been proven to cause severe poisoning. Toxicity to the liver by heavy metals is both concentration and period of exposure dependent, mainly because the liver is the primary target organ for elimination and metabolic balance. Structural changes less severe than those found in the gills suggested that the liver has a higher capacity and adaptability to counteract the metal-induced damage based on its stronger antioxidant system, thus preventing injuries induced by these toxins. The increased bioaccumulation of Zn in the gills of all analyzed species could be correlated with the observed desquamation of lamellae in sections G3 and G4, which is in tandem with the report of Albu *et al.* (2021).

Conclusion and Recommendation

Copper and Chromium are significantly high in the liver of fishes investigated in this study than in other organs. At the same time, pathology suggests necrosis and obliteration of hepatocytes in the liver and total desquamation of the gills. Despite the varying degrees of toxicological and pathological concerns in fish organs investigated for heavy metal toxicity in this study, the amount of intoxication is loudly insignificant to raise concerns. Therefore, fish farmers should always carry out water quality tests and ensure the water source does not cause heavy metal intolerance to fish organs. In addition, fish feeds formulators should ensure the concentration of heavy metals in feeds is within the normal limits.

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