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HEAVY METALS IN THE MYONEMATIC, HEPATIC AND RENAL TISSUES OF THE AFRICAN CAT FISH (*Clarias gariepinus*) FROM OGBA RIVER, BENIN CITY, NIGERIA.

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ABSTRACT

The concentrations of heavy metals (Pb, Cd, Zn, Cu, Cr and As) in the myonematic, hepatic and renal tissues of the African catfish (*Clarias gariepinus*) from Ogba river, Benin City, Nigeria, were determined using a Unicam 929 series atomic absorption spectrophotometer. The concentrations of the aforementioned metals were also determined in water. The mean concentration of Pb ranged from 0.19mg/kg (muscle) to 0.39mg/kg (liver) while the mean concentration of Cd ranged from 0.03mg/kg (muscle) to 0.09mg/kg (liver). The mean concentration of Zn ranged from 0.09mg/kg(muscle) to 0.33mg/kg (liver) while the mean concentration of Cu ranged from 0.23mg/kg (muscle) to 0.76mg/kg (liver). The mean concentration of Cr ranged from 0.19mg/kg (muscle) to 0.41mg/kg (liver) while the mean concentration of As ranged from 0.36mg/kg (kidney) to 0.54mg/kg (muscle). The mean concentrations of the heavy metals in water were Pb(0.09mg/l), Cd(0.04mg/l), Zn(0.01mg/l), Cu(0.53mg/l), Cr (0.38mg/l) and As(0.59mg/l). With the exception of Cu, the mean concentrations of the heavy metals in water were generally above the World Health Organization (WHO) maximum allowable limit for the respective metals in drinking water implying that water from the river is unfit for human consumption owing to heavy metal contamination. The mean concentrations of Cr and As exceeded the WHO maximum allowable limits for fish food. The direct implication of this finding is that people who consume fish from the river are liable to potential health hazards resulting from Cr and As contamination.

Key words: Heavy metals, *Clarias gariepinus,* Ogba river, Benin City, Nigeria

INTRODUCTION

The role of heavy metals in aquatic environments is increasingly becoming an issue of global concern at private and governmental levels with heavy metals biomagnifying and increasing in toxicity (Fonge *et al.,* 2011). Heavy metals are natural trace components of the aquatic environment, but background levels in the environment can be elevated especially in areas where industrial, agricultural and mining activities are widespread (Bryan and Langston, 1992). Most heavy metals released into the environment find their way into the aquatic environment as a result of direct input, atmospheric deposition and erosion due to rainwater. Aquatic animals may therefore become exposed to elevated levels of heavy metals due to their wide use for anthropogenic purposes (Mustafa, 2000).

In the aquatic environment, heavy metals in dissolved form are easily taken up by aquatic organisms, including fish, where they are strongly bound with sulfhydril groups of proteins and accumulate in their tissues (Shah, 2005). The accumulation of heavy metals in the tissues of organisms can result in chronic illness, mortality and potential decimation of populations (Senarathne and Pathiratne, 2007). Heavy metal exposure is known to induce changes in blood pH, hematocrit, red blood cell count and haemoglobin concentrations in fish through the disintegration of erythrocytes and damage of the hemopoietic system (Monteiro *et al.,* 2002). Urban contamination remains a problem in developing countries as many industries and residences have continued to contribute in a number of ways into the introduction of toxic materials to the environment (United States Department of Agriculture, USDA, 2001). Allochthonous and autochthonous influences can make the concentrations of heavy metals in the aquatic environment high enough to be of ecological concern (Oyewo and Don-Pedro, 2003).

In the aquatic environment, toxic metals are potentially accumulated in finfish and shellfish and subsequently transferred to man through the food chain. Aquatic products including fish, need to be carefully screened to ensure that high levels of heavy metals are not being transferred to man through ingestion (Ekeanyanwu *et al*., 2011). Nigeria is a large fish consuming nation with a total consumption of more than 1.2 million metric tons per year (Abdullah, 2011). It is against this backdrop that ecotoxicological studies such as the present study gains significance.

The Ogba river serves as a sink for Urban drainage and agro-industrial effluents in Benin city, Nigeria. It is also important in fisheries production as a large number of fishermen settlements along the river axis depend on it for their fish and other domestic uses. The river is also the source of potable water for urban Benin City as well as the government owned fish farm (Obasohan *et al.*, 2006). *Clarias gariepinus* is the most important Clarid species used in African aquaculture because of its fast growth, hardness and attainment of large size as adults (Fagbenro *et al*., 2000). It is a fish species of commercial importance in Benin City, valued for its tasty flesh. It is commonly found in swamps, streams, rivers and lakes. Fish are relatively sensitive to changes in their environment and have a relatively long lifespan. These animals can therefore give an indication of the effect of both short and long-term exposure periods to environmental pollution and toxicants.

This study was specially designed to determine the concentrations of Pb, Cd, Zn, Cu, Cr and As in the myonematic, hepatic and renal tissues of *Clarias gariepinus,* since it has been reported that these fish organs readily accumulate heavy metals owing to their physiological and metabolic roles (Allen, 1995). The study became imperative as the river is a sink for both organic and inorganic pollutants owing principally to the direct inflow of the Benin City master drainage system. Data generated from this study will help to update the polluted status of the Ogba river and will contribute towards protecting the health of man who may ultimately consume contaminated fish with resulting unwholesome health consequences.

MATERIALS AND METHODS

Study area:

Ogba river is one of the major sources of domestic water supply in Benin City, Nigeria and lies within Latitude 6.5° N and Longitude 5.8 °E (Oronsaye *et al*., 2011). The climate of the area is typical of the tropical rain forest belt with wet (April-October) and dry (November-March) seasons. Rainfall is bi-modal, usually peaking in July and September with a brief drop in August. Annual temperature ranges between 23 and 34° C while annual humidity is between 68 and 96%. The marginal vegetation in the area includes *Commelina, Ipomea, Emilia* and *Sonchifolia* species while the dominant macrophytes are *Azolla* and *Ceratophyllum* species. The sampling zone is a stretch of about 8 km along the river. The first sampling station (Drainage station) is located at the point of entry of effluents into the river through a master drainage channel, about 3.5 km from the river source. Further downstream are the Zoo and Bridge stations located about 4 km apart. The Zoo station receives effluents from the Benin City Zoological garden while at the Bridge station activities such as car washing bathing, fishing swimming, carpet washing and laundry of household clothes are carried out.

Collection of fish and water samples:

Collection of fish and water samples were done in July, September, November and December 2011.The wet months were July and September while the dry months were November and December. Fish samples were caught using baited hooks, traps and gill nets. The fishes were washed in flowing river water in order to remove adhering debris. They were transported to the Faculty of Agriculture main laboratory of the University of Benin, in an ice box within 24hours in order to preserve their integrity. In the laboratory they were stored frozen at -5^{0}C in a deep freezer prior to preparation and analysis. Water samples were collected at approximately 30 cm depth in 1 litre capacity polythene bottles with screw caps. The samples were acidified with 5 ml of concentrated nitric acid and stored frozen at -5^{0}C.

Digestion and analysis of samples:

Frozen fish samples were thawed at room temperature (27 ± 2^{0}C). The fish samples after defrosting were dissected with a stainless steel knife to obtain the muscle, liver and kidney. The extracted organs were oven-dried at 105 ± 10 ^{0}C to constant weight in a Gallenkamp hotbox oven. One gram of each sample was digested using 1:5:1 mixture of 70% perchloric acid, concentrated nitric acid and concentrated sulphuric acid at 80 ± 5^{0}C in a fume chamber, until a colourless liquid was obtained (Streedevi *et al*., 1992). Each digested sample was made up to 20ml with de-ionized water. Blank samples were handled as detailed for the samples. Water samples were digested using the pre-concentrated nitric acid method (Parker, 1972). Fifty (50) ml of water sample was measured into a 250 ml conical flask. 20ml of concentrated nitric acid was added and the mixture was heated over medium flame under a hood till the solution reduced to 10 ml. The digest was then transferred into 250 ml volumetric flask and made up to mark with distilled water. Blank samples were prepared using the same quantity of nitric acid. Heavy metal concentrations in the digested samples were eventually determined using a UNICAM 929 series atomic absorption spectrophotometer with solar software at Martlet laboratory, Benin City. The analytical procedure was checked using reference material (DORM 1, Institute of Environmental Chemistry, NRC, Canada). The concentrations of Pb, Cd, Zn, Cu, Cr and As were expressed in mg/kg and mg/l for fish organs and water respectively.

Computation of Bioaccumulation Quotient (BQ) for heavy metals:

The BQ for heavy metals in fish was computed in accordance with the method by Mazon and Fernandes (1999), as follows:

$$BQ = \frac{\text{Concentration of heavy metal in fish (mg/kg)}}{\text{Concentration of heavy metal in water (mg/l)}}$$

Statistically, all data were presented as means of duplicate determinations.

RESULTS

The heavy metal concentrations in the investigated matrices varied. As shown in Table 1, the mean concentration profile of the investigated metals in water in descending order was As>Cu>Cr>Pb>Cd>Zn. The mean concentrations of Cd and As followed the pattern in descending order; Drainage station> Zoo station > Bridge station while the mean concentration of Pb followed the pattern in descending order; Zoo station> Bridge station > Drainage station. The mean concentration of Cr followed the pattern in descending order; Bridge station > Zoo station > Bridge station. For Zn, the mean concentration of the metal was higher at the Drainage station while the same mean concentration of the metal was recorded at the Zoo and Bridge stations

Table 1: Mean heavy metals concentration (mg/l) in water from Ogba river

Metal	Drainage station	Zoo station	Bridge station	Mean concentration
Pb	0.01	0.23	0.04	0.09
Cd	0.05	0.04	0.03	0.04
Zn	0.02	0.01	0.01	0.01
Cu	0.66	0.38	0.56	0.53
Cr	0.54	0.32	0.28	0.38
As	0.72	0.64	0.42	0.59

As shown in Table 2, the mean concentrations of heavy metals in fish muscle at the Drainage station ranged from 0.01 mg/kg for Zn to 0.72 mg/kg for Cu while in the case of the liver, a range of 0.09 mg/kg for Cd to 0.95 mg/kg for Cu was observed. In the kidney, the mean concentrations of heavy metals ranged from 0.03 mg/kg for Cd to 0.44 mg/kg for As. As shown in Table 3, the mean concentrations of heavy metals in fish muscle at the Zoo station ranged from 0.01 mg/kg for Cd to 0.65 mg/kg for Cu while in the case of the liver, a range of 0.08 mg/kg for Cd to 0.70 mg/kg for Cu was observed. In the kidney, the mean concentrations of heavy metals ranged from 0.11 mg/kg for Cd to 0.27 mg/kg for As. As shown in Table 4, the mean concentrations of heavy metals in fish muscle at the Bridge station ranged from 0.02 mg/kg for Cd to 0.53 mg/kg for As while in the case of the liver, a range of 0.09 mg/kg for Cd to 0.62 mg/kg for Cu and Zn was observed. In the kidney, the mean concentrations of heavy metals ranged from 0.06 mg/kg for Cd to 0.72 mg/kg for Pb.

Table 2: Mean heavy metals concentration (mg/kg) in fish organs from the Drainage station

Organ	Pb	Cd	Zn	Cu	Cr	As
Muscle	0.02	0.06	0.01	0.72	0.25	0.65
Liver	0.24	0.09	0.15	0.95	0.19	0.51
Kidney	0.16	0.03	0.16	0.43	0.38	0.44

Table 3: Mean heavy metals concentration (mg/kg) in fish organs from the Zoo station

Organ	Pb	Cd	Zn	Cu	Cr	As
Muscle	0.15	0.01	0.13	0.65	0.18	0.43
Liver	0.37	0.08	0.23	0.70	0.46	0.39
Kidney	0.12	0.11	0.15	0.23	0.14	0.27

Table 4: Mean heavy metal concentrations (mg/kg) in fish organs from the Bridge station

Organ	Pb	Cd	Zn	Cu	Cr	As
Muscle	0.41	0.02	0.14	0.11	0.15	0.53
Liver	0.56	0.09	0.62	0.62	0.57	0.43
Kidney	0.72	0.06	0.15	0.16	0.16	0.38

Table 5: Mean heavy metal concentrations (mg/kg) fish organs for the study period.

Organ	Pb	Cd	Zn	Cu	Cr	As
Muscle	0.19	0.03	0.09	0.23	0.19	0.54
Liver	0.39	0.09	0.33	0.76	0.41	0.44
Kidney	0.33	0.07	0.15	0.27	0.23	0.36

As shown in Table 5, the mean concentrations of the heavy metals were highest in the liver with the exception of As while the mean concentrations of the metals were lowest in the muscle with the exception of As. As shown in Table 6, the BQ values for heavy metals revealed that Pb and Zn were bioaccumulated by all the investigated fish organs with the exception of As.

Table 6: The mean Bioaccumulation Quotient (BQ) of heavy metals in fish organs

Organ	Pb	Cd	Zn	Cu	Cr	As
Muscle	2.11	0.75	9.00	0.43	0.50	0.91
Liver	4.33	2.25	3.30	1.43	1.08	0.75
Kidney	3.67	1.75	1.50	0.51	0.60	0.61

Table 7: Comparison of observed heavy metal concentrations with recommended water quality standards for aquatic life in fresh water

S/N	Heavy Metal	Heavy Metal Concentrations in Water for this Study	*Recommended Water Quality Standard for Aquatic Life
1	Pb(mg/l)	0.09	0.03
2	Cd(mg/l)	0.04	0.004
3	Zn(mg/l)	0.01	0.05
4	Cu(mg/l)	0.53	0.1
5	Cr(mg/l)	0.38	0.005
6	Ni(mg/l)	N/D	0.02

*(Roberts, 1978) N/D= Not determined

DISCUSSION

Heavy metals have been used as indices of pollution because of their high toxicity to human and aquatic life (Omoigberale and Ogbeibu, 2005). The high concentrations of heavy metals in aquatic ecosystems have been linked with effluents from industries, sewage and refuse (Wogu and Okaka, 2011). In this study, the mean concentrations of the heavy metals in water were generally higher at the drainage station. This finding is not surprising considering the fact that the station is situated next to the entry point of mixed effluents from Benin City via the master drainage system. The mean concentrations of the heavy metals in water in this study generally exceeded the recommended water quality standards for aquatic life in freshwater, as shown in Table 7. From these finding, the state of health and survival of fish and other aquatic organisms in the Ogba River is conceivably under threat as physiological activities and biochemical processes may be adversely affected as corroborated by Canli, (1996).

With the exception of Cu, the mean concentrations of the heavy metals in water were generally above the World Health Organization (WHO) maximum allowable limit for the respective metals in drinking water. The direct implication of this finding is that water from the river is unfit for human consumption owing to heavy metal contamination. In this study, the mean concentration of the investigated heavy metals were highest in the liver. This finding is in accord with Peakall and Burger, (2003), who reported that heavy metals readily accumulate in metabolic organs such as the liver that stores metals by producing metallothioneins, which appears as a metal detoxification mechanism within the body. Metallothioneins are a family of cysteine-rich protein which has been proposed as biomarkers to indicate the presence of high levels of metals in the environment (Fonge et al., 2011). In this study, the mean concentrations of the heavy metals were lowest in the muscles followed by the kidney. It has been reported that fish in an ecosystem contaminated by heavy metals are known to accumulate significantly more metals in edible muscle tissues than do fish in an uncontaminated ecosystem (Wangboje and Oronsaye, 2012).

The low mean concentrations of heavy metals in the kidney can be linked to its excretory function. The kidney performs an important function related to electrolyte and water balance and the maintenance of a stable internal environment. The kidney excretes nitrogen-containing waste products of metabolism rich as ammonia, urea and creatinine. Pb and Zn were bioaccumulated by all the investigated fish organs. Cd bioaccumulated in the liver and kidney while Cu and Cr bioaccumulated in the liver. Metal accumulation in fish tissues varies according to the rate of uptake, storage and elimination, meaning that metals which have high uptake and low elimination rates in fish tissue are expected to be accumulated to higher levels. Metal uptake has been reported to be dependent upon the exposure concentration and period as well as other factors such as salinity, temperature, interaction with other metals and the complexing agents (Allen, 1995).

The accumulation of non-essential heavy metals such as Pb and Cd may occur at very low environmental concentration because fishes may not be able to regulate their levels (Bryan and Langston, 1992). The accumulation of essential metals such as Zn, Cu and Cr is normally smaller than the accumulation of non-essential metals and can be significant only beyond a threshold metal concentration in the surrounding medium (Heath, 1987). The different accumulation levels of metals in different tissues may be primarily due to different metabolic activities. Tissues like the liver, kidney, gills and spleen are highly active in fish metabolism and therefore may accumulate metals to higher levels than other tissues. Several factors influence the elimination of heavy metals from the tissues of fish such as duration, temperature, interaction with other heavy metals and metabolic activity as well as the tissue concerned (Douben, 1989).

The elimination routes of metals from fish are generally bile, urine, elimination from the gills and mucus (Riisgard *et al*., 1985). In this study, Arsenic (As) was the only heavy metal that was not bioaccumulated by fish tissues, an indication that the fish was able to effectively eliminate the metal from its body. It has been reported that once metals have accumulated in tissues, it is often difficult to eliminate them from the body especially the non-essential metals (Mustafa, 2000). Regarding health risk to man, the mean concentrations of Pb, Cd, Zn and Cu in fish did not exceed the WHO maximum allowable limits for these metals in fish food.

However, the mean concentrations of Cr and As exceeded the WHO maximum allowable limits (for fish food) of 0.015mg/kg and 0.01mg/kg respectively. The direct implication of this finding is that people who consume fish from the river are liable to potential health hazards resulting from Cr and As contamination. Possible sources of Cr include automobile parts, refuse, fertilizers, metallurgical and textile industry effluents. Cr is an essential metal needed in the body of man where it helps the body use sugar, protein and fats. It can however exert toxic effects when in excess of what is required, leading to both hepatic and renal problems in humans. Cr and its compounds are known to cause cancer of the lungs, nasal cavity and paranasal sinus and it is suspected to cause cancer of the stomach and larynx (Agency for Toxic Substances and Disease Registry, ATSDR, 2000).

Arsenic can be sourced from pigments, paints, alloys, pesticides, fertilizers and wood processing factories. The metal has been described as a highly toxic metalloid element that can wreak havoc in the body of man through the impairment of several internal organs including the liver and kidney (Pizzoro et al., 2003). Sources of Pb include, acid batteries, pigments, solders, alloys, rust inhibitors, ammunition and fossil fuels. Pb toxicity in man leads to anaemia, damage of the kidney and liver,low intelligent quotient in children and high blood pressure in adults (Ottaway, 1978). Pb has also been reported to reduce spermatogenesis in man owing to its negative effects on reproductive hormones and the gonads (Ashiru, 2012). Cd can be sourced from electroplating industry effluents, pigments, paints, electric batteries, electronic components, and inorganic fertilizers produced from phosphate ores. Cd has a range of negative physiological effects on organisms such as decreased growth rates and negative effects on embryonic development. The metal, just like Pb and As is not desirable in biological systems. Zn can be sourced from alloys, batteries, pesticides, pigments, fertilizers and solders. Excess intake of the metal has led to vomiting, dehydration, abdominal pain, nausea, dizziness and lethargy (Akan *et al*., 2010). Geomorphologically, Cd and Zn occur together and when processed, Cd is yielded as a by-product of Zn with a ratio of 3 kg Cd per 1 ton Zn (Mance, 1987). This tends to lead to the release of Cd into the environment whenever Zn is released (Sanders, et al,. 1997).

CONCLUSION

The negative effects of anthropogenic impact on the Ogba river manifested itself via the contamination of water and fish (*Clarias gariepinus*) with heavy metals to levels sufficient to raise serious health concerns in man. The results from this study proved that the activities of people within the metropolis might have been responsible for the elevated concentrations of heavy metals in the investigated ecosystem. In order to mitigate further negative impacts on the Ogba river and its resources, regular monitoring of heavy metal levels in fish and water has therefore become imperative as a matter of urgency.

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