JASEM ISSN 1119-8362

Full-text Available Online at www.ajol.info and www.bioline.org.br/ja

Vol. 21 (3) 406-410

Evaluation of Flesh and Serum Concentrations of Al, Zn, Mn and Sb in African Cat Fish (*Clarias gariepinus)* Reared in Plastic Ponds in Benin City, Nigeria.

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ABSTRACT: Fishing in both natural and artificial habitats is a major occupation in Nigeria. Fishing in plastic ponds is one of such artificial habitats. This study seeks to determine and compare the bioaccumulation of selected toxic and essential trace metals-Aluminum, Antimony, Manganese and Zinc in body, serum and water habitat of *Clarias gariepinus* obtained from five different plastic fish ponds in Benin metropolis, using fishes from Ikpoba River as control. Catfish were obtained from five different farms and the controls were from Ikpoba River. The concentrations of Aluminum, Antimony, Manganese and Zinc were determined using inductively coupled plasma mass spectrophotometer. Results revealed that pond A Mn concentration (75.7 ± 1.2 mg/g),was lowest, and followed by pond B (96.8 ± 1.5). The level of Zn in body of pond A, B, C, D were all high ($p<0.001$). High level of Antimony was observed in pond A, B, C and D ranging from (0.019 ± 0.001 to 0.020 ± 0.0005mg/g). Zinc level was high in pond A, C, D, E ranging from (118.8±3.4 to 124.8±1.0 mg/g. Sb is slightly high in pond B, C, D with a range of (0.018±0.001 to 0.021±0.001) but was low in pond A (0.002±0.000) when compared with control (0.003±0.000). The above results were all statistically significant at ($p<0.001$).This study demonstrated an increase in the body and serum levels of toxic metals in *Clarias gariepinus* across the various ponds and the control river. The concentrations of aluminum, antimony, manganese and zinc in the body and serum of *Clarias gariepinus* were above the WHO recommended limit for fish and fish products. Hence, it is concluded that these metals have been bio-accumulated in the tissues and organs of these fish and may therefore pose an immediate threat on the health of consumers of fish and fish products from these various plastic ponds within Benin metropolis. ©JASEM

https://dx.doi.org/10.4314/jasem.v21i3.1

***Keywords*: ** Toxic, Trace, Metals, Cat-fish, Plastic ponds

Metals and metal compounds such as lead, cadmium, chromium, mercury, zinc, aluminum and antimony have been used for many years in the manufacture of plastic products as pigments, fillers, UV stabilizers, and flame retardants. The metallic compounds added to plastics although encapsulated in polymer matrix, are usually not chemically bound to polymer molecules and consequently can be gradually released into the environment over the service life of a plastic made object. Therefore, in time they may potentially dislodge from the plastics matrix into the environment when used as ponds (Chalmin *et al*., 2006). Heavy metals including both essential and non-essential elements have a particular significance in ecotoxicology, since they are highly persistent and all have the potential to be toxic to living organisms (Storelli *et al*., 2005). For the normal metabolism of the fish, the essential metals must be taken up from water, food or sediments (Vahid *et al*., 2014).

The global consumption of fish and derived fish products has generally increased during recent decades (Farombi *et al.,* 2007). Fish provide high quality animal protein, vitamins, minerals and omega-3 fatty acids which have been associated with health benefits due to their cardio-protective effects (Farombi *et al.,* 2007) and Gamal *et al.,* 2010). Anthropogenic activities continuously increase the amount of heavy metals in the environment, especially in aquatic ecosystems. Increase in population, urbanization, industrialization and agricultural practices as well as lack of environmental regulations have further aggravated the situation (Gupta *et al.,* 2009). As the metal levels increase in aquatic ecosystems, they raise the concern of metal

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bioaccumulation through the food chain and related human health hazards (Wright *et al*., 2002) and (Agah *et al* 2009). Heavy metal contamination in aquatic ecosystems can be expressed by its high concentration in water, as well as in sediments and aquatic organisms (Pham *et al.,* 2007). Fish assimilate metals by ingestion of particulate materials suspended in water, ingestion of food, ion exchange of dissolved metals across lipophilic membranes for example the gills, and adsorption on tissue and membrane surfaces (Alison *et al.,* 2009).

Fish accumulates in its body tissues and organs toxic chemicals such as heavy metals directly from water and diet, and contaminant residues may ultimately reach concentrations hundreds or thousands of times above those measured in the water, sediments and food through bioaccumulation and biomagnification as they are positioned in higher trophic level in food chain (Osman *et al*., 2007). Heavy metals may affect human health if the level of concentration consumed are high. Among various causes of fresh water and riverine pollution, heavy metals are of considerable importance (Sthanadar *et al*., 2013). The presence of toxic metals in environmental matrices is one of the major concerns of pollution control and environmental agencies in most parts of the world especially in Nigeria (Taylor *et al*., 2008). Fish accumulate toxic chemicals such as heavy metals directly from water and diet, and contaminant residues may ultimately reach concentrations hundreds or thousands of times above those measured in the water, sediments and food (Osman *et al*., 2007). The objective of this study therefore is to evaluate the flesh and serum concentrations of four metals (Al, Zn,Mn and Sb) in African cat fish (*Clarias gariepinus)*, reared in different plastic ponds in Benin City,Nigeria.

MATERIALS AND METHOD

Study area: This study was carried out in Benin City, Edo State Nigeria. *Sample Collection and Preparation:* Specimens of the popular species *Clarias gariepinus* locally bred catfish reared in plastic ponds were purchased from five different local farmers in Benin metropolis (Edo state) in Nigeria. These species were locally reared in plastic ponds and are largely consumed by all class of individuals both in Benin and awide. It serves as a very high source of protein; the fishes were purchased from five fish farms namely: A, B, C, D, E farm. *Clarias gariepinus* from these different fish farms go into most markets around Benin City. The fishes were wiped dry with a piece of clean towel to avoid haemolysis due to water. Under proper sterile condition, the fishes were sacrificed using ceramic knife and blood was obtained. The fish was cut into trunk and tail, collected into polyethylene resealable zipper plastic bag. 20mL of each pond water sample from which each fish was harvested and the water sample was collected with a chemically clean container and filtered using of a Whatman No.1 filter paper into a sterile universal container respectively. Each fish, blood and water sample were labeled appropriately and transported in cooler containing ice packs from the fish farm location to a refrigerator (water and blood samples) and a freezer (tissue sample) at -20°C prior to laboratory analysis. Upon arrival at laboratory, the samples were kept in refrigerator until further analysis.

Sample Analysis: Sample Digestion**:** The fish body was carefully oven dried at 105°C to a constant weight. The dried fish samples were each ground to powder using a clean ceramic mortar and pestle. The homogenized samples (1.0g each) were digested in a digestion flask in pent plicate (n=5) according to FAO/SIDA manual part 8 (1983).
Fish samples were dried at 105°C for 2 hours using drying oven.
1.0g of the sample was taken into digestion flask
10ml of $HClO_4$ (Perchloric acid) and 10ml of HNO_3 (Nitric acid) was added.
It was swirled gently and heated under the fume cupboard at an increasing temperature for 10minutes.
Brown fumes of HNO_3 escaped.
A golden yellow liquid was obtained after 3 hours.
The liquid was cooled, filtered and transferred into 50ml volumetric flask and made up to 100ml with 2% HNO_3 solution.

A bi-acid digestion procedure was used for the sample preparation of *Clarias gariepinus* body and blood.

Sample Preparation: The fish body was carefully oven dried at 105°C to a constant weight. The dried fish samples were each ground to powder using a clean ceramic mortar and pestle. The homogenized samples (1.0g each) were digested in a digestion flask in pent plicate (n=5) according to FAO/SIDA manual part 8 (1983).

*Procedure***:** The concentration of Zinc, Aluminum, Zinc, Manganese, and Antimony in the muscles and blood and water samples were determined by Inductively Coupled Plasma Mass Spectrometer (Agilent 7500, Norwalk, U.S.A by Fong *et al.,* 2007). The data were reported in microgram per decilitre (µg/dl). All analytical procedures were carried out at the analytical laboratory of the International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria.

Quality control: Certified reference materials (CRMs) from Le Centre de toxicology du Quebec, were analyzed.
Before being used, all volumetric polyethylene (including the auto sampler cups) and glass material were cleaned by soaking in 20% (v/v) HNO_3 for 24 hours. They were finally rinsed with several washes of Milli-Q® water and dried in a polypropylene container.

Data Analysis

Transfer Factor (TF)
The transfer factor in fish tissues from the aquatic ecosystem was calculated according to Anim-Gyampo *et al* .(2013):

$$TF = \frac{\text{Metal Concentration in tissue}}{\text{Metal concentration in water}}$$

RESULTS AND DISCUSSION

Table: 1 Levels of measured parameters in the flesh and serum of *Clarias gariepinus* from different Ponds with the control (river)

		PONDS					
	River (cont.)	**A (mg/g)**	**B (mg/g)**	**C (mg/g)**	**D (mg/g)**	**E (mg/g)**	**p**
Mn (f)	97.4±0.50bc	103.2±0.70b	96.8±1.50a	75.7±1.20c	104.1±1.60d	123.7±3.80bc	0.001
Mn (s)	74.3±6.62	8.6±0.11	6.1±0.10	11.1±0.20	8.3±0.30	10.7±0.20	0.05
Zn (f)	88.0±0.80[a]	191.7±1.90[c]	176.2±1.90[b]	97.4±1.10[c]	178.6±1.70[d]	189.0±1.11[a]	0.001
Zn (s)	89.9±0.80	103.8±0.91	82.3±3.40	118.8±3.0	100.7±1.2	124.8±1.01	0.001
Al (f)	0.040±0.01[a]	0.037±0.00[a]	0.060±0.00[b]	0.080±0.00[c]	0.055±0.00[b]	0.038±0.00[a]	0.001
Al (s)	0.043±0.00	0.030±0.00	0.043±0.00	0.027±0.00	0.051±0.00	0.022±0.00	0.05
Sb (f)	0.006±0.00[b]	0.019±0.00[c]	0.032±0.00[d]	0.042±0.00[c]	0.029±0.00[b]	0.020±0.00[a]	0.05
Sb (s)	0.003±0.001	0.002±0.00	0.013±0.00	0.010±0.00	0.021±0.00	0.018±0.00	0.05

*SEM**=** Standard error of mean. (f): flesh, (s): serum

Fishes are one of the main protein sources besides meat which comprise a lot of omega-3 fatty acids, amino acids, and vitamins which are important in maintaining human health and well-being (Akani *et al.,* 2010). The beneficial minerals and vitamins include omega-3 fatty acids that are good for our body which are eicosapentenoic acid (EPA) and docohexenoic acid (DHA), that are able to lower cholesterol level, lowering blood pressure and maintain the heart in good condition (Akani *et al*., 2010). Exposure and ingestion of heavy metals can cause a myriad of physiological and neurological problems in both plants and animals and, ultimately deleterious effects in man and other higher consumers. More so, exposure to toxic metals has been associated with reduced IQ, learning disabilities, slow growth, hyper-activity, antisocial behaviors and impaired hearing (Dahiya *et al*., 2005). In this present study, serum toxic metals levels were assayed in the body of *Clarias gariepinus* from different ponds and Control River.

The table shows heavy metals concentration (mg/g) in the flesh and serum of fish (*Clarias gariepinus*) species reared in plastic ponds in Benin City, Edo state, Nigeria. The results of this study indicated that there were higher concentration of Manganese (Mn) present in the flesh of *Clarias gariepinus* in the various plastic ponds and control than in the serum ($p<0.001$). Similar Mn range of 0.14 - 3.36mg/kg for body of fish was reported by Turkmen and Ciminli (2007). This is particularly important because the body of fish contribute the greatest mass of the fish that is consumed as food.

The Zn level was found to be higher in the flesh of *Clarias gariepinus* from pond A, B, D and E than in the serum both in the various ponds and control ($p<0.001$). Similar higher levels in African Catfish muscles have been recorded in River Nile, Egypt (Osman *et al.,* 2010).This is in contrast with the level of zinc gotten from Eleyele lake in Ibadan having Zn concentration of *Clarias gariepinus* to be (46.4 ± 0.03) (Aleloja *et al.,* 2014). The main source of Zn pollution in aquatic environment is from fertilizers, sewage sludge, industrial wastes and mining (Bradi, 2005).

Aluminum was significantly increased in ponds B and C compared with the control river ($p<0.001$). However, there was no significant increase in the levels of aluminum obtained from pond A and E compared with the control river ($p>0.05$).

Also there was a significant ($p<0.001$) increased flesh antimony level across the different ponds A, B, C, D and E compared with the serum in both the ponds and control river. This result correlates with report from previous study (Nzeve *et al*., 2014; Ijeoma *et al*., 2015).

The increase in toxic metal levels may be due to discharge of industrial, sewage and agricultural wastes into aquatic environment. Ijeoma *et al*. (2015), reported a significant increase in the level of toxic metals in different organs of *Clarias gariepinus* purchased at three different locations in Nigeria. Also, Nzeve *et al*., 2014 reported an increased in bioaccumulation of heavy metals in *Clarias gariepinus* and *Oreochromis spirulus niger* from Masinga Reservoir, Kenya.

This present study demonstrated increased serum levels of toxic metals across the various ponds compared with the control river. However the highest serum level of zinc was recorded in pond E. serum level of aluminum was significantly ($p<0.001$) increased in pond D compared with the control river. However data indicated a significant decreased in the serum aluminum level of pond A, C and E. The serum level of Antimony was observed to increased statistically ($p<0.001$) across the various ponds (B, C, D and E), compared with the control river. However the highest level of antimony was observed in pond E (0.018±0.001). This finding is in agreement with reports from previous studies (Eneji *et al*., 2011; Nzeve *et al*., 2014; Ijeoma *et al*., 2015). Fish can act as bio indicator in polluted water as it tend to bio-accumulate the heavy metals and can be used to reflect the condition of surrounding environment (Jezierska and Witeska, 2006). Liver of fishes tends to accumulate higher concentration of heavy metals followed by gills and flesh (Asgedom *et al*., 2012). But according to Eneji *et al*.(2011), the accumulation of heavy metals is higher in kidney of *Clarias gariepinus*. In this study, the high levels of these toxic metals were found in cat fish cultivated in artificial plastic ponds instead of natural habitat. This is also supported by Victor *et al*. (2012) when he reported that cadmium and lead were also higher in kidney of the fishes studied. Besides, there were also reports that accumulation of heavy metals were high in the liver of fishes (Sthanadar *et al*.,2013). Nzeve *et al*., 2014 reported an increase in bioaccumulation of heavy metals in two species of fish (*Clarias gariepinus* and *Oreochromis spirulus niger)* from Masinga Reservoir, in Kenya. This study in Benin City ,Nigeria,has also demonstrated an increased in the level of zinc, manganese, aluminium and antimony more in the flesh of *Clarias gariepinus* than in the serum. In Afikpo freshwater ecosystem in Nigeria, lower mean Zn levels in *Tilapia zilli* have been recorded (Nwani *et al*., 2010). Zn levels recorded during this study in the different ponds and control river were above the 75 mg/kg recommended limit for Zn in fish and fish products (FAO, 2003).

Conclusion: This study demonstrated an increase in the body and serum levels of toxic metals in *Clarias gariepinus* across the various ponds and the control river. The concentrations of aluminum, antimony, manganese and zinc the body and serum of *Clarias gariepinus* were above the WHO recommended limit for fish and fish products. Hence, it is concluded that these metals have been bio-accumulated in the tissues and organs of these fish and hence possess an immediate threat on the health of consumers of fish and fish products from these various plastic ponds within Benin metropolis. The observed high levels of toxic metals in this study could be attributed to anthropogenic sources such as fossils fuels combustion, indiscriminate disposal of domestic waste and industrial effluents on land and water bodies and other human activities.

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