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Determination of Heavy Metals Concentration in the Muscles and Gills of two Species of Fish (*Oreochromis niloticus* and *Clarias gariepinus)*

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Abstract

Presence of heavy metals at high concentration in edible fish might lead to some health issues for consumers. The concentration of Pb, Cd contents were determined in muscles and gills of two common fishes Tilapia (*Oreochromis niloticus*) and Cat fish (*Clarias gariepinus*) collected from Bagwai River Kano state using atomic absorption spectroscopy after a modified Acid digestion process. The result showed that the concentration of the heavy metals were higher in the muscles than the gills for the two fish species. However, concentration of lead were significantly higher in gills than the muscles for both the two fish species while the concentration of cadmium was significantly higher in the muscles than the gills in both fish species. The maximum metal concentration was measured for Cd (0.886±0.012 mg/kg) in muscles of *clarias gariepinus*, followed by (0.791±0.524 mg/kg) in the gills of *clarias gariepinus*. The concentration of Pb in the fishes ranged from (0.283±0 mg/kg) in gills of *clarias gariepinus* to (0.016±0 mg/kg) in the muscles of *Oreochromis niloticus*. Analysis of variance result revealed a significant difference $p<0.05$ of concentrations of heavy metals Pb, Cd between two species and also between gills and muscles. Despite the detection of heavy metals in the edible tissues of investigated fish, the concentrations were within the recommended maximum residual level of FAO/WHO and EU.

***Keywords**: Heavy metals, Bioaccumulation, Pollution, Lead, Cadmium*

Introduction

Food safety is a major public health concern and the importance of the association with consumption of fishes contaminated by heavy metals has increased worldwide (Singh et al., 2010). Pollution of the aquatic environment with heavy metals has become an important health concern during recent years. Fishes are often at the top of aquatic food chain water ecosystems and fish living in the polluted waters may accumulate toxic trace metals (Mansour and Sidky, 2002). Heavy metals are non-biodegradable and persistent environmental contaminants, which may be deposited on the surfaces and then absorbed into the tissues of fishes (khan et al., 2013). Prolonged consumption of unsafe concentrations of heavy metals through foodstuffs may lead to the chronic accumulation of heavy metals in the kidney and liver of humans causing disruption of numerous biochemical processes, leading to cardiovascular, nervous, kidney and bone diseases (Jarup, 2003).

Rapid and unorganized urban and industrial developments have contributed to the elevated levels of heavy metals in the urban environment of developing countries such as China (Wong *et al*., 2003) and India (Tripathi *et al*., 1997; Khillare *et al*., 2004; Marshall, 2004; Sharma *et al.,* 2008). Several studies have also indicated that fishes are able to accumulate and retain heavy metals from their environment depending upon exposure, concentration and duration as well as salinity, temperature, hardness, and metabolism of the fishes (Allen, 1995). For instance in Nigeria, Georgr et al., (2013) have found above tolerable limits concentrations of cadmium and chromium contents in tongue sole (*Cynoglossus browne*) and Croaker (*Pseudotolithustypus)* procured from fishermen in Lagos and Delta States Nigeria. Similarly Abowei and Ogamba, (2013) studied the levels of heavy metals in fishes *Lagocephalus levigators* and *Tarpon atlantica* collected from Koluama area of Niger Delta and revealed higher concentrations of Pb>Cu>Cr>Zn>Cd respectively. Alinnor and Obiji (2010) also reported above permissible levels concentration of Fe, Cd, Mn, Cu, and Zn in fresh *Tilapia guineensis* from Nworie River, Nigeria.

Essential metals (Cu, Co, Zn, Fe, and Mg) are required in very trace quantities for the proper functioning of enzyme systems in humans. However, non-essentially heavy metals (Cd, and Pb) have no known beneficial role in human metabolism and are considered as chemical carcinogens even at very low levels of exposure (Khan et al., 2008). Therefore the main objectives of this study were to determine concentrations of heavy metals (Pb, Cd) in tissues (muscle and gills) of two species (*Oreochromis niloticus* and *Clarias gariepinus*) from Bagwai River in Kano. These fish species are regarded to be an essential part of diet in the region. In addition, metal concentrations in the muscle tissue and gills of these species were compared with the maximum permissible limits in order to ascertain whether this food could be considered suitable for human consumption.

Methodology

Study Area:

The Dam is located in the Sudan savannah zone of Northern Nigeria on latitude 11097'N and longitude 80.1'E with two distinct seasons (wet and dry). The rainy season period lasts from May to October while the dry season lasts from November to April. Bagwai Dam is approximately about 47 km away from Kano city along old Bichi-Gwarzo road in Bagwai, Bichi Local Governments Area of Kano State and about 1.1 km from Kabo town. It has an area of 17.22 km^2 (MANR, 1982).

Sample Collection and Preparation

A total number of 20 fish species of Tilapia (*Oreochromis niloticus*) and Cat fish (*Clarias gariepinus*) were captured from Bagwai River by professional fishermen in 2017 during fishery season, between August and October. The specimens were placed immediately in poly-ethylene bags with an ice and, then, brought to the biology laboratory at Bayero University Kano, Kano State. The body weight of each fish were measured respectively. The fish samples was prepared using the method described by Ozmen *et al*. (2004), the part of the fish sample (muscle and gills) needed for the analysis were separated using a high quality corrosion resistant stainless knife to cut the fish tissue (muscle) along the lateral line. The operculum was opened and the gills removed. After dissection, and oven dry at 80^0C for 48hrs. Both the samples were ground to powder using clean mortar and pestle. Fish sample (1.0 g) was placed in a 25 ml beaker. Afterwards, about 10 ml of concentrated HNO^3 (analytical grade, 69%w/w) was poured into the beaker. A watch glass was placed at the mouth of the beaker and the beaker was placed on a magnetic stirrer/hot plate. Initially, the temperature was kept at about 40 °C for one hour to prevent vigorous reactions. Then the temperature was maintained at 140 °C for another 3 hours. Once the digestion was completed all tissue samples were completely dissolved in the acid. Then the mixture was cooled to room temperature. Double distilled water was added into the vessel to dilute the mixture for AAS detection of heavy metals. The sample was filtered by filter paper (Whatman No.1 grade). The filtrates were stored at 4 °C until the metal determination by AAS (Plessis, 2012).

Statistical Analysis

The concentration of heavy metals (Pb, Cd) was presented in mg/kg for all the samples. One way ANOVA was employed to determine the significance mean difference between the concentration of heavy metals in two species of fishes and tissues (muscles and gills) using R statistical software 2014 version.

Result

The mean values of the investigated heavy metals cadmium and lead in the gills and muscles of *Oreochromis niloticus* and *Clarias gariepinus* are presented in (Table

1 and 2). The concentration of lead in the gills of *Oreochromis niloticus* varied from 0.049-0.098 mg/kg while in the gills *Clarias gariepinus* varied from 0.114-0.283 mg/kg. The concentration of lead was higher in the gills of *Clarias gariepinus* than in the gills of *Oreochromis niloticus* p. value <0.05. The mean concentration of lead in muscles of *Oreochromis niloticus* varied from 0.016-0.040 mg/kg while in the muscles of *Clarias gariepinus* varied from 0.019-0.126 mg/kg. The concentration of lead in the muscles was higher in *Clarias gariepinus* than in *Oreochromis niloticus* p.value <0.05. Lead concentration was higher in the gills than in muscles of both *Oreochromis niloticus* and *Clarias gariepinus* p. value <0.05 (Table 3). Lead concentration in all the investigated fish samples were less than the permissible limits of 2 mg/kg for lead by (FAO/WHO), 2011.

Table 1. Mean lead (Pb) concentration mg/kg in the gills and muscles of fishes *Oreochromis niloticus* and *Clarias gariepinus*

Oreochromis niloticus		*Clarias gariepinus*	
Gills	Muscles	Gills	Muscles
0.098 ±0.011	0.016±0	0.147±0.011	0.029±0.006
0.094±0.009	0.026±0.018	0.114±0.004	0.033±0.004
0.077±0	0.024±0	0.203±0.009	0.019±0.009
0.073±0	0.040±0.005	0.283±0	0.054±0.015
0.074±0.012	0.016±0.011	0.121±0.012	0.019±0
0.061±0.006	0.016±0.011	0.159±0.005	0.028±0.006
0.065±0	0.026±0.01	0.212±0.046	0.041±0.011
0.082±0.008	0.017±0.009	0.149±0	0.126±0.007
0.049±0.011	0.016±0	0.131±0.012	0.019±0
0.065±0.18	0.040±0.005	0.161±0.006	0.027±0.007
W.H.O limit	2		

Values are mean for n=3 and ±standard deviation

The mean values of cadmium in the gills of *Oreochromis niloticus* varied from 0.068-0.232 mg/kg while in the gills of *Clarias gariepinus* varied from 0.075-0.791mg/kg (Table 2). Cadmium concentration was higher in the gills of *Clarias gariepinus* than in the gills of *Oreochromis niloticus* p.value <0.05 (Table 3). The mean concentration of cadmium in muscles of *Oreochromis niloticus* varied from 0.46-0.818 mg/kg while in the muscles of *Clarias gariepinus* varied from 0.238-0.886 mg/kg (Table 2). The concentration of cadmium was generally higher in the muscles than gills of both fish species but higher concentrations was observed in muscles of *Clarias gariepinus* than in the muscles *Oreochromis niloticus* p.value <0.05 (Table 3, Figure 1). Cadmium concentration in all the investigated fish samples were less than the permissible limits of 1 mg/kg for cadmium by (FAO/WHO), 2011.

Table 2. Mean cadmium (Cd) concentration mg/kg in the gills and muscles of fishes *Oreochromis niloticus* and *Clarias gariepinus*

Oreochromis niloticus		*Clarias gariepinus*	
Gills	Muscles	Gills	Muscles
0.197±0.012	0.367±0.012	0.409±0.229	0.248±0.012
0.106±0.021	0.46±0.048	0.075±0.028	0.238±0.028
0.068±0.008	0.292±0.009	0.328±0.038	0.409±0.038
0.119±0.024	0.46±0.048	0.432 ±0.032	0.886±0.012
0.102±0.024	0.367±0.012	0.293±0.027	0.273±0.027
0.077±0.012	0.435±0.036	0.324±0.024	0.349±0.012
0.232±0.019	0.645±0.038	0.477±0.047	0.631±0.414
0.193±0.049	0.601±0.016	0.351±0.027	0.614±0.027
0.162±0.012	0.435±0.036	0.477±0.024	0.443±0.048
0.191±0.038	0.818±0	0.791±0.524	0.364±0.031
W.H.O limit	1		

Values are mean for n=3 and ±standard deviation

Table 3. Analysis of variance result of heavy metals between the fish species and tissues

Variables	Heavy metal	F.value	df	p. value
O. niloticus and C. gariepinus	Cd	4.846	1	0.030
Muscles and gills	Cd	37.673	1	4.11e-08
O.niloticus and C. gariepinus	Pb	114.47	1	68e-09
Muscles and gills	Pb	45.100	1	3.68e-09

P. value is significant at p<0.05

Discussion

In this study two heavy metals lead and cadmium were detected in the gills and muscles of all the investigated fish species. The concentration of lead were significantly higher in gills than the muscles for both the two fish species while the concentration of cadmium was significantly higher in the muscles than the gills in both fish species. However, lead concentration was significantly higher in the gills of *Clarias gariepinus* than in the gills of *Oreochromis niloticus*. Lead concentration was significantly higher in the muscles of *Clarias gariepinus* than in the muscles of *Oreochromis niloticus*. The concentration of lead in the gills varied from 0.049-0.098 mg/kg for *Oreochromis niloticus* and from 0.114-0.283 mg/kg in the gills *Clarias gariepinus*, while lead concentration in the muscles varied from 0.016-0.040 mg/kg for *Oreochromis niloticus* and 0.019-0.126 mg/kg for *Clarias gariepinus* respectively.

Lead can cause a large spectrum of physical effects such as neurological and gastro intestinal distress and oncogenic effects (Li et al., 2004a) Lead is neurotoxin that can affect almost every organ or system in the human body, reducing cognitive development and intellectual performance in children and damage kidneys and the reproductive system (Qin and Chen, 2010). Most of the accumulated Pb is sequestered in the bone and teeth (Tood, 1996) causing brittle bones and weaknesses in the wrists and fingers. Lead that is stored in the bones can re-enter the blood stream during periods of increased bone mineral recycling. Mobilized Pb can be re-deposited in the soft tissues of the body and can cause musculoskeletal renal, ocular, immunological and developmental effects (ATSDR, 1996). Lead exposure can cause severe health diseases such as headache, abdominal pain, stomach cancer, irritability and nerve damage (Steenland and Boffetta, 2000; Jarup, 2003).

The mean values of cadmium in the gills of *Oreochromis niloticus* varied from 0.068-0.232mg/kg while in the gills of *Clarias gariepinus* varied from 0.075 -0.791mg/kg. The concentration of cadmium was generally higher in the muscles than gills of both fish species but significant higher concentrations was observed in muscles of *Clarias gariepinus* than in the muscles *Oreochromis niloticus*. The maxiumum cadmium concentration was observed in muscles of *Clarias gariepinus* (0.886±0.012 mg/kg). The concentration of lead and cadmium in the tissues of the investigated fish species were all below the permissible limits sets by W.H.O, 2011. Cadmium exposure may cause chronic health diseases such as lung cancer, reproductive system impacts, gastro-intestinal, osteoporosis, prostate, endocrine disorder, cardio-vascular impacts, bone fracture, hypertention, anemia, injury of central nervous system and liver disease (Martinez-S_anchez *et al*., 2011; Prabu, 2009).

Similar results were reported by Elnabris et al., (2013) in six fish species collected from Gaza market, where as the concentration of Pb found in muscles ranged 0.0.55mg/kg and Cd 0-0.09 mg/kg . Recently, Dizman et al., (2017) in Turkey Rivers reported higher cadmium concentration in the tissues of *Salmo coruhensis* than those of *Oncorphynchus mykiss*, Cd levels ranged between 0.025mg/kg (muscle) and 0.039 mg/kg (gill and liver) in *Oncorphynchus mykiss* and 0.103mg/kg (gills) mg/kg in *Salmo coruhensis*. In Nigeria, Ayotunde *et al*. (2012) in Cross River reported lower lead level in gills of some fishes (0.02 to 0.04mg/kg). However, fish muscles present the most edible portion of commercial fish, but are not considered as active accumulation tissues for heavy metals (Bahnasawy et. al., 2009; Al-switi and Hasyen, 2013). But in highly polluted environments, the heavy metals might be accumulated in different fish tissues including muscles at levels exceeding the maximum acceptable limit (Kalay et al. 1999). The observed variability of metals levels in different species depend on feeding habits (Amundsen et al., 1997; Watanabe et al, 2003), ecological needs, metabolism (Canli and Kalay, 1998), age, size and length of fish (Al-Yousuf et. al., 2000) and their habitats (Canli and Atli, 2003). Despite the detection of heavy metals in the edible tissues of investigated fish, the concentrations were within the recommended maximum residual level of (WHO/FAO, 2010). Therefore, the investigated fishes might be considered safer for human consumption.

Conclusion

This study showed that the levels of heavy metals in muscles and gills of the two fish species (*Clarias gariepinus* and *Oreochromis niloticus*) from Barwai River Kano are lower than the acceptable limit recommended by WHO/FOA. Moreover, concentration of lead were significantly higher in gills than the muscles for both the two fish species while the concentration of cadmium was significantly higher in the muscles than the gills in both fish species. The concentration of cadmium was generally higher in the muscles than gills of both fish species but significant higher concentrations was observed in muscles of *Clarias gariepinus* than in the muscles *Oreochromis niloticus*. Although the heavy metal levels found in this study did not exceed the recommended maximum level of FAO/WHO and EU, there should be restraint in the chronic consumption of these fish species.

Reference

Abowei, J. F. N., Ogamba, E. N. (2013). Effects of Water Pollution in Koluama Area, Niger Delta Area, Nigeria Fish Species Composition, Histology, Shrimp Fishery and Fishing Gear Type. *Research Journal of Applied Sciences, Engineering and Technology*, 6(3): 373-381.

Alinnor, I. J., Obiji, I. A. (2010). Assessment of Trace Metal Composition in Fish Samples from Nworie River. *Pakistan Journal of Nutrition*, 9(1):81-85.

Al-Switi, I.N., Hasayen, K.A. (2013). The comparison of heavy metal concentration in commercial marine fish collected from Red sea and Arabian Gulf in between 2009-2013, with reference to national and international standards, *Ultra scientist*, 25(1): 75-84.

Amundsen, P.A., Staldvik, F.J., Lukin, A.A., (1997). Heavy metal contamination in Freshwater fish from the border region between Norway and Russia, *Sci Total Environ 201:211-24.*

Ayotunde E.O., Offen, B.O. (2012). Heavy metals profile of water, sediment and freshwater catfish *Chrysichthys nigrodigtalus* (Siluriformes, Bagridae) of Cross River, Nigeria. *Revista de Biologia Tropical*, 60(3): 12- 20.

Al-Youful, M.H., El-shahawis, M.S., Al-Ghais, S.M. (2000). Trace metals in liver, skin and Muscles of Lethrinus lentjan fish species in relation to body length and sex. *Sci Total Environ.* 256:87-96.

Bahnasawy, M., Khidr, A., Dheina, N. (2009). Seasonal variations of heavy metals Concentrations in Mullet, Mugil cephalus and Liza ramada (Mugilidae) from Lak Manzala, Eqypt. *Journal. Appl. Sci.Res*., 5:845-852.

Canli, M., Kalay, M. (1998). Levels of heavy metals (Cd, Pb, Cu, Cr and Ni) in tissue of *Cyprinus carpio*, *Barbus capito* and *Chondrostoma regium* from the Seyhan River Turkey. *Zoological* 22:149-57.

Canli, M., Atli, G. (2003). The relationship between heavy metal (Cd,Cr,Cu,Fe,Pb,Zn) Levels and the size of six Mediterranean fish species, *Environ Pollut*, 121:129-36.

Dizman, S., Filiz, K.G.,Recep, K.(2017).Assessment of human health risk from heavy Metals levels in water and tissues of two trout species (*Oncorphyncus mykiss* and *Salmo coruhensis*) from the Fir2tina and Guneysu Rivers in Turkey, *Toxin Reviews*, DOI: 10.1080/15569543.2017.131245.

Elnabris, K.J., Muzyed, S.M., El-Ashgar, N. M. (2013). Heavy metal concentrations in Some commercially important fishes and their contribution to heavy metals Exposure in Palestinian people of Gaza strip (Palestine). *Journal of Association Arab Universities for Basic and Applied Sciences*, 13:44-51

EU, (2001). European Union Commission Regulation as regards heavy metals, Directive 2001/22/EC, No:466.

FAO (2010). The state of World Fisheries and Aquaculture http:// www.fao.org/docrep/013/i1820e/i1820e.pdf

Jarup, L., (2003). Hazards of heavy metals concentration. *BR. Med. Bull*. 68

Kalay, M., Aly, O., Canil, M., (1999). Heavy metal concentrations in fish tissues from the Northeast Mediterranean Sea. *Bull. Environ. Contam. Toxicol*., 63:673-681.

Khan, S., Cao, Q., Zheng, Y.M., Huang, Y.Z., Zhu, Y.G., (2008). Health risk of heavy metals in contaminated soils and food crops irrigated with waste water in Beijing, China. *Environ. Pollut*. 152 (3), 686–692.

Khan, M.U., Malik, R.N., Muhammad, S. (2013a). Human health risk from heavy metal through Crops consumption with wastewater irrigation practices in Pakistan. *Chemosphere* 93:2230-9

Khillare, P.S., Balachandran, S., Meena, B.R., (2004). Spatial and temporal variation of heavy metals in atmospheric aerosols of Delhi. *Environ. Monit. Assess*. 90,1–21.

Li, X., Lee, S.L., Wong, S.C., Shi, W., Thornton, I., (2004a). The study of metal contamination in urban soils of Hong Kong using a GIS-based approach. *Environmental Pollution*:129

Marshall, (2004). Enhancing food chain integrity: quality assurance mechanism for air pollution impacts on fruits and vegetables systems. Crop Post Harvest.

Mansour, S.A, Sidky, M. M. (2002). Ecotoxicological studies of Heavy metals contaminating water and fish from Fayoum Gov., Egypt. *Food Chem* 78:15–22.

Martinez-Sanchez, M., Martinez-lopez, S., Carcia-Lorenzo, M.L., Martinez-Martinez, L.B., Perez-Sirvent, C. (2011). Evaluation of arsenic in soils and plant uptake using various Chemical extraction methods in soils affected by old mining activities. *Geodermal* :160

Plessis A, (2012). Quick method to digest fish tissues for heavy metals analysis. Method to digest fish tissue for heavy metal analysis.

Tripathi, R.M., Ragunath, R., Krishnamurty, T.M., (1997). Dietary intake of heavy metals in Bombay City, India. *Sci. Total Environ*. 208, 149–159.

Prabu, P.C., (2009). Impact of heavy metal contaminations of Akaki river of Ethiopia of soil and metal toxicity on cultivated vegetable crops. *Electron. J. Environ. Agric Food Chem*:9

Qin, F., Chen, W., (2010). Lead and copper levels in tea samples marketed in Beijin. China Bull *Environ. Contam*. Toxicol:78

Sharma, R.K., Agrawal, M., Marshall, F.M., (2008). Heavy metals (Cu, Cd, Zn and Pb) contamination of vegetables in Urban India: a case Study in Varanasi. *Environ. Poll*. 154, 254–263.

Steenland, K., Boffetta, P., (2000). Lead and cancer in humans: where are we now? *Am. J. Ind. Med*. 38,

Sing, A., Rajesh, K.S., Madhoolika, A. (2010). Health risk assessment of heavy metals through Dietary intake of food stuffs from the wastewater irrigated site of a dry tropical area of India. Food *Chem Toxicol* 48:611-9.

Wang S, Shi X. (2003) Molecular Mechanism of metal toxicity and carcinogenesis Mo cell *Biochem*.; 222: 3-9.

Watanabe, K.H., Desimone, F.W., Thiyagarajah, A. (2003). Fish tissue quality in the Lower Mississippi River and health risks from fish consumption. *Sci Total Environ* 302:109-26.