Determination of Heavy Metals Bioaccumulation, Non-Carcinogenic and Carcinogenic Risk of Muscle Tissue of Catfish (*Clarias gariepinus*) Wild and Cultured from Unwana River and Pond

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ABSTRACT

Heavy metals bioaccumulations have been associated with carcinogenic and non carcinogenic toxic effect on certain tissues and organs in humans and animals. The present study aimed at the determination of bioaccumulation of heavy metals and their possible risk of carcinogenic and non carcinogenic toxicity in catfish muscle. The wild and cultured catfish were obtained from Unwana river and Cikano fish pond respectively. All from Unwana Afikpo North L.G.A. The fish samples were digested by the aqua regia digestion standard method and analyzed using AAS. The result obtained showed Zn (0.01, 0.01), Se (0.02, 0.01), Cu (0.09, 0.06), Cd (0.01, 0.01), Pb (0.12, 0.09) and Cr (0.01, 0.01) for wild and cultured respectively, Target Hazard Quotients (THQ) obtained Zn (0.0013, 0.0017), Se (0.0012, 0.002), Cu (0.0011, 0.0012), Cd (0.06, 0.06), Pb (0.02, 0.015) and Cr (0.004, 0.004) for wild and cultured respectively, Hazard Index (HI) of 0.083 and 0.073 for wild and cultured respectively and Cancer Risk (CR) for Pb (6.0, 4.5) and Cd (22.0, 22.0) all in 10^-7 for wild and cultured respectively. Low concentration of heavy metals suggest low contamination of their aquatic habitats and low THQs and HI far below one could also indicate possible no or low risk of non carcinogenic toxicity. More so, the values of CR being well below one also could indicate no or low possible risk of cancer. Finally, it could be concluded that the levels of these heavy metals in the fish muscle may possibly pose no or low carcinogenic and non carcinogenic toxic effects.

Keywords: *THQ, HI, CR, EDI, Carcinogenic and Non carcinogenic*

INTRODUCTION

Heavy metals are frequently highlighted due to their basic features of potential toxicity, bioaccumulation, non-degradability, persistence and biomagnifications at various trophic levels in the aquatic ecosystems (Kortei et al. 2020; Yu et al. 2020). Continuous deposition heavy metals in the aquatic ecosystem through natural (e.g., weathering, erosion) and anthropogenic activities (e.g., domestic waste, deforestation, usage of chemical fertilizers and pesticides) in recent decades increased the level of heavy metals uptake by aquatic organisms such as fish. Fish stand at a higher trophic level and may bioaccumulate a high level of these metals via either gills or diet (alimentary tract). Depending on the species of the fish, the risk of bioaccumulation and biomagnifications is highest with the omnivores because of their broad range of diet within the aquatic environment. Furthermore, bioaccumulation of toxic compounds in different fish species and their tissue depends on several factors which include physiological condition, their habitat, fat content, capacity to adapt and biometric characteristics (Fazio, et al 2020). This is one of the reasons why fishes have persistently been used as good bioindicators of heavy metal pollution of their ability to accumulate metals by direct absorption from aquatic systems (Akila et al., 2022 and Burger et al 2000).

Fish has been a long standing source of affordable high quality protein with high level of polyunsaturated omega-3 fatty acids (PUFAs), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), vitamins, minerals and other essential amino acids (Ullah et al. 2017; Annette et al. 2018).

However, bioaccumulation of heavy metals to high levels in fish might counter the potential benefits of fish consumption. Heavy metals can be grouped into nutritional or essential (Fe, Cu, Mn, Zn, etc.) and potentially toxic (As, Pb, Cd, Hg, etc.). Nevertheless, all the heavy metals can pose a serious health threat when exceeding the permissible limit regardless of their classification.

The study of heavy metals is helpful in three main aspects. Firstly, from the point of view of public health; to measuring the heavy metal accumulation that has serious health hazards to humans, such as As, Cd, Pb, and Hg with no biological function and can be carcinogenic. In addition, they may lead to decreased mental health and cognitive development in children while in adults they can result in the rise of cardiovascular diseases; in addition, the renal and reproductive malfunction. Secondly, from the perspective of the aquatic environment to detect and stop sources that endanger the ecological balance (Ashraf et al., 2012; Salam et al., 2019). Thirdly, from the economic point of view; whereas, this affects the fish quality, productivity, and fish marketability. Consequently, on the economy

Several risks of toxic effects are associated with the consumption of fish that are contaminated with heavy metals, including neurological effects, lung fibrosis, and various types of cancer, defective bone mineralization, renal failure and reproductive problems (Bosch et al. 2016; Titilawo et al. 2018; Mwakalapa et al. 2019; Alipour et al. 2021).

Clarias gariepinusis the most commonly harvested and consumed fresh water fish species in tropical waters especially in Nigeria. The consumption rate of fish in Nigeria is 13.3Kg/person/year (36.43g/person/day) (Bradley et al., 2020).They are highly adaptable and are easily cultured to increase food production. This obviously point out the need for regular monitoring of heavy metal bioaccumulation in the fish and prevent its health consequences (Adhikari et al., 2006 and Nsikak et al. 2007). In 2022, life expectancy at birth in Nigeria was about 61.33 years. More specifically, this figure equaled 60 years for males and 63 years for females. Life expectancy at birth in Nigeria is among the lowest in Africa as well as in the world (Doris, 2022).

Aim: This study aimed at determining the heavy metals concentration in the muscle tissue of catfish (wild and cultured) as well as the risks of non-carcinogenic and carcinogenic toxicity upon their consumption.

Objectives:

- To determine bioaccumulation of heavy metals by quantitatively analyzing Lead (Pb), Cadmium (Cd), Chromium (Cr), Zinc (Zn), Copper (Cu), and Selenium (Se) in muscles of wild and cultured catfish.
- To determine the risk of non-carcinogenic effect by determining estimated daily intake (EDI), target hazard quotient (THQ) and hazardous index (HI).

- To determine the risk of carcinogenic effect by the carcinogenic risk (CR) index.

MATERIALS AND METHODS

Source: Matured wild and cultured catfish were obtained from a local fisherman at Unwana river Afikpo and the fish farmer at Cikano fish pond in Unwana. The fish was washed with deionised water and identification was conducted based on the guideline listed by Kottelat (2013). All the fish samples were kept frozen at –20°C prior to analyses.

Sample Digestion: The samples were crushed into a fine powder using a porcelain mortar and pestle which was pre-washed with 10% nitric acid (HNO3). The digestion methods suggested by Radojevic and Bashkin (2006) and EPA Method 3051A (EPA 2007) were used in this study. The aqua regia solution was prepared by mixing 150 mL of HCI solution (130 mL concentrated HCI + 120 mL of mili-Q water) with 50 mL of concentrated HNO3. The fish samples were placed in the digestion tube and immersed overnight in 5 mL of aqua regia solution. Then the samples were digested in duplicate using a microwave digester. The temperature was set at 180°C for 9.5 min and allowed to cool at room temperature in the microwave. The digested samples were filtered with a 0.45 μm Whatman filter paper into a 50 mL volumetric flask. The total of 0.25 M HNO3 was added up to the mark before analysed using Perkin Elmer Optima 5300 DV ICP-OES for the presence of trace elements. The trace elements content were determined based on dry weight (mg.kg–1 d.w) and later converted into wet weight (mg.kg–1 w.w) using moisture content for each sample. The precision of the analyses was assessed using certified reference material of fish protein (DORM 4). The recovery rates were ranged from 89.4% to 107%, thus confirming the accuracy of the analyses.

Sample Analysis: The filtrate was analyzed for the following parameters: Lead (Pb), Cadmium (Cd), Chromium (Cr), Zinc (Zn), Copper (Cu), and Selenium (Se), using AAS.

Estimated Daily Intake (EDI) The EDI of the trace elements was calculated based on the equation below (Ashraf et al. 2012): EDI = C × IR /BW Where: C = mean concentration of heavy metals in foodstuff (mg.kg–1) IR = daily ingestion rate in Malaysia = 168 g.day–1 (Nurul Izzah et al. 2016) BW = body weight (64 kg for Malaysian adults) (Anual et al. 2018)

Target Hazard Quotient (THQ) The THQ is the estimation of the non-carcinogenic level due to the exposure of the pollutants. If the value of THQ is below 1, it means that there is no possible health threat, whereas if the value of THQ is ≥ 1, it means that there is a possible health threat and that corrective measures should be taken (Kortei et al. 2020). THQ index can be calculated using the equation below:

THQ = (EFr × ED × FiR × C / RfD × BW × TA) × 10–3 Where: EFr = the total exposure frequency, which is equivalent to 365 days.year–1

ED = the exposure duration (63 years) FiR = the rate of fish ingestion (g.day–1)

C = the mean concentration of trace elements in foodstuff (mg.kg–1)

RfD = the oral reference dose according to USEPA BW = the mean of body weight (61.3 kg)

TA = the mean of exposure time (365 days.year–1 × ED)

The oral reference dose (RfD) for the trace elements Cd is 0.0001 mg.kg–1.day–1, Cr is 1.5 mg.kg–1.day–1, Cu is 0.04 mg.kg–1. day–1, Pb is 0.0035 mg.kg–1.day–1, Se is 0.005 mg.kg–1.day–1 and Zn is 0.3 mg.kg–1.day–1

Hazardous Index (HI) The hazardous index (HI) is summative of the THQ for all trace elements to which an individual might be exposed. The HI was calculated using the equation below (Łuczyńska et al. 2018). The value of HI ≥ 1, reveals the possible non-carcinogenic risk to humans. HI = Σ THQ = THQ (Cd) + THQ (Cr) + THQ (Cu) + THQ (Se) + THQ (Pb) + THQ (Zn)

Carcinogenic Risk (CR) This index assesses the probability of an individual developing cancer over the lifespan due to exposure to carcinogenic metal(loid)s. Assessment of CR was conducted on Pb and As only where the cancer slope factors (CSF) for Pb and Cd were 0.0085 mg.kg–1 day–1 and 0.038 mg.kg–1.day–1, respectively (Ullah et al. 2017). According to USEPA (2018), the value of CR ≤ 10–6 is considered an acceptable range, whereas the value of CR is ≥ 10–3 is considered intolerable. The calculation of CR was given by the following equation: CR = CSF × EDI Where: CSF = Cancer slope factor by USEPA (mg.kg–1.day–1) EDI = Estimation Daily Intake

RESULTS

TABLE 1: A table showing the concentrations of heavy metals and their WHO and NAFDAC permissible limits

HEAVY METALS	SAMPLES (mg/kg) WILD	CULTURED	WHO (mg/Kg)	NAFDAC (mg/Kg)
Pb	0.12±0.08 [a]	0.09 ± 0.01 [a]	0.03	1
Zn	0.01 ± 0.00 [b]	0.01 ± 0.00 [b]	100	50
Cu	0.09 ± 0.01 [a]	0.06 ± 0.01 [a]	1.0	1.5
Cd	0.01 ± 0.00 [b]	0.01 ± 0.00 [b]	0.05	0.1
Se	0.02 ± 0.01 [b]	0.01 ± 0.05 [c]	0.5	0.5
Cr	0.01 ± 0.00 [b]	0.01 ± 0.00 [b]	0.6	0.5

TABLE 2: A table showing the results of EDI, THQ, HI, RfD and CR.

ELEMENTS/ HI	EDI (10^-3) WILD	EDI (10^-3) CULTURED	THQ (WILD)	THQ (CULTURED)	RfD (mg/Kg)	CR 10^-7 (WILD)	CR 10^-7 (CULTURED)
Pb	0.071	*0.053*	0.020	0.015	0.0035	6.0	4.5
Cd	0.006	0.006	0.06	0.06	0.0001	22	22
Cr	0.006	0.006	0.004	0.004	1.5		
Zn	0.40	0.35	0.0013	0.0017	0.3		
Cu	0.053	0.036	0.0011	0.0012	0.04		
Se	0.006	0.01	0.0012	0.002	0.005		
HI			0.083	0.079			

DISCUSSION

Accumulations of heavy metals which is dependent on several factors such as the affinity of these elements to bind with different molecular groups within the cells of difference tissues and organs, the uptake and removal rates of these elements, the intensity of fish metabolism and the physiological roles of each organ (El-Sadaawy et al. 2013; Aissaoui et al. 2017).

This bioaccumulation has been associated with multi-system toxicity affecting many organs and tissues. These toxicity effects are broadly categorized into carcinogenic and non carcinogenic. From this study that aimed at determining the health risk for both carcinogenic and non carcinogenic toxicity, the bioaccumulated quantities of heavy metals obtained in the muscle tissue of wild and cultured catfish were within the permissible limits. The low bioaccumulation of these heavy metals is an indication of low presence these metals in their aquatic environments.

Pb and Cd have been defined as non nutritional associated with non carcinogenic and carcinogenic health risk to both humans and animals (Buha, et al., 2018 and Zhang et al., 2019). The values of THQs of Pb and Cd in this study are lesser than one suggesting no risk of non carcinogenic risk. This implies that the consumption of the fish (cultured and wild). CR which is the index for prediction of risk of of cancer obtained for Pb and Cd from this study have values less than 10^-6, suggesting possible no risk of cancer from these elements. Similar findings of low concentration of heavy metals in the muscle tissues of freshwater fish were also reported with low or no risk of non carcinogenic and carcinogenic (El-Sadaawy et al. 2013, ; Mwakalapa et al. 2019 and Mohd, et al., 2022)

Cu, Se, Cr and Zn are the nutritional trace elements needed in small quantities for normal health. They serve as cofactors of many metalloenzymes in the body that are involved in diverse metabolic activities. (Mohd, et al., 2022). However, excessive nutrition of these trace elements beyond their required daily allowance (RDA) could lead to excessive accumulation and derangement of normal body physiology. These manifest as signs and symptoms of toxicity (Coad, 2010). The values of EDI were lower than the established reference dose and THQ were lesser than one. This indicates low or no risk of non carcinogenic health challenge. Similar report was made by (Mathias, et al., 2022) on a study done on smoked fish.

In conclusion, the low bioaccumulation of heavy metals analyzed in this study below the permissible limits present low EDI, THQ, HI and CR and hence low or no risk of non carcinogenic and carcinogenic toxicity. Consumption of the fish muscles at the level of these elements could be said to be safe without possible carcinogenic and non carcinogenic toxicity.

REFERENCES

Aissaoui A, Sadoudi-Ali Ahmed D, Cherchar N and Gherib A. (2017). Assessment and biomonitoring of aquatic pollution by heavy metals (Cd, Cr, Cu, Pb and Zn) in Hammam Grouz Dam of Mila (Algeria). International Journal of Environmental Studies 74(3): 428–442.

Akila, M.; Anbalagan, S.; Lakshmisri, N.M.; Janaki, V.; Ramesh, T.; Jancy Merlin, R.; Kamala-Kannan, S. (2022). Heavy metal accumulation in selected fish species from Pulicat Lake, India, and health risk assessment. Environ. Technol. Innov., 27: 102744.

Alipour M, Sarafraz M, Chavoshi H, Bay A, Nematollahi A, Sadani M, Fakhri Y, Vasseghian Y and Khaneghah A M. (2021). The concentration and probabilistic risk assessment of potentially toxic elements in fillets of silver pomfret (Pampus argenteus): A global systematic review and meta-analysis. Journal of Environmental Sciences (China) 100: 167–180.

Azaman F, Juahir H, Yunus K, Azid A, Kamarudin M K , Mohd Ekhwan T, Mustafa A K, et al. (2015). Heavy metal in fish: Analysis and human health-A review. Jurnal Teknologi 77(1): 61–69.

Bosch A C, O'Neill B, OSigge G, Kerwathb S E and Hoffman L C. (2016). Heavy metals in marine fish meat and consumer health: A review. Journal of the Science of Food and Agriculture 96: 32–48.

Bradley, B., Byrd, K. A., Atkins, M., Isa, S., Akintola, S. L., Fakoya, K. A., Ene-Obong, H. and

Buha, V., Matovic, B. and Antonijevic, G (2018). Overview of cadmium thyroid disrupting effects and mechanisms, international Journal of Molecular Science, 19 (5): 1501.

Burger, J.; Gaines, K.F.; Shane Boring, C.; Stephens, W.L.; Snodgrass, J.; Dixon, C. (2022). Metal levels in fish from the Savannah River: Potential hazards to fish and other receptors. Environ. Res. 89: 85–97

Coad, B.W. (2010). Freshwater Fishes of Iraq, 1st ed.; Pensoft: Sofia, Bulgaria, 86–89, ISBN 978-954-642-530-0

Doris, D. S. (2022). Life expectancy at birth in Nigeria 2022, by gender, September, 2022.

El-Sadaawy M M, El-said G F and Sallam N A. (2013). Bioavailability of heavy metals in fresh water Tilapia nilotica (Oreochromis niloticus Linnaeus, 1758): Potential risk to fishermen and consumers. Journal of Environmental Science and Health, Part B 48(5): 402–409.

Fakhri Y and Sarafraz M. (2021). Refer to human exposure to trace metals and arsenic via consumption of fish from River Chenab, Pakistan and associated health risks by Alamdar et al. (2017). Chemosphere 267: 129002–129002.

Fazio, F.; D'Iglio, C.; Capillo, G.; Saoca, C.; Peycheva, K.; Piccione, G.; Makedonski, L. (2020). Environmental Investigations and Tissue Bioaccumulation of Heavy Metals in Grey Mullet from the Black Sea (Bulgaria) and the Ionian Sea (Italy). Animals, 10: 1739.

Kortei N K, Heymann M E, Essuman E K, Kpodo F M, Akonor P T, Lokpo S Y, Boadi N O, Ayim-Akonor M and Tettey C. (2020). Health risk assessment and levels of toxic metals in fishes (Oreochromis noliticus and Clarias anguillaris) from Ankobrah and Pra basins: Impact of illegal mining activities on food safety. Toxicology Reports, 7: 360–369.

Kottelat M. (2013). The fishes of the inland waters of Southeast Asia: A catalogue and core bibliography of the fishes known to occur in freshwaters, mangroves and estuaries. The Raffles Bulletin of Zoology 27(Suppl.): 1–663.

Łuczyńska J, Paszczyk B and Łuczyński M J. (2018). Fish as a bioindicator of heavy metals pollution in aquatic ecosystem of Pluszne Lake, Poland, and risk assessment for consumer's health. Ecotoxicology and Environmental Safety 153: 60–67.

Mathias N. B., Tijjani S. I., Idris Umar Z. (2023). Determination of Heavy Metals Contamination on Smoked Fish Sold at Some Fish Markets in Borno State, Nigeria, Journal of Chemical Health Risk, 13(1): 135-143.

Mwakalapa E B, Simukoko C K, Mmochi A J, Mdegela R H, Berg V, Bjorge Müller M H, Lyche J L and Polder A. (2019). Heavy metals in farmed and wild milkfish (Chanos chanos) and wild mullet (Mugil cephalus) along the coasts of Tanzania and associated health risk for humans and fish. Chemosphere 224: 176–186.

Thilsted, S. H. (2020). Fish in food system in Nigeria: A Review. Penang Malaysia: WorldFish.Program Repot: 2020-06.

Titilawo Y, Adeniji A, Adeniyi M and Okoh A. (2018). Determination of levels of some metal contaminants in the freshwater environments of Osun State, Southwest Nigeria: A risk assessment approach to predict health threat. Chemosphere 211: 834–843.

Töre Y, Ustaoğlu F, Tepe Y and Kalipci E. (2021). Levels of toxic metals in edible fish species of the Tigris River (Turkey); Threat to public health. Ecological Indicators 123: 107361.

Ullah A K M A, Maksud M A, Khan S R, Lutfa L N and Quraishi S B. (2017). Dietary intake of heavy metals from eight highly consumed species of cultured fish and possible human health risk implications in Bangladesh. Toxicology Reports 4: 574–579.

USEPA. (2018). Reference Dose (RfD): Description and use in health risk. https://www.epa.gov/iris/reference-dose-rfd-description-and-use-health-risk-assessments (Accessed on 10 February 2019). Ullah A K M A, Maksud M A, Khan S R, Lutfa L N and Quraishi S B. (2017). Dietary intake of heavy metals from eight highly consumed species of cultured fish and possible human health risk implications in Bangladesh. Toxicology Reports 4: 574–579.

Varol M and Sünbül M R. (2018). Multiple approaches to assess human health risks from carcinogenic and non-carcinogenic metals via consumption of five fish species from a large reservoir in Turkey. Science of the Total Environment 633: 684–694.

WHO. (1985). Guidelines for the study of dietary intakes of chemical contaminants. WHO Offset Publication No. 87. Geneva: WHO.

Yu B, Wang X, Dong K F, Xiao G and Ma D. (2020). Heavy metal concentrations in aquatic organisms (fishes, shrimp and crabs) and health risk assessment in China. Marine Pollution Bulletin 159(August): 111505.

Zhang, S., Hao, Z. and Qiu, E (2019). Cadmium disrupts the DNA damage response by destabilizing RNF168, Food and Chemical Toxicology, 133: 1107.

Zhong W, Zhang Y, Wu Z, Yang R, Chen X, Yang J and Zhu L. (2018). Health risk assessment of heavy metals in freshwater fish in the central and eastern North China. Ecotoxicology and Environmental Safety 157(January): 343–349.