ISSN 2522-9400 **European Modern Studies Journal** Vol 5 No 3
available at journal-ems.com

Histotoxicity Associated with Some Heavy Metals in Tilapia (*Oreochromis niloticus*) from Eleyele River, South-Western Nigeria

Kenneth C. Onyegbula[1, 2]*, Gideon T. Oluwaloye[2], Ayodele A. Okesola[2]
[1] Department of Biomedical Laboratory Science, Faculty of Basic Medical Sciences, College of Medicine, University of Ibadan, Ibadan, Nigeria
[2] Department of Medical Laboratory Science, School of Public and Allied Health, Babcock University, Ilishan-Remo, Nigeria

Abstract. We determined the concentration of Iron, Zinc, Copper, Lead and Cobalt in water, gills, liver, intestine and muscle of Tilapia (*Oreochromis niloticus*) obtained from Eleyele River, South-western Nigeria with the objective of assessing risk to fish and human health by estimating the pattern of metal-bioaccumulation, hazard quotient and histopathology. The result reveals that Iron was the most abundant in both water and fish tissues. Mean concentration of Iron in water was above all permissible limits used for comparison. The liver exhibited a higher capacity for metal bioaccumulation. Histological deformations observed in the tissues and the high hazard quotient of Lead indicated risk to fish and human health. These findings therefore call for urgent interventional actions by government and non-governmental organizations interested in issues of safe water to channel all efforts at identifying the source(s) of these pollutants in order to stem their release into the reservoir to avoid possible future Lead poisoning.

Keywords: Bioaccumulation, gills, liver, intestine, muscle

Introduction

Air, water and soil are continuously being contaminated by countless harmful materials notable among which are heavy metals that get discharged into the environment without proper treatment as a result of extensive urbanization and industrialization witnessed during this century thereby leading to serious environmental problems worldwide. A great quantity of these contaminants ends up in drinking water sources consequently becoming a threat to plant and animal lives (Mayan-Kutty *et al*., 1995; Dra *et al*., 2019).

Pollution of terrestrial and aquatic ecosystems by heavy metals has long been recognized as a serious environmental concern due largely to their non-biodegradability and tendency to accumulate in plant and animal tissues (Uphadi *et al*., 2013; Wei *et al*., 2019). Metal bioaccumulation is therefore a major route through which increased levels of the pollutants are transferred across food chains/web, creating public health problems wherever man is involved in the food chain. Therefore, it is important to always determine the bioaccumulation capacity for heavy metals by organisms especially the edible ones, in order to assess potential risk to human health (Otitoloju *et al*., 2009; Ahmed *et al*., 2019; Ali *et al*., 2019).

Histopathological alterations can be used as indicators for the effects of various anthropogenic pollutants on organisms and are a reflection of the overall health of the entire population in the ecosystem. These histopathological biomarkers are closely related to other biomarkers of stress since many pollutants have to undergo metabolic activation in order to be able to provoke cellular change in the affected organism (Khoshnood *et al*., 2010).

The rate of bioaccumulation of heavy metals in aquatic organisms depend on the organisms ability to digest the metals, concentration of the metals in water, concentration of the heavy metals in the surrounding sediment, as well as feeding habit of the organism. Fish,

*Corresponding author: kennethchukwudionyegbula@yahoo.com; Tel. +234 80 3423 9571; ORCID id: 0000-0001-8177-3440.

particularly of the Tilapia family is the most abundant, cheap and accessible source of protein in the study area, as such its contamination can easily affect humans which are the ultimate consumers (Uphadi *et al*., 2013).

In a previous study, Onyegbula & Okesola (2016) while investigating the effect of Magnesium, Nickel, Manganese and Cadmium on the gills, liver, intestine and muscle of Tilapia (*Oreochromis niloticus*) obtained from Eleyele River in Nigeria, observed that even though several histopathological alterations were elicited in the target organs suggesting inherent risk to fish health, however, values of hazard quotient indicated that there was no health risk of fish consumption to humans. It is in furtherance of this research that the scope of heavy metals being investigated was extended to include Iron, Zinc, Copper, Lead and Cobalt.

The study site is the Eleyele water reservoir located along Eleyele-Ologuneru road in Ibadan. It serves as a major source of drinking water for over 20% of the population of Ibadan and receives effluents from several sources. Ibadan is renowned for being the largest city in the West African sub-region falling within the coordinates: 7°23′47′′N - 3°55′0′′E -/- 7.39639°N 3.916667°E in Nigeria with an area of 828km^2 and a population of approximately 2.6 million by the 2006 census (Onyegbula & Okesola, 2016; Akinyemi *et al*., 2014).

The mean concentration of five heavy metals (Iron, Zinc, Copper, Lead and Cobalt) were determined in water and tissues (liver, gills, intestine and muscle) of fish obtained from Eleyele River, Ibadan, Southwest, Nigeria during the rainy month of June, 2016. Furthermore, the impact of these metals on histological structures was also investigated.

Materials and Methods

Collection of Water

Water samples from the upstream and downstream points of the reservoir were collected in plastic bottles of 1 liter capacity that had been preconditioned by washing with metal-free detergent solution, rinsed with distilled water and rinsed again with water from the reservoir. The water samples were preserved by acidifying with 5 ml of 6N HNO_3 (pH 2.0) for subsequent estimation of heavy metal concentration (Onyegbula & Okesola, 2016; Javed & Usmani, 2013).

Collection of Fish

Several live samples of Tilapia (*Oreochromis niloticus*) of varying sizes, weights and maturity were collected and transferred into buckets containing water from the reservoir for onward transmission to the laboratory for analysis. Fishes were dissected and organs of interest harvested for estimation of heavy metal concentration and histotoxicity.

Heavy Metal Analysis in Water and Fish

The concentrations of Iron, Zinc, Copper, Lead and Cobalt were estimated in water and fish tissue using Atomic Absorption Spectrophotometer (Perkin Elmer, Analyst A800) as per the standard protocols of APHA (2005).

Estimation of Heavy Metal Bioaccumulation in Fish

Metal bioaccumulation in fish tissues was evaluated by estimating the bioaccumulation factor using the following equation.

$$\text{BAF}=\frac{\text{Concentration of heavy metal in fish tissue}}{\text{Concentration of the same heavy metal in water sample}}$$

Assessment of Health Risk to Humans

The risk for human health of consuming Tilapia from Eleyele water reservoir, Southwest, Nigeria was assessed by calculating the estimated daily intake (EDI) using the following equation (Onyegbula & Okesola, 2016; Onsaint *et al*., 2010).

$$\text{EDI}=\frac{\text{C}\,fish\ \text{X D}\,fish}{\text{BW}}$$

Where C fish is the mean concentration of metal in fish muscle (ppm dry weight), D fish is the global mean daily consumption (g/day) which is 6.5 g/person for Nigerians (Williams & Unyimadu, 2013) and BW is the average body weight (kg) considering an adult average body weight to be 70 kg (USEPA, 2000). The hazard quotient (HQ) was then calculated by dividing the estimated daily intake (EDI) by the established reference dose (R*f*D) to assess the health risk from fish consumption. There would be no obvious risk if the HQ for each metal investigated were less than 1.00 (Onsaint *et al*., 2010).

Statistical Analysis

The results are presented as mean of duplicate determinations.

Histological Examination of Fish Tissue

Selected fishes were dissected; target tissues (gills, liver, intestine and muscle) were harvested, fixed, dehydrated, embedded in paraffin wax, sectioned and stained with Hematoxylin and Eosin for examination under light microscope. All sections in triplicates were examined and photographed using SONY cybershot DSC-W800 digital camera attached to a Leitz binocular microscope (Onyegbula & Okesola, 2016).

Results and Discussion

Table 1.Mean concentration of heavy metals in tissues of Tilapia (*Oreochromis niloticus*) and Eleyele water in comparison with water quality standards (ppm)

Fish tissue/water quality	Iron	Zinc	Copper	Lead	Cobalt
Gills	1200.00	270.00	100.00	10.00	100.00
Liver	13,100.00	1930.00	60.00	70.00	40.00
Intestine	10,700.00	1010.00	90.00	20.00	60.00
Muscle	1200.00	700.00	420.00	30.00	50.00
Eleyele water	1.70	0.59	0.04	0.16	0.07
SON (2007)	0.30	3.00	NA	0.01	NA
WPCL (2004)	0.45	4.25	NA	0.01	NA
CCME (1999)	NA	NA	NA	5.00	NA
WHO (2003)	0.30	3.00	NA	0.05	NA
USEPA (2002)	0.50	5.00	NA	0.05	NA
EC (1998)	NA	NA	NA	10.00	NA

Note. Key: SON (Standards Organization of Nigeria), WPCL (Water Pollution Control Legislation), CCME (Canadian Council of Ministers of the Environment), WHO (World Health Organization), USEPA (United States Environmental Protection Agency), EC (European Commission). NA (Not Available), ppm (Parts Per Million)

Table 2.Bioaccumulation factor of heavy metals in different tissues of Tilapia (*Oreochromis niloticus*)

Metal	Gill	Liver	Intestine	Muscle
Iron	705.88	7705.88	6294.11	705.88
Zinc	457.62	3271.18	1711.86	1186.44
Copper	2500.00	1500.00	2250.00	10,500.00
Lead	62.50	437.50	125.00	187.50
Cobalt	1428.57	571.42	857.14	714.28

Table 3.Health risk of fish consumption

Metal	Concentration in muscle (ppm)	Permissible limits (mg/kg dry weight in fish muscle)	EDI (mg/kg b.w/day)	RfD (mg/kg b.w/day)	Hazard quotient (EDI/RfD) USEPA, 2005
Iron	1200.00	1.0-4.5 (WHO, 1982)	111.43	700	0.16
Zinc	700.00	50 (FAO, 1983)	65	300	0.22
Copper	420.00	30 (FAO, 1983)	39	40	0.98
Lead	30.00	0.5 (FAO, 1983)	2.79	0.05	55.8
Cobalt	50.00	0.01 (USFDA, 1993)	4.64	20	0.23

Note. Key: ppm (parts per million), EDI (Estimated Daily Intake), RfD (Reference Dose), USEPA (United States Environmental Protection Agency).WHO (World Health Organization), FAO (Food and Agricultural Organization), USFDA (United States Food and Drugs Administration)

Concentration of Heavy Metals in Water and Fish Tissues

The mean concentration of Iron, Zinc, Copper, Lead and Cobalt in Eleyele water are presented in Table 1. The comparative differences in the mean concentration of Iron, Zinc, Copper, Lead and Cobalt in the gills, liver, intestine and muscle of Tilapia (*Oreochromis niloticus*) are also presented in Table 1. The pattern of metal accumulation in the various tissues examined is presented in Table 2 while Table 3 shows the human health risk attached to consumption of fish harvested from Eleyele water reservoir polluted with Iron, Zinc, Copper, Lead and Cobalt.

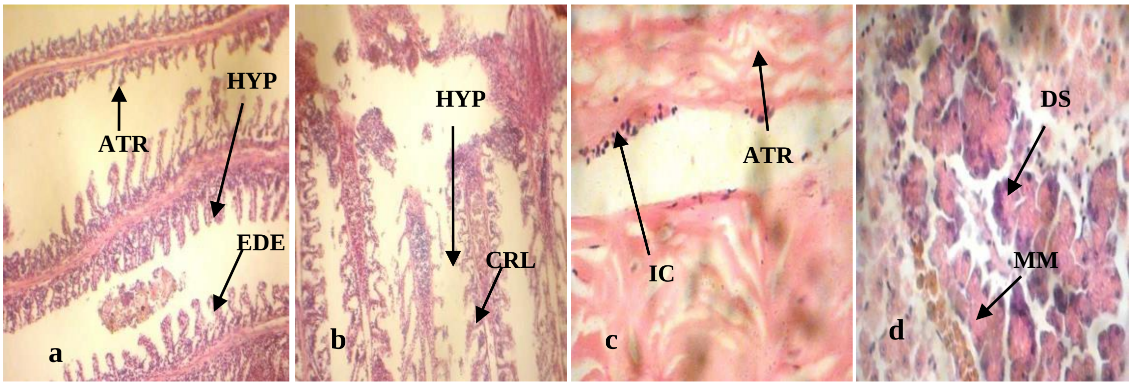
While we observed that Iron has the highest mean concentrationand Copper the lowest in Eleyele water, Anifowose & Oyebode (2019) reporting on Osun River observed that Iron was also the highest but Cobalt was the lowest. Previous studies on these heavy metals in Eleyele water showed variation in trend. While working on the concentration of Lead, Cobalt and Copper in Eleyele water, Etim & Adie (2012) observed that Lead has the highest concentration while Copper has the lowest. In another study on the concentration of Zinc, Copper and Lead in Eleyele water, Akinyemi *et al.*, (2014) observed that Zinc has the highest concentration while Lead has the lowest. Also working on the concentration of Zinc, Copper and Lead, Ayeloja *et al.*, (2014) observed that Lead was the highest and Zinc was the lowest. This variation in trend was also observed in studies from other geographical areas. In the waters of Lake Nasser (Egypt), Mohamed (2008), reported that the concentration of Iron was the highest, while Cobalt was the lowest. Javed & Usmani (2013, also reported a similar trend in the waters of a rivulet situated at Kasimpur that receives waste water from Harduaganj thermal power plant in India. This disparity could be attributable to factors such as time of sampling, sampling site, depth at which sampling was done and the type of waste entering the water body.

We also observed that the mean concentration of Iron in Eleyele water exceeded the available local/international water quality standard values, whereas, Zinc was below the available local/international water quality standard values. While there were no local/international water quality standard values for both Copper and Cobalt for comparison with Eleyele water, we however observed that the mean concentration of Lead in Eleyele water in comparison with local/international water quality standard values was higher in some cases and lower in other cases.

On the comparative differences in the mean concentration of Iron, Zinc, Copper, Lead and Cobalt in the gills, liver, intestine and muscle of Tilapia (*Oreochromis niloticus*), generally the concentrations of these metals were several times higher in the fish tissues than in Eleyele water and the available local/international water quality standard values. Our observations show that Iron was the highest and Lead was the lowest in the gills, intestine and muscle. However, a slight difference in trend was observed in the liver with Iron being the highest and Cobalt the lowest. In a study by Mohamed (2008), it was reported that Iron was the highest and Cobalt the lowest in the liver, gills, intestine and muscle of *Oreochromis niloticus*. The report of Javed & Usmani (2013) also shows that Iron was the highest and Cobalt the lowest in the gills, liver and muscle of *Mastercembelus armatus*. Reporting on *Tilapia zilli*, Eneji *et al*. (2011) observed that Iron was the highest and Lead the lowest in the gills, intestine and muscle. Furthermore, it was reported by Kaoud & El-Dashan (2010) that Copper was the highest and Lead the lowest in *Oreochromis niloticus*. These variations may be related to differences in geographical area and activities around the sampling site.

On the pattern of metal accumulation in the various tissues examined, our results show that while Iron, Zinc and Lead were preferentially accumulated in the liver, Copper and Cobalt on the other hand were preferentially accumulated in the muscle and gills respectively. Results from study conducted by Mohamed (2008) showed that while Cobalt was preferentially accumulated in the gills and muscle, Copper and Iron were preferentially accumulated in the liver and intestine respectively. In their own study, Javed & Usmani (2013) reported that Zinc was preferentially accumulated in the gills, liver and muscle. Eneji *et al*. (2011) also reported that Zinc was preferentially accumulated in the gills, intestine and muscle, while Kaoud & El-Dashan (2010) reported that Lead was preferentially accumulated in the muscle and liver.

On the human health risk attached to consumption of fish obtained from Eleyele water reservoir polluted with Iron, Zinc, Copper, Lead and Cobalt, our result reveals that the mean concentration of these metals in the fish muscle were extremely higher than the permissible limits, however, it was observed that while Iron, Zinc, Copper and Cobalt had hazard quotient less than 1.00, indicating no health risk to humans, Lead on the other hand with an hazard quotient greater than 1.00, strongly indicates risk to human health being the consequence of contamination with this metal.



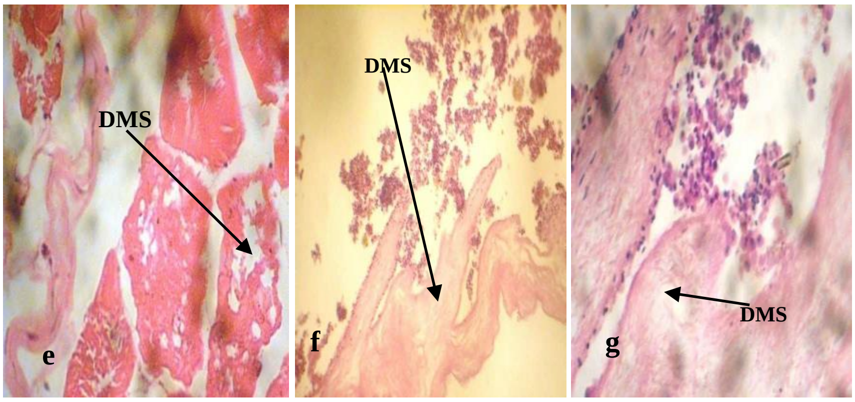


Figure 1.Photomicrographs of dissected organs of Tilapia (*Oreochromis niloticus*) from Eleyele River. (a) Section of gills showing atrophy **ATR**, edema **EDE** and hyperplasia **HYP** in secondary lamella (x100); **(b)** Section of gills showing hyperplasia **HYP** in gill filament and curling of secondary lamella **CRL** (x100); **(c)** Section of smooth muscle fibers showing inflammatory cells **IC** and atrophy **ATR** (x200); **(d)** Section of liver showing dilated sinusoids **DS** and melanomacrophages **MM** (x200); **(e)** Section of skeletal muscle fibers showing degeneration of mucosal surface **DMS** (x50); **(f)** Section of intestinal tract showing degeneration of mucosal surface **DMS** (x50); **(g)** Section of intestinal tract showing degeneration of mucosal surface **DMS** (x200)

Pattern of Histological Alterations

The pattern of histological derangements observed in the gills, liver, intestine and muscle of Tilapia (*Oreochromis niloticus*) harvested from Eleyele water reservoir are presented in Figure 1(a-g).

Histological examination of the target tissues showed a wide range of pathological derangements indicative of tissue toxicity associated with the high concentration of heavy metals. Sections of gills show atrophy, edema and hyperplasia in secondary lamella (Figure1a), hyperplasia in gill filament and curling of secondary lamella (Figure 1b). Sections of smooth muscle fibers show inflammatory cells and atrophy (Figure 1c), while section of liver shows dilated sinusoids and melanomacrophages (Figure 1d). Furthermore, section of skeletal muscle fibers shows shrinkage and degeneration (Figure 1e), while sections of the intestinal tract shows degeneration of mucosal surface at different magnifications (Figures 1f-g).

In our previous study (Onyegbula & Okesola, 2016), we reported similar findings for Magnesium, Nickel, Manganese and Cadmium. Conclusively, accumulation of heavy metals to abnormal levels with a concomitant production of several histopathological derangements in the target tissues and the high hazard quotient of Lead is suggestive of danger to fish health and risk to humans who primarily consume the fish respectively.

Conclusion

We have been able to show that the following heavy metals; Iron, Zinc, Copper, Lead and Cobalt are present in excessive quantity in the water of Eleyele River, Southwest, Nigeria. We have also shown that these metals excessively accumulate in the tissues of Tilapia fish (*Oreochromis niloticus*) harvested from this river. Furthermore, of the metals investigated in this study, health risk to human consumers of Tilapia (*Oreochromis niloticus*) was associated to Lead and several histological derangements were elicited in the tissues of Tilapia (*Oreochromis niloticus*) obtained from this river.

Acknowledgements

This research did not receive any form of funding from funding organizations.

Competing Interests

Authors declare that there are no competing interests.

Authors' Contributions

KCO was responsible for the concept, design, control and supervision of the project; Data collection and processing were done by KCO, GTO and AAO; Analysis and interpretation of results was done by KCO; Literature review and writing of article was done by KCO; All authors have critically reviewed and approved the final draft and are responsible for the extent and similarity index of the manuscript.

Availability of Data and Materials

Data used in this study were presented in the main text.

References

Ahmed, A.S.S., Sultana, S., Habib, A., Ullah, H., Musa, N., & Hossain, M. B. (2019). Bioaccumulation of heavy metals in some commercially important fishes from a tropical river estuary suggests a higher potential health risk in children than adults. *PLoS ONE, 14*(10), e0219336. Doi.org/10.1371/journal.pone.0219336.

Akinyemi, L.P., Odunaike, R.K., Daniel, D.E., &Alausa, S.K. (2014). Physicochemical parameters and heavy metals concentrations in Eleyeleriver in Oyo state, Southwest of Nigeria. *Int. Jour. of Environ. Science and Toxicology Research, 2*(1), 1-5.

Ali, H., Khan, E., &IIlahi, I. (2019). Environmental chemistry and ecotoxicology of hazardous heavy metals: Environmental persistence, toxicity and bioaccumulation. *Journal of Chemistry, 2019,* 6730305. Doi.org/10.1155/2019/6730305.

American Public Health Association. (2005). Standard methods for the examination of water and wastewater analysis (21st ed.). Washington D.C.: American Water Works Association/Water Environment Federation.

Anifowose, A.J., &Oyebode, A.W. (2019). Studies on heavy metals contents of Osun River at the pre-urban settlement and across Oshogbo city. *Journal of Taibah University for Science, 13*(1), 318-323.

Ayeloja, A.A., George, F.O.A., Shorinmade, A.Y., Jimoh, W.A., Afolabi, Q.O., &Olawepo, K.O. (2014). Heavy metal contamination in selected fish species from Eleyele reservoir in Ibadan, Oyo state, Southwestern Nigeria. *African Journal of Environmental Science and Technology, 8*(7), 422-427.

CCME. (1999). Canadian water quality guidelines for the protection of aquatic life: CCME water quality index 1.0; Technical report, Canadian Council of Ministers of the Environment, Winnipeg, MB, Canada.

Dra, A., Gaidoumi, A.E., Tanji, K., Benabdallah, A.C., Taleb, A., &Kherbeche, A. (2019). Characterization and quantification of heavy metals in QuedSebou sediments. *The Scientific World Journal, 2019,* 7496576. Doi.org/10.1155/2019/7496.

EC. (1998). Council directive 98/831.Ec of November 1998 on the quality of water intended for human consumption L. 338/32, European Commission.

Eneji, I.S., Sha'Ato, R., &Annune, P.A. (2011). Bioaccumulation of heavy metals in fish (*Tilapia zilli* and *Clariasgariepinus*) organs from river Benue, North-central Nigeria. *Pak. J. of Anal. Environ. Chem., 12*(1 and 2), 25-31.

Etim, E.U., & Adie, G.U. (2012). Assessment of qualities of surface water, sediments and aquatic fish from selected major rivers in Southwestern Nigeria. *Research J. of Environmental and Earth Sciences, 4*(12), 1045-1051.

FAO. (1983). Compilation of legal limits for hazardous substances in fish and fishery products. FAO Fish Circular 464, 5-100.

Javed, M., &Usmani, N. (2013). Assessment of heavy metal (Cu, Ni, Fe, Co, Mn, Cr, Zn) pollution in effluent dominated rivulet water and their effect on glycogen metabolism and histology of *Mastacembelusarmatus*. *SpringerPlus, 2*, 390-402.

Kaoud, H.A., & El-Dashan, A.R. (2010). Bioaccumulation anghistopathological alterations of the heavy metals in *Oreochromisniloticus* fish. *Nature and Science, 8*(4), 147-156.

Khoshnood, Z., Mokhlesi, A., &Khoshnood, R. (2010). Bioaccumulation of some heavy metals and histopathological alterations in liver of *Euryglossaorientalis* and *Psettodeserumei* along North coast of the Persian Gulf. *African Journal of Biotechnology, 9*(41), 6966-6972.

Mayan-Kutty, P.C., Nomani, A.A., Al-Sulami, S., & Al-Rabeh, A. (1995). *Monitoring of trace metals in desalinated drinking water and their permissible limits.* Paper presented at the IDA Conference, Abu-Dhabi 18-24 Nov., 1995.

Mohamed, F.A.S. (2008). Bioaccumulation of selected metals and histopathological alterations in tissues of *Oreochromisniloticus* and *Latesniloticus* from lake Nasser, Egypt. *Global Vertirinaria, 2*(4), 205-218.

Onsaint, S., Ke, C., Wang, K.J., & Wang, W.X. (2010). Trace elements in two marine fish cultured in fish cages in Fujian province, China. *Environmental Pollution, 158*, 1334-1345.

Onyegbula, K.C., &Okesola, A.A. (2016). Histotoxicological assessment of four heavy metals in Tilapia (*Oreochromisniloticus*) from Eleyele water reservoir in South-western Nigeria. *African Journal of Cellular Pathology Scientists of Nigeria, 7*, 22-29.

Otitoloju, A.A, Ajikobi, D.O., &Egonmwan, R.I. (2009). Histopathology and bioaccumulation of heavy metals (Cu and Pb) in the giant land snail, *Archachatinamarginata* (Swainson). *The Open Environmental Pollution and Toxicology Journal, 1*, 79-88.

Standards Organization of Nigeria. (2007). Nigerian standards for drinking water quality. ICS 13.060.20. NIS 554:1-30.

Upadhi, F., Wokoma, O.A.F., &Edoghotu, J.A. (2013). Levels of bioaccumulation of some heavy metals in fish (*Tilapia zilli*) and their concentration in water and sediment of Owubu creek, Niger Delta, Nigeria. *Resources and Environment, 3*(3), 59-64.

USEPA. (2000). Guidelines for assessing chemical contaminant data for use in fish advisories VI: Fish sampling and analysis (3rded.). Office of Science and Technology, Office of Water, Washington DC, Chap. 1, pp. 1-22.

USEPA. (2002). Region 9 preliminary remediation goals, Region 9. PRGs Table 2002 update, United States En. Pro. Agen. 2002.

USEPA. (2005). Risk based concentration table, April, 2005, USEPA, Region3, Philadelphia, PA.

USFDA. (1993). Food and drug administration guidance document for Nickel in shell fish. DHHS/PHS/FDA/CFSAN/office of sea food, Washington D.C.

Wei, J., Duan, M., Zhang, J., & Li, Y. (2019). Concentration and pollution assessment of heavy metals within surface sediments of the Raohe Basin, China. *Scientific Reports, 9*, 13100. Doi.org/10.1038/s41598-019-49724-7.

WHO. (1982). Toxicological evaluation of certain food additives: copper, zinc. WHO Food Additives Series, Number 17.

WHO. (2003). Background document for preparation of WHO guidelines for drinking water quality. World Health Organization (WHO/SDE/WSH/03.04/103). Geneva.

Williams, A.B., &Unyimadu, J.P. (2013). Organochlorine pesticide residues in muscle tissues of *Ethmalosafimbriata*and*Psettiassebae* from Lagos lagoon, Nigeria. *Int. J. of Academic Research, 1*, 166-172.

WPCL. (2004). Land based water quality classification. Official Journal, 25687, Water Pollution Control Legislation, Turkey.