Water and fish samples were collected from river Kaduna at seven sampling locations (Malali, Anguwan Rimi, Makera, Nassarawa, Kudandan, Anguwan Mu'azu, and Kabala West) as shown in Figure 1. After which, 50 ml of thoroughly shaken water sample was measured accurately into a beaker and digested with 5 ml of conc. HNO_3. The solution was allowed to cool again then filtered with 125 mm filter paper into 100 ml standard flasks and made to the mark with de-ionized water.

Fish samples were dissected and the muscle and liver were collected and washed with deionized water and sun dried for a week. Thereafter, dried in the oven for 3 hours at 80^{o}c. Fish samples (muscle and liver) weighing 2-10 g were transferred into 50 ml quartz crucible and dried in an oven at 120 ^{0}C. One gram of the dried sample was placed in a muffle furnace and ashed in a quartz crucible over night at temperature of 450 ^{0}C. The next day, the sample was removed from the furnace and cooled to room temperature, and then 1ml of concentrated nitric acid was added and dried on a hot plate. The sample was placed in a muffle furnace for the second time and the temperature was raised to 450 ^{0}C and kept for about 1 hour, until the ash became carbon free. The sample was removed from the muffle furnace and cooled at room temperature, and the ash was dissolved in 20 ml of 1 N hydrochloric acid and heated on a hot plate to further make the metals soluble. The residue obtained was dissolved in 50 ml de-ionized water, and filter into a 100 ml volumetric flask and made up to the mark with de-ionized water.
Concentration of mercury, arsenic and copper in samples were determined in triplicates using atomic absorption spectroscopy (Thermo scientific iCE 3000 series AA spectrometers were used). Concentration each heavy metal was expressed in mg/kg dry weight.

2.2 Data Analysis

Data obtained were subjected to analysis of variance using SPSS software. Mean separation was done using least significant difference.

3. Results and Discussion

Results reveal that for each fish species analyzed there was no significant difference in the concentration of mercury (Figure 2) and arsenic (Figure 6) in the muscle and in the liver. This could be to the fact that in each of these two fish species the muscle and the liver contain similar amount of metallothioneins specific to these heavy metals. Metallothioneins are metal binding proteins which play important role in metal homeostasis and protection against heavy metal toxicity **[2].** Das *et al.* **[14]** reported that metallothioneins play a key role in homeostasis of Cu and Zn. A significant relationship was obtained between metallothionein and hepatic Zn levels in selected fish samples **[15].** On the contrary; concentration of Cu in the muscle and in the liver varies significantly only in *C. gariepinus*. Mean values obtained were: 37.74 mg/kg dry weight, 60.23 mg/kg dry weight (muscle and liver respectively of *C. gariepinus*) as shown in Figure 10. The liver accumulated more copper (32.13 mg/kg dry weight) than the muscle (21.34 mg/kg dry weight) as shown in Figure 12 signifying higher level of Cu-specific metallothioneins in the former than the latter. This shows that bioaccumulation of Cu in *C. gariepinus* is tissue dependent. Akan ***et al.*** **[16]** reported that a significant variation in bioaccumulation of some heavy metals within each fish species analyzed occurred.

O. niloticus has higher affinity for Hg than *C. gariepinus* as shown by the over-all mean concentration (25.61 mg/kg dry weight and 17.79 mg/kg dry weight for *O. niloticus* and *C. gariepinus* respectively) in Figure 3. Both fish species had bioaccumulation factor (BAF) of more than 1 for Hg. But *O. niloticus* had higher BAF for Hg than *C. gariepinus* (Figure 5). *C. gariepinus* accumulated more arsenic (0.70 mg/kg dry weight) than *O. niloticus* (0.47 mg/kg dry weight) as shown in Figure 7. The over-all mean concentration of copper in *C. gariepinus* (48.99 mg/kg dry weight) was significantly higher ($P < 0.05$) than in *O. niloticus* (4.48 mg/kg dry weight) as shown in Figure 11. BAF values for arsenic (Figure 9) and copper (Figure 13) were above 1. *C. gariepinus* had higher BAF than *O. niloticus* for both arsenic and copper. The observed variability of Hg, As, and Cu concentrations in these fish species must be seen in the wide perspective of factors such as habitat, seasonal variations, and individual affinity for metal uptake. Differences in life history patterns that influence exposure (including trophic levels and geographic distribution of life stages) are possible explanations for the variation observed in Hg, As, and Cu concentration in the two fish species. It is pertinent to mention that the over-all mean concentration of Hg in both fish species exceeded the maximum levels recommended by Codex alimentarius commission **[17]** which is ≤ 0.5 mg/kg.

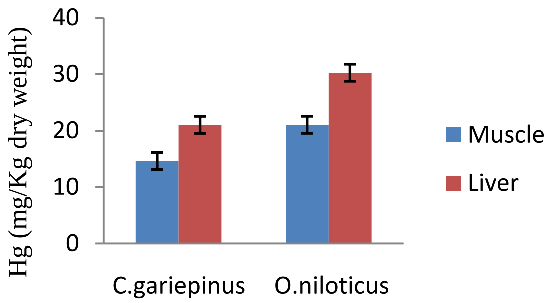

Figure 2: Concentration of mercury (mg/Kg dry weight) in different tissues of two fresh water fish species from Kaduna.

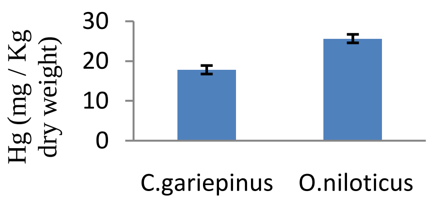

Figure 3: Concentration of Mercury in two fresh water fish species from Kaduna.

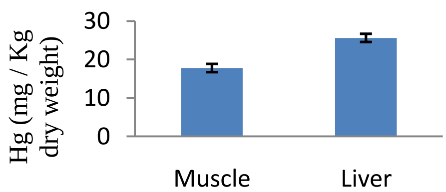

Figure 4: Concentration of mercury in different tissues of two fresh water fish species from Kaduna.

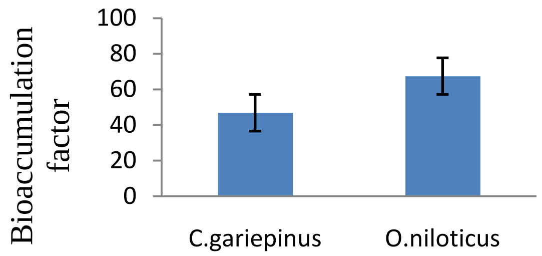

Figure 5: Bioaccumulation factor for mercury of two fresh water fish species from Kaduna.

Therefore it is unsafe for human consumption. Excessive exposure to Hg is associated with damage to the central nervous system (neurotoxicity) and the kidney **[18]**. Concentration of As was within permissible limits in both fish species according to Australia New Zealand food standards code **[19]** which is 2 mg/kg. According to FAO **[20]** maximum limit for Cu is 30 mg/kg. This value was exceeded only in *C. gariepinus* making it unsafe for human consumption.

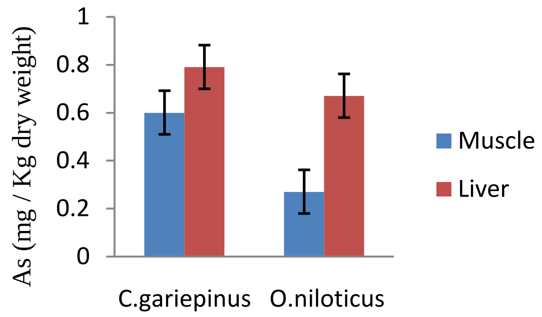

Figure 6: Concentration of arsenic (mg/Kg dry weight) in different tissues of two fresh water fish species from Kaduna

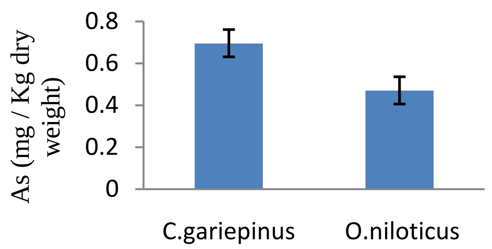

Figure 7: Concentration of arsenic in two fresh water fish species from Kaduna.

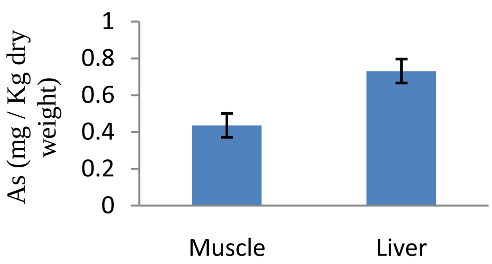

Figure 8: Concentration of arsenic in different tissues of two fresh water fish species from Kaduna.

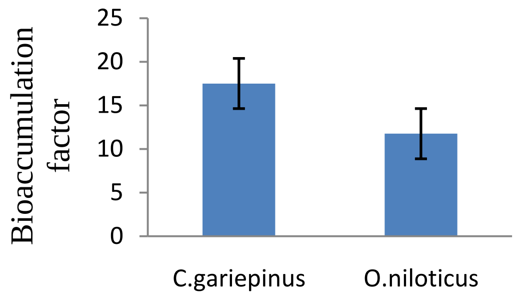

Figure 9: Bioaccumulation factor for arsenic of two fresh water fish species from Kaduna.

Copper in the blood exists in two forms: bound to ceruloplasmin (85–95%), and the rest "free", loosely bound to albumin and small molecules. Free copper causes toxicity, due to its ability to generate reactive oxygen species such as superoxide, hydrogen peroxide, and the hydroxyl radical. These damage proteins, lipids and DNA **[21]**.

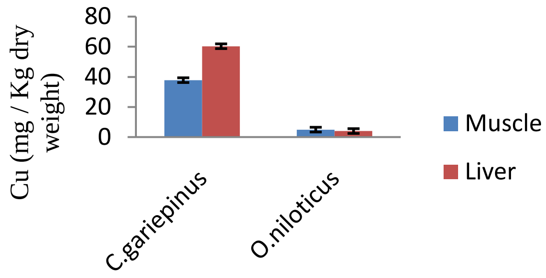

Figure 10: Concentration of copper (mg/Kg dry weight) in different tissues of two fresh water fish species from Kaduna.

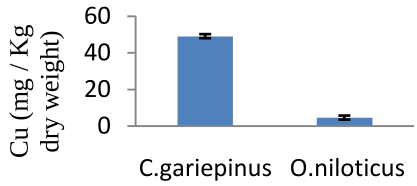

Figure 11: Concentration of copper in two fresh water fish species from Kaduna.

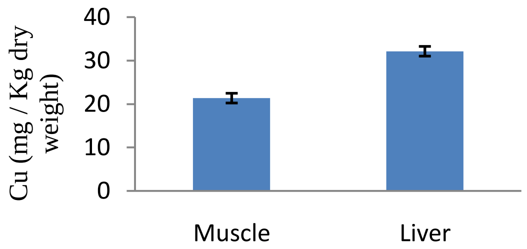

Figure 12: Concentration of copper in different tissues of two fresh water fish species from Kaduna

Results show that the over-all mean concentration of each of the heavy metal (Hg, As, and Cu) in the liver is significantly higher than in the muscle as shown in Figure 4,8 and 12. Reason being that the liver is the main tissue for metabolism of xenobiotics. Hence, it should have higher level of metallothioneins than the muscle. Papagiannis *et al.*, **[2]** reported that the liver accumulated more copper and zinc than the muscle in the four fresh water species analyzed. Kaoud and El-Dahshan **[22]** reported that the liver of *O. niloticus* obtained from river Nile accumulated amount of Cu than the muscle. Ekpo *et al*. **[7]** reported that gills and liver of selected fish species contained the highest concentration of the studied heavy metals, while muscles appeared to be the last preferred site for the bioaccumulation of heavy metals. The presence of high levels of Hg, As, and Cu in *C. gariepinus* and *O. niloticus* shows that river Kaduna is highly contaminated with these heavy metals. This is not surprising because farmlands and industries are cited close to the river (Figure 1). Run-off from farmlands could be a contributing factor to the pollution of this river due to application of inorganic fertilizers, pesticides and herbicides.

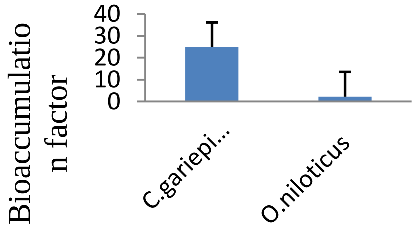

Figure 13: Bioaccumulation factor for copper of two fresh water fish species from Kaduna.

Improper discharge of industrial wastes could also one of the major causes of pollution of river Kaduna **[5]**. One of the ways to circumvent this challenge is by judicious use of chemicals on farmlands which can be achieved by using more of organic materials (organic manure, organic pesticides, biological control of pests and diseases, cultural practices in weed control) and proper industrial wastes management.

4. Conclusion

This research has established that River Kaduna is polluted with Hg, As, and Cu. *C. gariepinus* and *O. niloticus* contain Hg and Cu (only *C. gariepinus*) at levels above permissible limits hence, are unsafe for human use. Both fish species are good bioaccumulators of these heavy metals however, *C. gariepinus* is a better than *O. niloticus*. Furthermore the liver accumulated these heavy metals more than the muscle. Therefore, there is an urgent need for development and implementation of policies to reduce the pollution of river Kaduna for the good wellbeing of consumers of these two fish species.

5. Future Studies

Future research will focus on the effect of these heavy metals on consumers of the fish species. Also, relationship between these heavy metals and metallothionein levels will be investigated.

6. Acknowledgements

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References

[1] Kucuksezgin FA, Kontas, O, Altay, E, Uluturhan DE (2006) Assessment of marine pollution in Izmir Bay; Nutrient heavy metal and total hydrocarbon concentrations. Environment International 32: 41-51.

[2] Papagiannis I, Kagalou I, Leonardos J, Petridis D, Kalfakakou V (2004) Copper and zinc in four freshwater fish species from lake Pamvotis (Greece). Environment International 30: 357-362.

[3] Krishna PV, Madhusudhana Rao K, Swaruparani V, Srinivas Rao D (2014) Heavy metals concentration in fish *Mugil cephalus* from Machilipatnam coast and possible health risks to fish consumers. British biotechnology journal 4: 126-135.

[4] Opaluwa OD, Aremu MO, Ogbo LO, Magaji JI, Odiba IE, Ekpo ER (2012) Assesment of heavy metals in water, fish and sediments from UKE stream, Nasarawa state, Nigeria. Current World Environment 7: 213-220.

[5] Emere MC, Dibal DM (2013) Metal accumulation in some tissues/organs of a freshwater fish (*Clarias Gariepinus*) from some polluted zones of River Kaduna. Journal of Biology Agriculture Healthcare 3: 112–117.

[6] Ali N, Onyie AN, Balarabe SJ, Auta J (2005) Concentration of Fe, Cu, Cr, Zn and Pb in maker drain, Kaduna Nigeria. chem class journal 2: 69-73.

[7] Ekpo FE, Agu NN, Udoakpan UI (2013) Influence of heavy metals concentration in three common fish, sediment, and water collected within quarry environment, Akamkpa L.G. Area, Cross River State, Nigeria. European Journal of Toxicologicl Sciences 2013, 1-11.

[8] Shah SL (2006) Hematological Parameters in tench *Tinica tinica* after short term exposure to lead. Journal of Applied Toxicology 26: 223-228.

[9] Pouomogne V. Capture-based aquaculture of Clarias catfish: case study of the Santchou fishers in western Cameroon. In A. Lovatelli & P.F. Holthus (eds.). Capture based aquaculture. Global overview. FAO Fisheries Technical Paper FAO, Rome, Italy 2008; 508:3-108.

[10] Safriel O, Bruton, MN (2006) A cooperative aquaculture research programme for South Africa. South African National Scientific Programmes Report 89 CSIR, Pretoria, 1984, p. 79.

[11] Van der Waal BW (1984) Survival strategies of sharptooth catfish *Clarias gariepinus* in desiccating pans in the northern Kruger National Park. Koedoe - African Protected Area Conservation and Science 41: 131-138.

[12] Boyd, E.C. 2004. Farm-Level Issues in Aquaculture Certification: Tilapia. Report commissioned by WWF-US in 2004. Auburn University, Alabama 36831.

[13] Picker MD, Griffiths CL (2011) Alien and Invasive Animals – A South African Perspective. Randomhouse/Struik, Cape Town, South Africa p. 240.

[14] Das K, De Groof A, Jauniaux T, Bouquegneau J (2006) Zn, Cu, Cd and Hg binding to metallothioneins in harbor

porpoises Phocoena phocoena from the southern north sea. BMC ecology 6: 2.
[15] Bervoets L, Knapen D, De Jonge M, Van Campenhout K, Blust R. Differential hepatic metal and metallothioneins levels in three feral fish species along a metal pollution gradient. PLos ONE 8(3): e60805. doi:10.1371/journal.pone.0060805.
[16] Akan JC, Mohmoud S, Yikala BS, Ogugbuaja VO (2012) Bioaccumaulation of some heavy metals in fish samples from river Benue in Vinikilang, Adamawa State, Nigeria. American Journal of analytical chemistry 3: 727-736.
[17] Codex Alimentarius Commission. Joint FAO/WHO food standards programme. CODEX committee on contaminants in food Eighth Session. The Hague, The Netherlands, 31 March – 4 April 2014.
[18] Food Safety Authority of Ireland. Mercury, Cadmium, Lead, Tin and Arsenic in food. Issue No 1, 2009.
[19] Australia New Zealand Food Standards Code - Standard 1.4.1 - Contaminants and Natural Toxicants. Federal Register of Legislative Instruments F2011C00542.
[20] FAO: Compilation of legal limits for hazardous substances in fish and fishery products. FAO Fishery Circular 1983; 464:5-10.
[21] Brewer GJ (2010) Copper toxicity in the general population. Clinical neurophysiology 121: 459-460.
[22] Kaoud HA, El-Dahshan AR (2010) Bioaccumulation and histopathological alterations of the heavy metals in *Oreochromis niloticus* fish. Nature and science 8: 147-156.

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