A bi-annual journal published by the Faculty of Science, University of Lagos, Nigeria
http://jsrd.unilag.edu.ng/index.php/jsrd

Heavy metals investigation in *Sarotherodon melanotheron* obtained from Ayetoro River, Ilaje, Ondo State

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((Received 21 March 2022/Revised 01 August 2022/Accepted 11 August 2022)

Abstract:
This study was conducted in Ayetoro River in Ilaje local government area in the southern part of Ondo State, Nigeria, for five months (May 2021 - September 2021) to investigate the concentration of heavy metals in *Sarotherodon melanotheron*. This species of fish is commonly consumed by a large section of the population of Ilaje people, being that they live close to Ayetoro river; whereby 95% of the populace are fishermen. The accumulation of heavy metals in fishes is an important problem because many fishes tend to accumulate the non-essential metals in their organs. The accumulation above the recommended limit for both essential and non-essential metals may pose health hazard to consumers. Sixty (60) *Sarotherodon melanotheron* were collected using gill-net during the wet season. The level of heavy metals copper (Cu), lead (Pb), cadmium (Cd) and iron (Fe) in the muscle tissue of the fish were determined using Atomic Absorption Spectrophotometry technique (AAS). Only cadmium was not detected out of the metals examined. Bioaccumulation of iron was found to be higher in the month of July (0.890 ± 0.241ppm) compared to other months. Copper showed a significant difference in concentration across the months of investigation, most especially in the month of June (0.181 ± 0.058ppm). Lead also revealed constant concentration (0.002 ± 0.001ppm) across the studied months. The results obtained from this study showed the values of the examined metals (Pb, Cu, Fe, Cd) in the *S.melanotheron* fish samples are high. Fe, Cu and Pb were found to be higher than the permissible limits ($p < 0.05$, 0.03, and 0.001ppm respectively) recommended by Food and Agricultural Organization (FAO) and World Health Organization (WHO). This can be attributed majorly to anthropogenic activities like release of industrial wastes and the exploration activities that is being carried out in the region. The accumulation of heavy metals in the tissues of organisms can result in chronic illness and cause potential damage to the population. Therefore, regular monitoring of heavy metals concentration in S. *melanotheron is necessary and* members of this community need to be conscious of the dangers of long-term exposure to these heavy metals through the consumption of this sample specie.

Keywords: *Heavy metals, Sarotherodon melanotheron, Ayetoro, River, Bioaccumulation, Anthropogenic*

Introduction

In the last decades, between 2000 and 2020, the rapid development of industries and agriculture as resulted in increased heavy metals pollution, which is a significant environmental hazard for invertebrates, fish, and humans (Uluturhan and Kucuksezgin, 2007). Heavy metals are found to exist in nature and are very important to life. Nonetheless, they can become toxic through accumulation in organisms (Keke *et al*., 2015; Padrilah *et al*., 2018). In aquatic environments, heavy metal pollution arises from direct atmospheric deposition, geologic weathering or through the discharge of agricultural, municipal, residential or industrial waste products, also through wastewater treatment plants. Heavy metals concentrate in water and entered into the food chain. The contamination by heavy metals and metalloids in water and sediment, can pose a serious threat because they can occur in high level due to their toxicity, persistence, bioaccumulation and bio magnification in the food chain [Alhashemi *et al*., 2012; Mauriya, 2019]. Fishes are regarded as the most indicative biomonitors in aquatic systems for the measurement of metal pollution level [Rashed, 2001; Authman, 2008; Keke *et al*., 2020], Fishes suffers the danger of heavy metal contamination given that water environment is their natural habitat where they get supplied with what is necessary for life, health, and growth (Ahmed *et al*., 2020). Heavy metals enter the aquatic environment from both natural pathways and a variety of anthropogenic sources, and they can have a negative impact on aquatic ecosystems, the food chain, and human health. The concentration of heavy metals in biological compartments, such as fish muscle, is a complex combination of biological and ecological variables. In fish, these elements can cause disturbances in growth and reproduction, as well as histopathological alterations in the skin, gills, liver, spleen, and kidneys (Vitek et, *et al*., 2007). In addition, some metals may decrease the plasticity of the cardio respiratory responses, reducing the survival chances of fish under hypoxic conditions, which has been frequently observed in their wild habitats (Monteiro *et al*., 2013). In humans, heavy metals accumulation has hazardous effects on the brain, liver, kidneys, lungs, and muscles (Petera *et al*., 2011; Youn-Joo, 2013). For these reasons, evaluation of heavy metal levels in fish is important from a toxicological perspective, verifying whether there is a significant health risk arising from fish consumption. Currently, some physiological and histopathological biomarkers of fish have been extensively used to document and quantify the effects of pollutants in aquatic environments, such as follicular atresia, heat shock proteins, apoptosis, metallothionein, vitellogenin in males, hormonal disturbances, melano-macrophage centres (MMCs), fibrosis, and steatosis in teleosts (Santos *et al*., 2014). Many studies suggest that the general function of the MMC is the centralization of destruction, detoxification, or recycling of endogenous and exogenous materials. An increase of MMC numbers and size has often been associated with degraded environmental conditions (Agius and Roberts 2003; Ribeiro *et al*. 2011).

Many fish species are susceptible to the deleterious effects of heavy metals, as reflected in the tissue changes, including anaemia, eosinophilia, lymphocytosis, necrosis, alterations in erythrocyte morphology, bronchial and renal lesions. However, information on the adverse effects of copper, lead, cadmium and zinc on Sarotherodon melanotheron, in Ayetoro river region in Ilaje, is very scarce. Hence, this work determines the presence and concentration of cadmium, copper, iron and lead on tissue of Sarotherodon melanotheron in Ayetoro River.

Materials and Methods

Hydrogen Trioxonitrate (v) acid (HNO_3 65% suprapure), perchloric acid ($HClO_4$ 70%), hydrogen fluoride (HF, 47%, Fisher Scientific), Hydrogen Chloride (HCl, 99%, Sigma aldrich), All chemicals and standards solutions used in the study were obtained from Merck, except otherwise stated and were of analytical grade. Atomic Absorption spectrophotometer (AAS).

A total of sixty (60) samples of *Sarotherodon melanotheron* fishes were caught using gill-net during the wet season over a period of six months from May 2021 to September 2021 and transported daily to the laboratory. The total weight (g) of fishes were measured. Each sample collected from the Ayetoro river seasonally was dissected for its muscle tissues. Sample preparation and analysis were carried out according to the procedure described by UNEP Reference Method (1984).

The tissue was digested with concentrated hydrochloric acid and nitric acid (3:1) at 30℃ for 2 hours and all samples were diluted with distilled water. Following acid digestion, analyzed for four elements by atomic absorption spectrometer (Brant AAS 320N). Fe, Cu, Pb and Cd were determined and analyzed in an air-acetylene flame with an auto sampler. All digested sample were analyzed three times per month for each metal. The standard addition method was used to correct for matrix effects. The instrument was calibrated with standard solution prepared from commercial materials.

Study Area

The study was conducted in Ayetoro, Ilaje local government area of Ondo State, Nigeria. Ilaje is made up of several communities settled along the rivers and estuaries, in which Ayetoro is one of the major communities. The primary occupation in the area is fishing and noted for sea foods which are consumed within and outside the state. The choice of the sampling area was based on the possible anthropogenic inputs from activities of oil exploration, transportation, farming practices, domestic and industrial discharges into rivers and streams which finally emptied into Atlantic Ocean in the southern part. The climate is tropical, with annual rainfall exceeding 1500 mm/annum, temperature between 29-35 ℃ and the seasonal flooding makes it productive in terms of biodiversity and agricultural uses. The coordinate of the area is latitude 6^{o} 15'N and longitude 4^{o} 30'E. There are commercial and domestic wastes, oil spillage, drainage and raw sewage from the settlement areas.

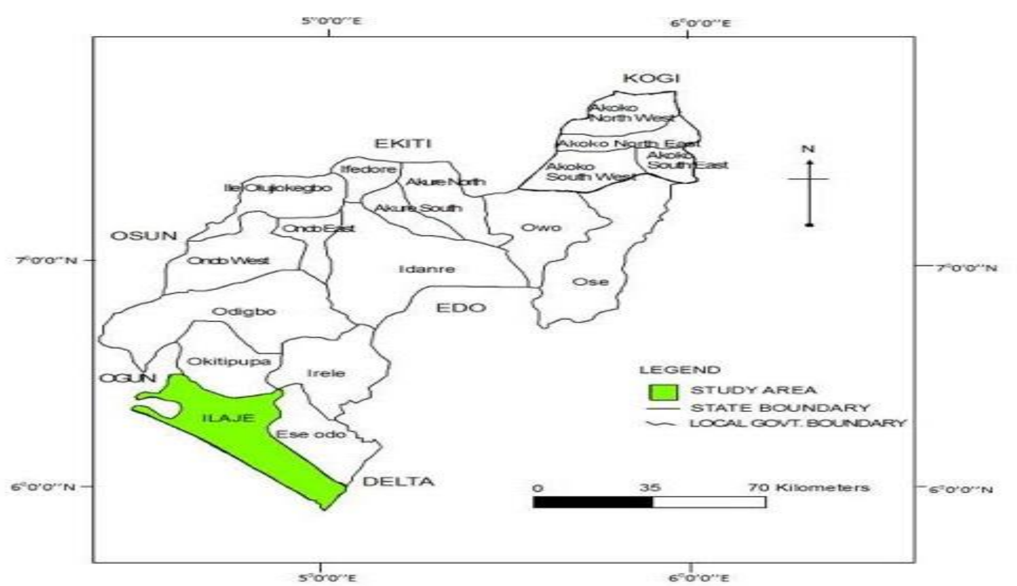

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Figure 1: Map of Ondo State showing Ilaje local Government Area.

Sample Collection and Identification

The blackchin tilapia (*Sarotherodon melanotheron*) used for this study were collected with the assistance of local fishermen. The blackchin are the preponderant fish in the river and highly consumed by people in the communities bordering the river. The samples obtained were immediately transported alive in ice chest to the laboratory where they were identified using FAO species distribution sheet and their sexes differentiated by visual observation of the gonads and by the presence or absence of papillae.

Weighing and Dissection of Fish Samples

Fish samples were weighed using digital weighing balance, and values were recorded to the nearest grams. The weighed samples were dissected with dissecting tools to separate the muscle from head/viscera and bone (Nnaji*et al.,* 2007).

Digestion of Samples and Determination of Metals

The fish samples were digested following established procedure (Olaifa *et al.,* 2004). A portion from each fish sample was ground and weighed (5g) into 10mL Teflon crucible. Freshly prepared concentrated HCl/HNO_3 (aqua-regia) in ratio3:1 was then added to the sample in the Teflon crucibles. The crucibles were covered with watch glass for initial reaction to subside. The samples in the crucibles were then heated in the oven at a temperature of 30 ℃, for 2 hours until the solution became clear and completely digested. The samples were subsequently cooled and 10mL of distilled water was added to each sample. The samples were filtered using Whatman's filter paper (No.1) into 250mL volumetric flasks and made up to mark with distilled water. The digest obtained was then used for metal analysis using Atomic Absorption spectrophotometer (AAS). Cadmium, iron, lead and copper were determined.

Statistical Analysis

Data obtained were analyzed using IBM SPSS statistics, version 22. Descriptive tool was employed to show distribution of copper, lead and zinc concentrations examined in the muscle tissues with relative weights of the fish. Analysis of variance was conducted and multiple comparison among treatment means achieved using Post Hoc Duncan Multiple Range. Degree of association between the weights of the sampled fish and heavy metal concentrations determined *in vivo* was explored using Pearson-moment correlation analysis as adopted by Okafor, (2000). The probability of significance was measured at alpha error of $P<0.05$ and 95% confidence limit. Physico-chemical analyses were determined.

Results and Discussion

The concentrations of the metals in the fish from the study location were all above safe limits stipulated by Food and Agricultural Organization (FAO) and World Health Organization (WHO) for fish except for cadmium which was not detected in any fish samples (Table 2). Similarly, the concentrations of the sampled, heavy metals in the fish, weight of the fish showed moderate positive and significant associations with amounts of copper and lead observed at r =0.581 and 0.510, respectively, while iron concentrations assumed an inverse and negative association with the weight of test *S. melanotheron* species (r = - 0.218). The bioaccumulation of iron in the samples (Figure 1) was significantly higher in the month of July (0.890±0.241ppm) when compared with other months. Also, the bioaccumulated copper (Figure 1) in the sampled fish was significantly different across the months, especially in the month of June.

The results also showed the bioaccumulation of Pb in the samples (Figure 3) to be relatively constant in the studied months (0.002±0.001ppm). Though, iron, copper and lead were all above FAO and WHO permissible limits (0.05, and 0.03 and 0.001ppm) across the months respectively.

For iron (Figure1); may, august, and sept. ═ a $\leq p < 0.05$,(b) june $p \leq 0.5$, (c) july ≤ 1.0

For copper (Figure2); may (a), july (c), august (d), sept. (e) < $p < 0.2$, june (b) > 0.2

For lead (Figure3); may (a) ≤ june (a) ≤ july (a) ≤ august (a) ≤ sept. (a) ≤ $p < 0.002$

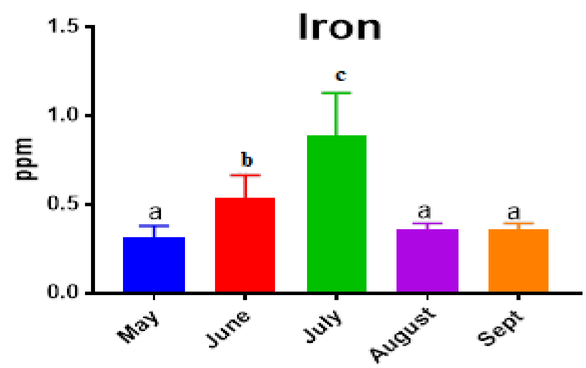

Figure 2: Bioaccumulation (ppm/ wet weight) of Iron in tissue of *S. melanotheron* Mean and standard deviation. The different alphabet mean significant differences ($p < 0.05$)

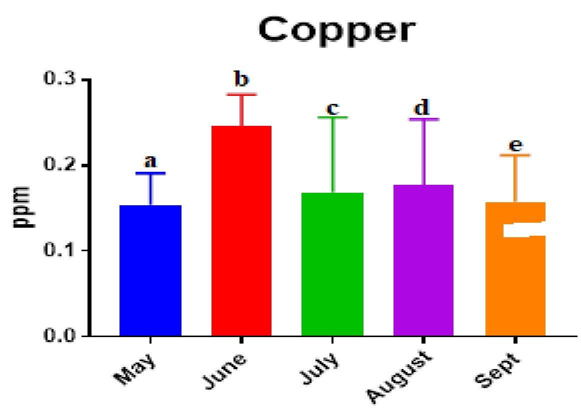

Figure 3: Bioaccumulation (ppm/ wet weight) of Copper in tissue of *S. melanotheron* Mean and standard deviation. The different alphabet mean significant differences ($p < 0.05$)

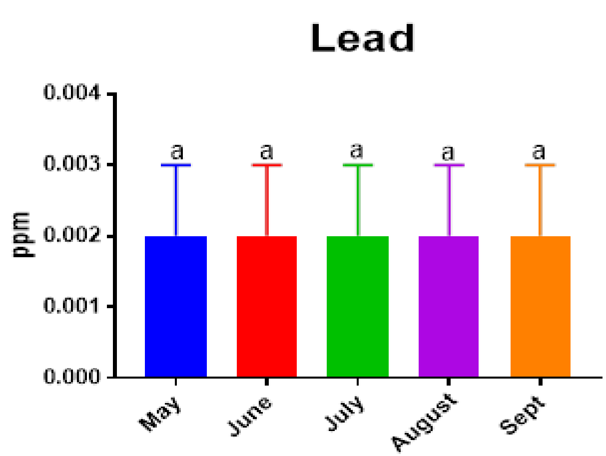

Figure 4: Bioaccumulation (ppm/ wet weight) of Lead in tissue of *S. melanotheron* Mean and standard deviation. The different alphabet mean significant differences ($p < 0.05$)

Table 1: Heavy metal concentrations in muscle tissues of *S. melanotheron* sourced from River Ayetoro.

	May (ppm)	June (ppm)	July (ppm)	August (ppm)	Sept (ppm)	FAO (ppm)	WHO (ppm)
Cd	ND	ND	ND	ND	ND	0.2	0.5
Fe	0.312±0.069[a]	0.536±0.128[b]	0.890±0.241[c]	0.384±0.194[a]	0.333±0.153[a]	0.2	0.05
Cu	0.154±0.037[a]	0.246±0.037[b]	0.169±0.087[c]	0.177±0.077[d]	0.158±0.054[e]	0.03	0.05
Pb	0.002±0.001[a]	0.002±0.001[a]	0.002±0.001[a]	0.002±0.001[a]	0.002±0.001[a]	0.001	0.001

ND = Means not Detected.
Values are presented in Mean±Standard Deviation, values are in part per million (ppm)
Mean values bearing the same superscript along the same column are significantly different (P<0.05).

Table 2: Mean ±SD (n=3) concentrations of heavy metals and FAO safe limits in the tissues of the *S. melanotheron* sourced from River Ayetoro.

Heavy metal	Concentrations (ppm)	FAO safe limit (ppm)	r^2
Cadmium	0.000±0.000	0.200	
Iron	0.491±0.157		-0.218
Copper	0.181±0.058	0.030	0.581
Lead	0.181±0.058	0.001	0.512

Note: r^2 coefficient of correlation

Table 3: Weight (mg/g) of *S. melanotheron* sourced from River Ayetoro.

Month	Mean (n=3)	Standard Deviation	Minimum	Maximum
May	12.53	1.72	10.30	15.40
June	10.73	0.97	9.20	11.80
July	10.53	0.93	9.20	11.60
August	11.16	1.15	9.80	13.10
Sept	12.11	1.95	10.30	18.20

The minimal amount of oxygen is (1-6 mg/L), while shallow water fish need high levels (4-15 mg/L). There was high DO level in the month of June and July due to diffusion and aeration caused by heavy rain than other months.

Temperature (℃): July experienced the lowest temperature, it is because there was high rain fall, the higher the rain fall, the lower the temperature.

Conductivity (mS/cm): September had the highest, it is because there was high sunshine in the month (when compared to other months) which leads to evaporation.

Salinity (%): The higher the evaporation the higher the salinity, Sept. has the highest because of the high level of sunshine when compared to other months.

Hardness (ppm): The water was moderately hard because it fell between 60-120 ppm. In general, water with less than 60 ppm can be considered soft and water with greater than 120 ppm is hard.

pH: June and July experienced low pH, it is because there was high level of waste discharged to the river that could make the river acidic.

Table 4: Monthly variation of physico-chemical parameters of Ayetoro River between May and September 2021

Parameters	May	June	July	Aug.	Sept.	Mean
DO (mg/L)	7.45	7.80	7.81	7.50	7.76	7.66
Tempt.(*C)	28.90	28.00	27.99	28.90	29.00	28.56
Salinity (%)	6.60	6.58	5.90	8.89	9.90	7.57
Conductivity (mS/cm)	39.10	38.00	38.00	39.10	40.00	38.82
Hardness (ppm)	78.10	80.00	80.10	82.00	84.10	80.86
pH	7.15	6.60	6.50	6.68	7.00	6.79

Bioaccumulation of heavy metals in fish has been of significant importance in monitoring aquatic pollution. Measurements of copper, zinc and lead in the muscles of *S. melanotheron* showed their bioaccumulation in noticeable amounts. Heavy metal bioaccumulation from aquatic environment is highly dependent on a lot of variables that play crucial roles for the entire element uptakes. The factorial facilitators include total amount of bioavailability of each metal in the environmental medium and route of uptake, storage and excretion mechanisms (Chapman *et al.,* 2016). Thus, the less available it is, the less it will be accumulated. Aquatic lives are exposed to single and multiple contaminants primarily by oral and dermal routes with surrounding water constituting the principal medium of uptakes of heavy metals for fish, which occurs either passively or via facilitated uptake (Regan, 1993).

There was significant accumulation of iron and copper in the muscle tissue of all the samples of the S. malanotheron from used. The high accumulation of copper could be attributed to anthropogenic activities such as; agriculture, antifouling paint used for ship hulls, buoys, industrial effluents. Iron (Fe) accumulation could also be ascribed to the domestic activities around the region, such as corrosion of iron, steel pipe or transported run-off from industries, municipalities and urban areas; since most metals can end up accumulating in sediments of water bodies. This could be why there is substantial accumulation of copper in the muscle tissues of the fish, *Sarotherodon malanotheron.*

According to Nsikak (2007) and; Nnaji *et al.* (2007), it can be surmised that, the extent of availability of metals in the water determines the extent of bioaccumulation which in turn is related to the level of concentration of any heavy metal in the fish. In their study, they recorded high concentration of zinc, and low concentration of copper in the muscle of *Tilapia* species, and they attributed this to the availability of the metals in the water. In this study, high level of copper and low level of lead (Pb) was observed in the muscle tissue of the fish. The sources of copper in natural waters may be from geological rock weathering or from human activities such as industrial and domestic waste-water discharges where it forms constituent functions in maintaining cytoplasmic veracity (Shah and Altindag, 2015). The concentration of Cu in the tissue of *Sarotherodon malanotheron* sourced from River Ayetoro (0.181±0.058ppm) was significantly higher than FAO and WHO permissible limits (0.03 and 0.05 ppm, respectively). Copper and lead were observed to be dependent on the bodyweight of the fish sampled. Parallel to reports that copper and lead concentration in fish displayed significant association with their individual body sizes, that is, the concentration of heavy metals in smaller fish is always higher than the bigger fish (Rahmawati *et al* 2008). This study is in agreement with the findings of Selda and Nurşah, (2012) in Isikli lake, Turkey; Ibrahim and Sa'id (2010) on River Jakara and Kasalla Dam, Kano State. At high concentrations, copper exerts adverse effects on fish thus resulting in structural damage, which affects the growth, improvement and survival of fish. Copper accumulates in the gills of fish and this designates a depressing effect on tissue respiration leading to death by hypoxia (Siti *et al* 2018). Lethal and sub-lethal level of copper has been known to unfavourably affect hatchability, existence and hematological structures of fish (Olaifa *et al* 2004). Copper could cause sub-acute effects that change fish behaviours including deficiency of balance since most fins are stationary in the affected fish; restless swimming; air guzzling; periods of dormancy and death (Shah and Altindag, 2005). From this study, it has been observed that lead (Pb) concentrations in fish showed significant association with their body sizes (r = 0.5120). This work is in agreement with findings of the Silene and Sandra, (2009) on River Benue and Ibrahim and Sa'id, (2010) on River Jakara and Kusalla Dam, Kano State, Nigeria. Lead has been of particular concern due to its toxicity and ability to bio-accumulate in aquatic ecosystems, as well as persistence in the natural environment (Miller *et al.*, 2012). Lead is known to accumulate in fish tissues such as bones, gills, liver, kidneys and scales, while gaseous exchange across the gills to the blood stream is reported to be the major uptake mechanism (Oguzie, 2012; Tawari-Fufeyin and Ekaye, 2007). The bioaccumulation of Pb in *Sarotherodon malanotheron* sourced from River Ayetoro (0.002±0.001ppm) was higher than FAO and WHO permissible limits (0.001ppm and 0.001ppm). Some effects of Pb if consumed by human via contaminated fish include deficiency in cognitive function due to destruction of the central nervous system, abdominal pain and discomfort, formation of weak bones as Pb replaces calcium and causes anaemia due to reduction of enzymes concerned with synthesis of red blood cells, (Lars, 2003). Lead also leads to decreased fertility, causes cancer and other minor effects like vomiting, nausea, and headache (Lars, 2003; WHO, 2011).

Cadmium may enter into the atmosphere from zinc, lead or copper smelter (Buyun Du *et al* (2020). It can enter water through disposal of the wastes from households or industries. Fertilizers often contain some cadmium. Cd was not detected in this study; this might be of no smelting factories in the settlement. While bodyweight is a factor which drives accumulation of pollutants in resident aquatic organisms, however, the results of this study show that not all accumulation of heavy metals is dependent on body weight of fish, as seen in lead which show inverse association. Trophic transfer of heavy metals can occur with consequential high accumulation of the substances in respective organisms at that tropic level with a particular functional group. This phenomenon could not be ruled out with the observations made in this study. Fostner and Wittman (1981) in earlier studies described that aquatic micro flora and fauna which constituted fish food are capable of incorporating and accumulating heavy metals in their living cells from their environment. As such, higher concentrations of the heavy metals are potentially expected at higher tropic levels. However, this would evidently depend on the organisms and metals involved. Since many factors have been identified to influence bioaccumulation of heavy metals to toxic levels in organisms (Chapman *et al.,* 2006), it may be considered that the variation in the heavy metals concentrations in the fish samples might have been largely influenced by the contamination levels of the study site, implying that high concentrations of metals recorded is probably as a result of the anthropogenic activities and transported run-off from industries, municipalities. Consumptions of heavy metal contaminated fish and other dietary elements are a potential threat to public health, even at a low concentration. Their accumulation can reach toxic levels in the biological systems, because heavy metals are non-biodegradable. Although, some authors have maintained that there is no uniform standard for most metal concentrations in fish tissues except mercury (Burger and Gochfeld, 2005), however, differences on fish consumption as a measure of socio-economic factors and budding risk to community health have been documented in developed countries (Watanabe *et al.,* 2003). Moreover, the concentrations of the selected heavy metals were markedly above safe limits established by Food and Agriculture Organization (FAO) for fish meant for human consumption (FAO, 2013). The implications remain that the continued consumption of *S. melanotheron* from Ayetoro River poses a serious health risk. Public health effects of dietary intake of fish poisoned with heavy metals have been earlier demonstrated (Ogwuegbu and Mahanga, 2005). With the observed concentrations of copper and lead from the study area, measures are required to reduce the various activities that could lead to further increase in the river if the safety of the indigenes not to be compromised.

Conclusion

The results of this study showed that, lead (Pb), iron (Fe), copper (Cu) and cadmium (Cd) were found in the muscle tissue of blackchin tilapia fish from Ayetoro river. The presence of these heavy metals in the blackchin tilapia fish were above FAO and WHO permissible limit. The accumulation of heavy metals in the tissues of organisms can result in chronic illness and cause potential damage to the population. Aquatic animals have often been used in bioassays to monitor water quality of effluent and surface water. The bioaccumulation of these heavy metals led to contamination in the fishes. The bioavailability of both anthropogenic and natural input that could cause harm to the aquatic organisms and humans through the food chain determines the bioaccumulation of these metals by the fishes in the river Ayetoro. Therefore, there should be moderate consumption of this species of fish from this river.

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