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Heavy Metal Bioaccumulation Potential of Liquid Habitat from River Challawa to *Tillapia zilli*

Ovye Akyengo[1, 2], Bawa Inalegwu[1], Ogo A. Ogo[1,3], Jacob Aondongusha Jato[1*]

[1]Department of Biochemistry, College of Science, University of Agriculture Makurdi, P.M.B 2373; 970001 Makurdi, Benue State.

[2]Directorate of Research and Development, Nigerian Institute of Leather and Science Technology, Zaria. P.M.B 3329, Kaduna State

[3]Department of Biochemistry, College of Health Science, Benue State University, Km 1, Gboko Road Makurdi, P.M.B 102119; 970001 Makurdi, Benue State

*Corresponding Author: jatojack@gmail.com

Abstract: **Contamination of water bodies by heavy metals is a serious health hazard not only to the aquatic biota but also to their predator. The degree of contamination is a function of bioavailability. This work was aimed at assessing the differential bioaccumulation of heavy metals (Zinc, Lead, Cadmium, Chromium, Aluminium, Copper and Iron) in liver and skeletal muscles of Tilapia zilli. The liver was used for its role in detoxification and the skeletal muscle for been the part of fish mostly consumed. Tilapia zilli was exposed to three liquid habitats for a period of 72 hours. Station A situated upstream of river Challawa, Station B situated downstream of river Challawa, a control obtained from Tigga Dam where test models were obtained from. Analysis of liquid habitats showed that all the liquid habitats had levels of heavy metal above limits set by the Federal Ministry of Environment for Surface water, except in few cases where the control had levels below set limits. Results of Bioaccumulation factor show that heavy metals were bioaccumulated in the following order: Zn>Fe>CU> Cd>Al>Cr>Pb, Liver had higher bioaccumulation potential as compared to the skeletal muscles of Tilapia zilli. Despite the low levels of heavy metals bioaccumulated by the skeletal muscle as compared to the liver, continuous consumption of contaminated fish still poses a serious threat to consumers. The observed level of heavy metals show that the Challawa River is highly polluted and continuous monitoring and proper effluent treatment must be observed to reduce the level of deterioration of the aquatic ecosystem.**

Keywords: **bioaccumulation, tilapia zillii, heavy metals, aquatic habitat.**

I. INTRODUCTION

Bioaccumulation means an increase in the level of a chemical/toxicant in biological organism, especially when compared to the level of the chemical/toxicant in the environment [1]. Bioaccumulation occurs when an organism absorbs a potentially toxic substance in a rate faster than that at which the substance is lost by catabolism or excretion. Thus, the longer the biological half-life of a toxic substance the greater the risk of chronic poisoning, even if environmental levels of the toxin are not high [2].Methods which involve the monitoring of uptake and retention of agrochemical and metal in the tissues of organs of plants or animals such as fish are referred to as bioaccumulation measurement [3]. Such studies are often restricted to a group of metal called heavy metals. Heavy metals are generally referred to as those metals that possess a specific density of more than 5 g/cm^3 and adversely affect the environment and living organisms [4]. These metals are of biochemical and physiological benefit in living organisms at low concentrations; however, they become noxious when threshold concentrations are exceeded. Despite existing knowledge on the numerous adverse health effects that last for a long period of time, exposure to heavy metals is on the increase in many parts of the world [5], [6], due in part to increased human population with resultant increased demand for several products whose production processes require utilization of these metals either directly or indirectly [7], [8]**.** Heavy metals are significant environmental pollutants and their toxicity is a problem of increasing significance for ecological, evolutionary, nutritional and environmental reasons [9], [10]. The most commonly found heavy metals in waste water include: Arsenic, Cadmium, Chromium, Copper, Lead, Nickel and Zinc, all of which cause risks to human health and the environment [11].

Sources of heavy metals contamination to rivers and other large water bodies includes: soil erosion, natural weathering of the earth's crust, mining, urban runoff, sewage discharge, insect or disease control agents applied to crops, industrial effluents, and many others [12]. Although most of these metals have crucial biological functions in plants and animals, their deleterious effects to these organisms is evident [13], [14]**.** Chemical coordination and oxidation-reduction are identified properties that enable these metals escape control mechanism such as homeostasis, transport, compartmentalization and binding to required cell constituents. These metals bind to protein sites after displacing the original organometals from their natural binding sites, thereby, causing malfunctioning of cells and ultimately cause toxicity [15]. It has been reported that oxidative deterioration of biological membranes is primarily due to binding of heavy metals to the DNA and nuclear proteins [16], [17]. Heavy metal accumulation in the aquatic environment could result in toxicity to both aquatic life and man. Edible fishes form an important member of the food chain originating from the aquatic habitat and could serve as vectors of metallic ions when these metals accumulate in their tissues. Man as consumers of fish, directly or indirectly is reached by these metallic ions in the aquatic medium with a potential for toxicity [18].

Muscle tissues and the liver are good storage sites for metals. However, the liver accumulates higher concentrations irrespective of the uptake in that; it is the site of metabolism and detoxification of these metals. Thus it is often considered a monitor of water pollution because liver metal accumulation and its concentration represent storage and substantially contribute to the overall metal load in the fish [15], [19]. Though muscle metal accumulation is relatively low as compared to other organs, muscles constitutes a greater potential risk for predator fishes, birds and mammals feeding on contaminated fish [19]. The accumulation of metals in fish in sub-lethal exposure is time dependent. Usually, in the initial period of exposure, metals are absorbed and accumulated at a high rate, and then the levels stabilize when equilibrium of metal uptake and excretion rate is attained. Metal distribution in various organs is also time-dependent. Accumulation of metals in the fish organs is a function of uptake and elimination rates and metal concentrations in various organs may change during and after exposure, according to various pattern. The effect of time on metal distribution within organism is a complex issue due to different affinity of various metals to the tissues of various species [20].

Interest in this study is necessitated by the threat posed on human health from consumption of fish from polluted waters. The bioaccumulation of heavy metals in fish is an important factor with reference to the degree of fish contamination and by extension the risk level to man. Therefore, accurate assessment of bioaccumulation of these metals to muscles and liver tissue would shed light to the key question: what extent of risk is posed to human population consuming these fish? The answer to this question would improve man's remedial option in the phase of increasing heavy metal pollution in the aquatic habitat which affects the whole ecosystem at the long run. The aim of this study is to assess the differential bioaccumulation of heavy metals (Pb, Cr, Cd, Zn, Cu, Al and Fe) in the liver and muscle tissues of *Tilapia zilli* exposed to different parts of river Challawa, Kano state.

II. MATERIALS AND METHODS

A. *Study Area*

Challawa River is located in Challawa industrial estate in Kumotso local government area of Kano State. Kano is located in an area extending from latitude $12^0 40$'and $10^0 30$'and longitude $7^0 40$ and $9^0 40$ in the northern part of Nigeria. Challawa River is one of the receiving rivers of effluents from tanneries and textile, foods and packaging industries located in challawa industrial estate, the effluent from the industries are connected by canal and channelled directly into River Challawa.

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B. ***Experimental Animals***

Tilapia zilli used in this study belongs to the cichlid family and is widely found in Africa and the Middle East, it is known for its good taste which makes it attractive. The choice of *Tilapia zilli* as a model animal for this study was based on the following advantages of it over other species: (1) Resistance to harsh conditions such as temperature and disease, (2) resistance to poor quality water, (3) Rapid growth rate, (4) Ease of breeding, (5) low cost of production [21] and (6) wide acceptability for consumption in Kano state and Nigeria at large.

C. ***Experimental Design***

90 fishes (*tilapia zilli*) were obtained from Tigga Dam in Kano, weighing 60-100g. The fishes were dived into three groups namely: station 1 (30 fishes), Station 2 (30 fishes) and control (30 fishes). Station 1 was located upstream of River Challawa, before the effluent channel while station 2 is located downstream of the same River with a flow direction of upstream to downstream (figure 1). Fishes from the two groups were placed in polyvinyl chloride (PVC) tanks containing liquid habitat obtained from the respective stations, and were fed with commercial fish feed (Alltech® Coppens). The control group had the same breeding conditions as the treatment group, except for the habitat which was liquid obtained from Tigga Dam.

D. ***Sample Collection***

Before the period of exposure, samples of the different liquid habitat was collected and the heavy metal concentration as well as physiochemical analyses were carried out to ascertain the concentration of the metals (Pb, Cr, Cd, Al, Zn, Cu and Fe) in the liquid habitat with reference to FME standards [22] and other parameters. Heavy metals analysis was determined using atomic absorption spectrophotometer, while physiochemical analysis was carried out by the method described by APHA [23]. After the period of exposure, the different groups were collected separately with the aid of a gill net. Obtained fishes from the various groups were dissected for collection of the liver and muscle tissue with 95% ethanol sterilized equipment and new latex gloves for each fish. The muscle tissues were collected from the left side of the fishes above the lateral line and between the dorsal fin and the caudal fin; this is to prevent contamination by contents of the abdominal cavity as contained in POPs kits [24]. The liver was subsequently collected prepared along with the muscle tissues and analysed.

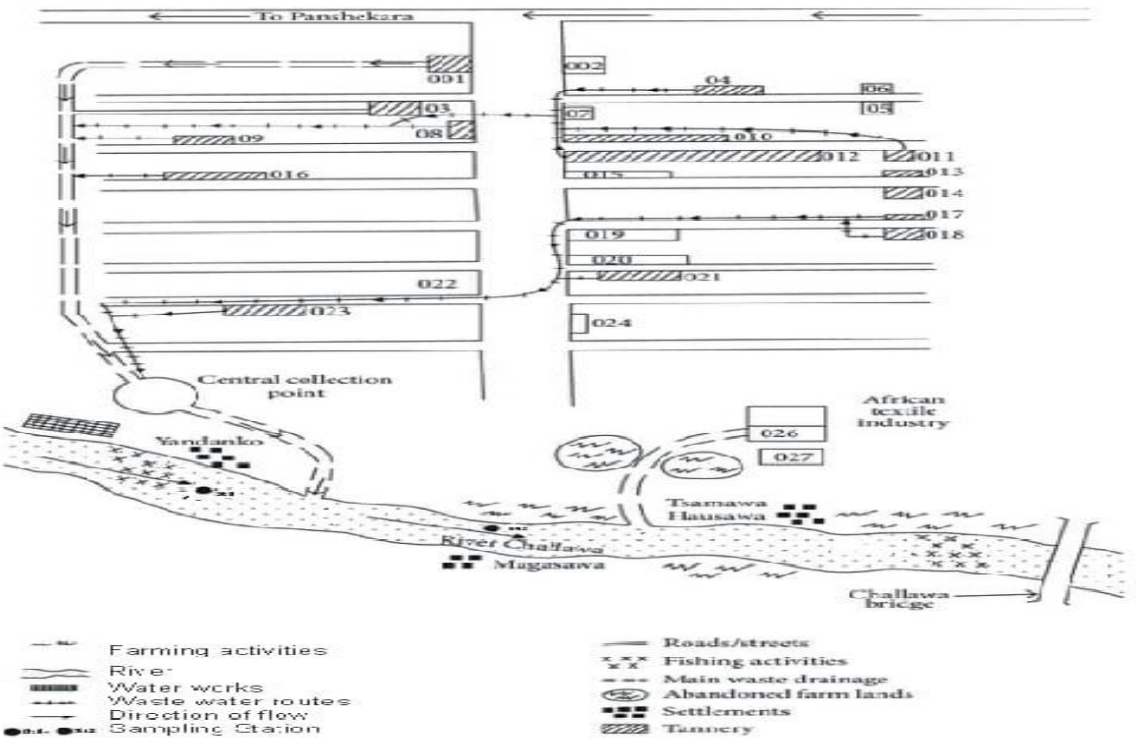

Figure 1: Schematic Representation of River Challawa Study locations and Environmental Activities

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E. *Sample Preparation*

Collected liver and muscle tissues were dried separately for 24hours to a constant weight in a thermal electrostatic drying oven (DHG-9202-40) at 105^{0}C. Dried samples were then acid digested by and aqua regia method as described by Ang and Lee, [25]. To 1g of grounded samples, 18ml of a fresh mixture of hydrochloric acid and nitric acid in the ratio of 3:2 was added; the mixture was boiled over a HK15-18B-2 digital thermostatic water bath at 95^{0}C. After complete digestion, the residue was made up to 50ml with distilled water. Digested sample was stored in pre-cleaned polyethylene sample bottles until analysis using atomic absorption spectrophotometer.

F. *Bioaccumulation Factor*

Bioaccumulation factor is the ratio of the concentration of a particular chemical in the organism or tissue of an organism to the concentration in the environment and is calculated by the expression:

$$BAF = \frac{Concentration\ chemical\ in\ organism\ or\ tissue}{concentration\ of\ chemical\ in\ the\ environment} \qquad \text{------ (Eqn 1)}$$

G. *Statistical Analysis*

Data was analysed using Duncan multiple variance test using SPSS statistical software version 21 and Microsoft Excel® version 2010. Results were presented as mean± SD an considered significantly different at P<0.05

III. RESULTS

The results of physiochemical conditions of the liquid habitats are presented in table 1. They show that temperature ranged from 30.60 ± 1.000 to 30.70 ± 1.000 ^{0}C, while electrical conductivity had values as low as 990 µs/ms in the control liquid habitat to as high as 2530 µs/ms in the downstream liquid habitat. Total suspended solute were higher than the maximum permissible limits, and ranged from 68.00 ± 5.000 mg/l to 1324.00 ± 50.00 mg/l. Dissolved oxygen ranged from 0.71 ± 0.020 to 2.80 ± 0.010 mg/l. Elemental analysis showed that, the liquid habitats had concentration of the elements chlorine, sulphate and phosphate ranging from 5.25 ± 0.002 to 397.010 ± 0.001 mg/l. Generally, temperature and Ph were the only parameters that met the permissible limits by the federal ministry of environment, showing that liquid habitat physiochemical conditions were way above maximum permissible limits.

Table 1: Physiochemical analysis of the studied liquid habitats of *Tilapia zilli* and Maximum permissible limits by Federal Ministry of environment Nigeria.

Parameters	Control	Upstream	Downstream	MPL by Federal Min. of Environment.
Temperature (^{0}C)	30.60± 1.000	30.70 ± 1.000	30.60 ± 1.000	40
Conductivity (µs/ms)	990.00 ± 2.000	2530.00 ± 0.000	3270.00 ± 1.000	1000
TSS (Mg/L)	68.00 ± 5.000	172.00 ± 10.000	1324.00 ± 50.000	30
Chloride (Mg/L)	179.250 ±0.002	214.750 ± 0.002	397. 010 ± 0.001	350
Sulphate (Mg/L)	53.98 ± 0.010	177.74 ± 0.020	235.79 ± 0.010	500
Phosphate (Mg/L)	6.15 ± 0.020	5.25 ± 0.020	9.93 ± 0.010	3.5
Ph	6.33 ± 0.000	6.82 ± 0.010	6.49 ±0.010	6.5-8.5
DO (Mg/L)	2.80 ± 0.010	1.80 ± 0.010	0.71 ± 0.020	Minimum of 6

Legend: values are presented as mean ± SD of data obtained. MPL= Maximum permissible limit; DO= dissolved oxygen; TSS = total suspended Solute

In figure 2, the mean values of biochemical oxygen demand (BOD) of the various stations, control upstream and downstream is plotted against total dissolved solute (TDS). The plot show a positive linear relationship, presenting an equation of y=44.238x-385.19; R^2=0.9973. This indicates that the higher the TDS, the higher the BOD as was the case in test habitats (control < Upstream < downstream), BOD also increase concomitantly.

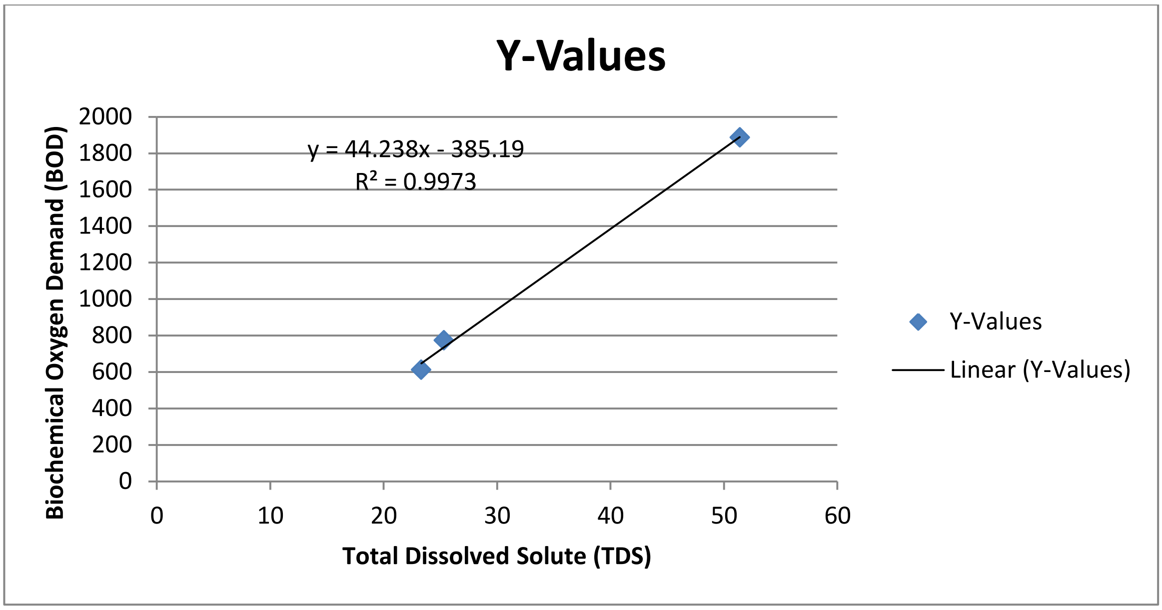

Fig 2: Regression Analyses for BOD/TDS for Control, Upstream, and Downstream liquid Habitats

Legend: values are mean values of BOD and TDS in the order control-upstream-downstream (left to right on X-axis and bottom to top on Y-axis)

In table 2, the results of heavy metal concentrations of the liquid habitats of *Tilapia zilli* are presented. The results show that zinc had the highest concentration with a concentration of 06.189 ± 0.002 ppm, 06.791 ± 0.000 ppm, 11.588 ± 0.002 ppm for the control, upstream and downstream respectively while iron ranged from 05.380 ± 0.000 ppm to 06.872 ± 0.000 ppm. Copper had concentrations which ranged from 01.204 ± 0.000 ppm to 08.198 ± 0.001 ppm, and lead had the least concentrations with 00.781 ± 0.001 ppm, 01.091 ± 0.000 ppm and 01.825 ± 0.000 ppm being concentrations for control, upstream and downstream respectively.

Table 2: Heavy Metal concentrations of the studied liquid habitats of *Tilapia zilli* and Maximum permissible Limits set by Federal Ministry of environment Nigeria.

Heavy metal	Control (ppm)	Upstream (ppm)	Downstream (ppm)	MPL by Federal Min. of Environment.
Cadmium	0.288 ± 0.001	1.890 ± 0.007	2.008 ± 0.000	0.010
Chromium	0.048 ± 0.000	1.061 ± 0.000	2.584 ± 0.002	0.500
Lead	0.781 ± 0.001	1.091 ± 0.000	1.825 ± 0.000	0.100
Aluminium	0.210 ± 0.002	5.529 ± 0.001	8.411 ± 0.001	0.900
Iron	5.380 ± 0.000	5.593 ± 0.002	6.872 ± 0.000	0.500
Zinc	6.189 ± 0.002	6.791 ± 0.000	11.588 ± 0.002	0.200
Copper	1.204 ± 0.000	3.150 ± 0.002	8.198 ± 0.001	0.010

Lengend: *Values are mean* ± SD of metal concentrations. MPL= Maximum permissible limit, PPM= Parts per Million

The result of heavy metal concentrations in the liver and muscle tissues of *Tilapia zilli* after 72 h breeding in test liquid habitats are present in the table 3. Results show that zinc had the highest concentration 4.517 ± 0.002 (liver), 0.467 ± 0.000 (muscle) for the control; 6.859 ± 0.002 (liver), 5.205 ± 0.000 (muscle) for the upstream and 11.488 ± 0.002 (liver), 5.429 ± 0.001 (liver) for the downstream. The order of concentration in tissues was Zn>Fe>Cu>Cd>Al>Cr>Pb. Generally, each heavy metals analysed had a significant ($p<0.05$) statistical difference in its concentration in the liver and muscle tissues in the upstream and downstream stations when compared to the control group.

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Table 3: Bioaccumulation of Heavy Metals in (mg/Kg) in liver and Muscle Tissues of *Tilapia zilli* exposed to three liquid habitats.

Group and Fish Sample	Cu (mg/Kg)	Cd (mg/Kg)	Cr (mg/Kg)	Zn (mg/Kg)	Pb (mg/Kg)	Al (mg/Kg)	Fe (mg/Kg)
Liver-CTL	0.655 ±0.001[a]	0.010 ±0.002[a]	0.002 ±0.001[a]	4.517 ±0.002[a]	0.043 ±0.000[a]	0.017 ±0.001[a]	5.476 ±0.000[a]
Muscle-CTL	0.224 ±0.000[a]	0.012 ±0.001[a]	0.024 ±0.002[a]	0.467 ±0.000[a]	0.064 ±0.001[a]	0.006 ±0.000[a]	4.678 ±0.001 [a]
Liver-USM	3.205 ±0.000[b]	1.248 ±0.000 [b]	0.511 ±0.001[b]	6.859 ±0.002[b]	0.427 ±0.000[b]	0.355 ±0.001[b]	4.859 ±0.001[b]
Muscle-USM	1.446 ±0.002[b]	0.678 ±0.001[b]	0.231 ±0.002[b]	5.205 ±0.000[b]	0.146 ±0.001[b]	0.198 ±0.000[b]	2.894 ±0.001[b]
Liver-DSM	8.683 ±0.000[c]	2.248 ±0.001[c]	2.648 ±0.000[c]	11.488 ±0.002[c]	0.873 ±0.002[c]	6.416 ±0.002[c]	5.276 ±0.00[c]
Muscle-DSM	4.164 ±0.002[c]	0.644 ±0.000[c]	0.848 ±0.0002[c]	5.429 ±0.001[c]	0.347 ±0.001[c]	3.211 ±0.000[c]	2.857 ±0.002[c]

Legend: CTL= control, USM= Upstream, DSM =Downstream, Cu= Copper, Cd= Cadmium, Cr= Chromium, Zn= Zinc, Pb= Lead, Al= Aluminium, Fe= Iron. Values are presented as mean ± SD.Values with different alphabetical superscript are significantly different down the column, for same body tissue at $p<0.05$ using Duncan test.

Figure 3 presents a plot of the concentration of liver heavy metals in the liver against the muscle tissues in the upstream. The plot in order of decreasing concentration (Zn>Fe>Cu>Cd>Cr>Pb>Al) showed a positive linear relationship between liver and muscle tissue heavy metal concentration. An equation for this relationship is $y = 1.3297x + 0.4437$; $R^2 = 0.9616$.

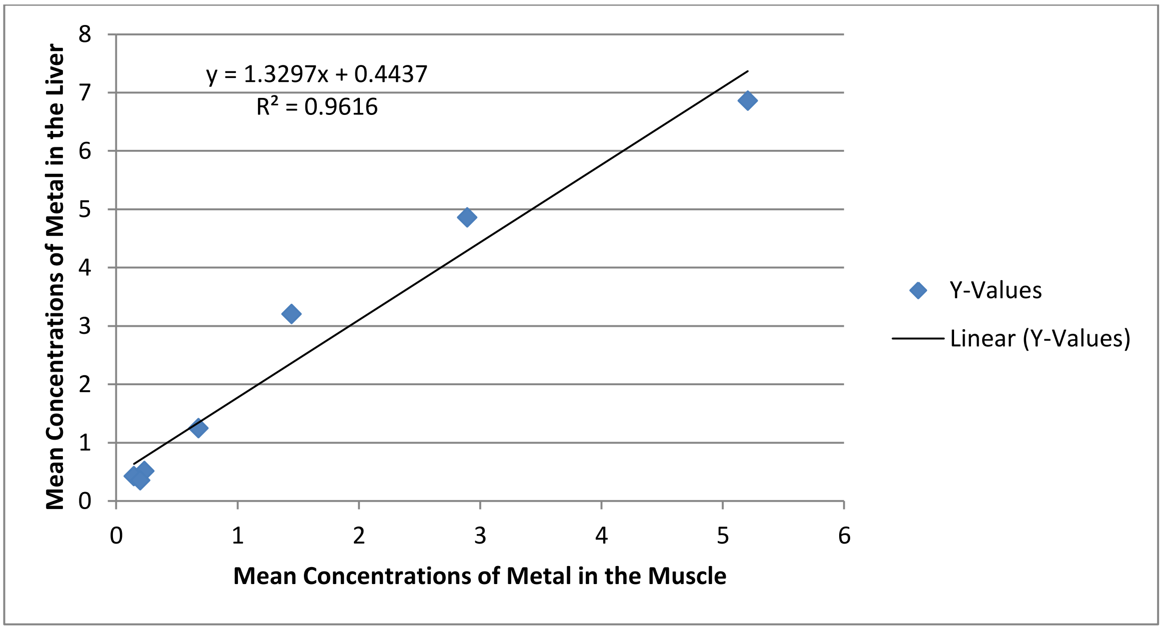

Figure 3: Regression Analyses of Metal concentration in the Liver and Muscles Tissues of the Upstream Station

Legend: Order of mean metal concentration entry was in decreasing concentration Zn>Fe>Cu>Cd>Cr>Pb>Al (left to right on X-axis and bottom to top on Y axis)

Figure 4 presents a plot of the concentration of liver heavy metals in the liver against the muscle tissues in the downstream. The plot in order of decreasing concentration (Zn>Cu>Al>Fe>Cr>Cd>Pb) showed a positive linear relationship between liver and muscle tissue heavy metal concentration. An equation for this relationship is $y = 1.3297x + 0.4437$; $R^2 = 0.9616$.

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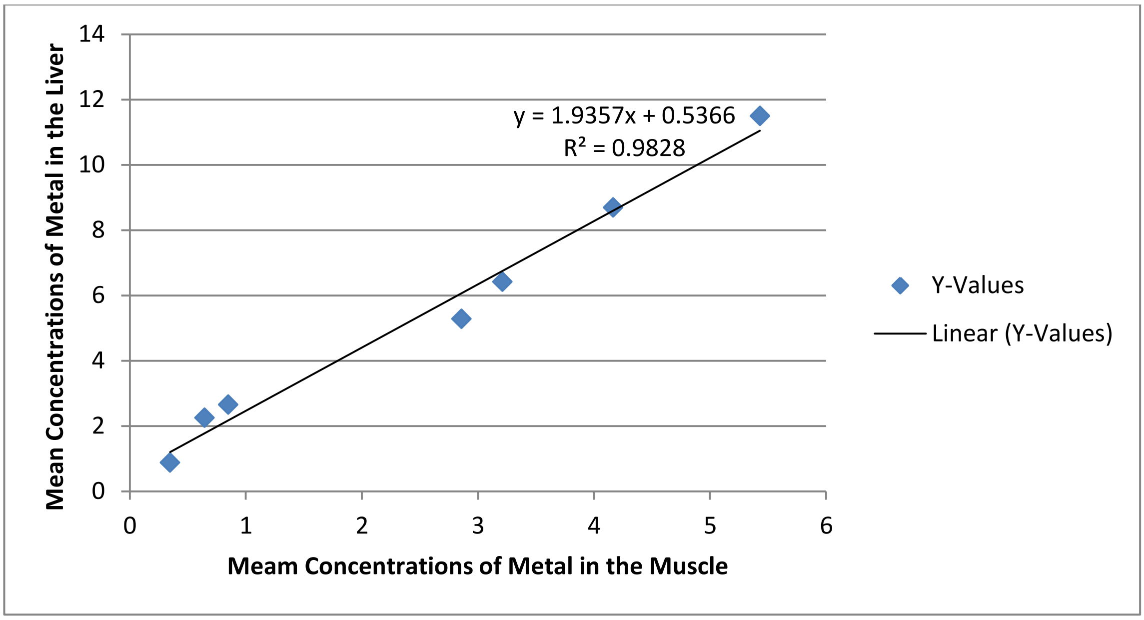

Figure 4: Regression Analyses of Metal concentration in the Liver and Muscles Tissues of the Downstream Station

Legend: Order of mean metal concentration entry was in decreasing concentration Zn>Cu>Al> Fe>Cr>Cd>Pb (left to right on X-axis and bottom to top on Y axis)

The result of bioaccumulation of heavy metal in the liver and muscle tissues of *Tilapia zilli* by determination of bioaccumulation factor (eqn 1) are presented in fig 5. Results show that the liver has greater bioaccumulation factor than the skeletal muscles, also the fishes in the downstream had greater bioaccumulation factor than the control and upstream. By metal consideration, copper had the highest bioaccumulation factor while lead was the least.

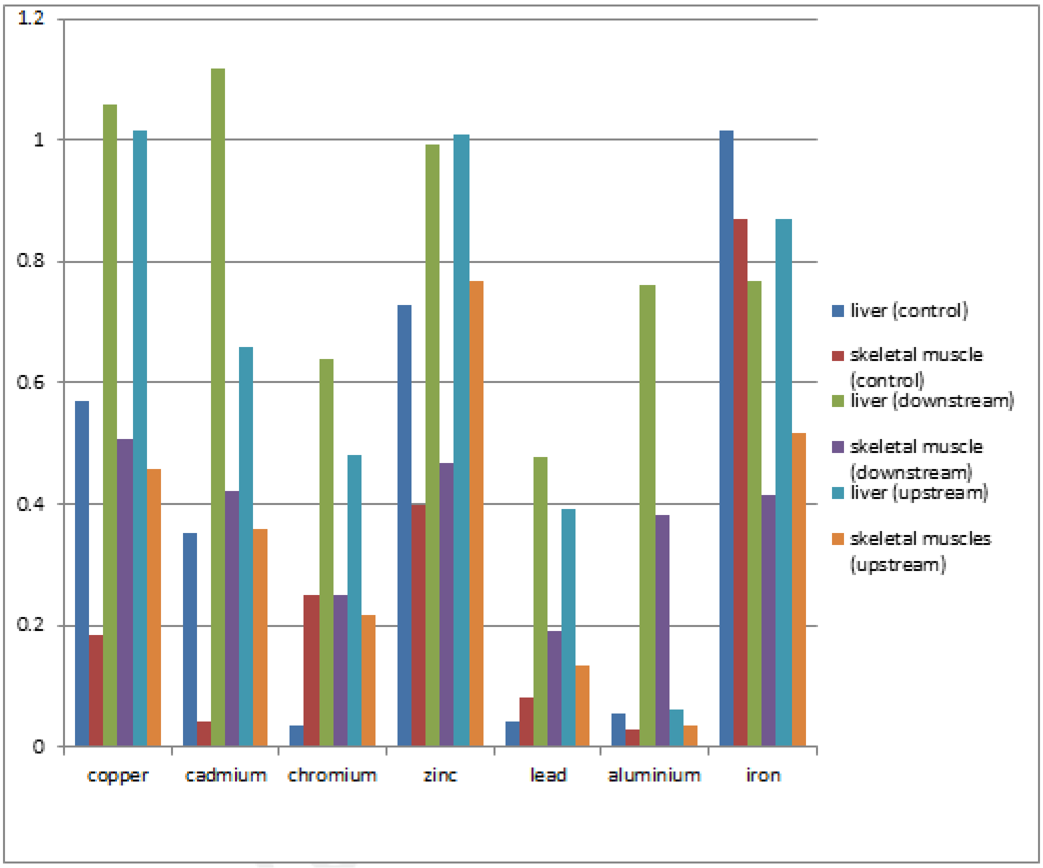

Figure 5: Bioaccumulation factor for liver and skeletal muscles tissues of tilapia zillii exposed to the test liquid habitats for a duration of 72 hours

IV. DISCUSSION

The temperature for all liquid habitats was not beyond the MPL of Federal ministry of environment in Nigeria. Increase in temperature increases the rate of bioaccumulation of heavy metals by increasing the solubility of heavy metals, tissue permeability and metabolic rate [18]. In addition, because oxygen is needed for combustion, respiration and energy production, high levels of temperature will lead to more demand for oxygen. We observed that though temperatures were within the MPL, the temperatures were high. This generally could account for DO and other physiochemicals like BOD, TDS and TSS not meeting set standards. These physiochemical factors has been reported to affect the uptake and accumulation of heavy metals in tissues of fishes, as increase in most of them directly or indirectly affects the rate of accumulation of heavy metals in fish [26].

BOD_5 and TDS had positive linear relationship $y=44.238x-385.19$; $R^2=0.9973$. This is consistent with a study conducted in River Challawa by [27]. The greater the TDS the higher the BOD, this is because TDS and TSS play significant role in the total concentration of DO in the liquid habitat. The higher levels of TDS was indicative of high level of organic matter in the various habitats which serve as a suitable medium for microorganisms which compete with fishes and other aquatic animals for the limited available oxygen [28].

Generally all the heavy metals where above the maximum permissible limit stipulated by Federal Ministry of Environment, except in few cases where the control liquid habitat had aluminium and chromium concentration below the limit set. Liver tissues of the test model generally showed higher level of bioaccumulation across all the heavy metal tested for when compared to their corresponding skeletal muscle tissues, while the tissues exposed to downstream liquid habitat generally had higher level of bioaccumulation compared to corresponding tissues exposed to other test liquid habitats and thus a threat faces the survival of aquatic life in these habitat, this results is collaborated with findings by [28].

Heavy metals analysis of the liver and skeletal muscles tissues of *tilapia zillii* revealed that the liver had a higher accumulation of these metals when compared to the skeletal muscle across all the three liquid habitats considered, this pattern is in tandem with observation made by other authors [6], [15], [29], [30]. The results showed that there was a statistically significant ($p<0.05$) difference in the concentrations of heavy metals in liver and muscle tissues across test habitats as compared to the control habitat; this finding is consistent with [28]. Analysis also showed that the more a heavy metal was accumulated in the liver the more the skeletal muscles also accumulated such an metal. It is therefore proposed that though heavy metal detoxification activity by the liver could account to some extent for high concentrations in it, based on the relationship in figures 3 and 4, heavy metals are first accumulated in the liver and then excesses are transported to the skeletal muscles for storage.

Bioaccumulation factor calculated for whole tissues, liver tissues and skeletal muscles tissues of *Tilapia zilli* exposed to the three liquid habitats, of the three habitat in which *Tilapia zilli*, the liquid habitat downstream had higher bioaccumulation factors with the exception of iron where the control had higher values, these pattern was also maintained in the bioaccumulation factors of heavy metals in the tissues of the liver and skeletal muscles of *Tilapia zilli*, this observed pattern is elucidated by the assertion by [26] that the more the concentration in the environment the more they can be taken up and accumulated in fish tissue. Various metals are accumulated in aquatic organism body in different amount, these differences results from differences resulting from different affinity of metals to the tissues, different rate of uptake, deposition and rate of excretion.

The liver bioaccumulated more heavy metal than the skeletal muscles in the results of the bioaccumulation factor of heavy metals for the two tissues across the three test habitats. In two separate studies, muscle tissues have been reported to exert a weak accumulating potentials and thus accumulated lower level of heavy metals when compare to the liver [31, 32]. Conversely, the liver is considered to have a high accumulating ability as a result of the activity of metal binding proteins such as metallothioneins, which can bind with heavy metals, and thus reducing their toxicity and allowing the liver to accumulate high concentration of these toxic heavy metals [29], [32].

V. CONCLUSION

The level of Cr, Cd, Fe, Cu, Al, and Zn in both the livers and skeletal muscles was high in samples exposed to liquid habitat. The liver showed higher bioaccumulating potential from results of the bioaccumulation factor when compared with the skeletal muscle in *Tilapia zilli* exposed to all test liquid habitat. Despite the low heavy metals bioaccumulated by the skeletal muscles, it still poses a serious threat to the consumers in that, continuous consumption of heavy metals contained in skeletal muscles of fish could increase the level of toxicity of these metals to predators of *Tilapia zilli*. Continuous monitoring of contamination in River Challawa as well as proper effluent treatment is necessary to prevent further deterioration of the pollution level and contamination of the aquatic biota and a trace on the heavy metal accumulating preference in tissues of aquatic animals is recommended.

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