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Pilot Testing on the Feasibility in A Study on the Accumulation of Heavy Metals in Tissues of *Tilapia Zilli* Exposed to Liquid Habitat from River Challawa

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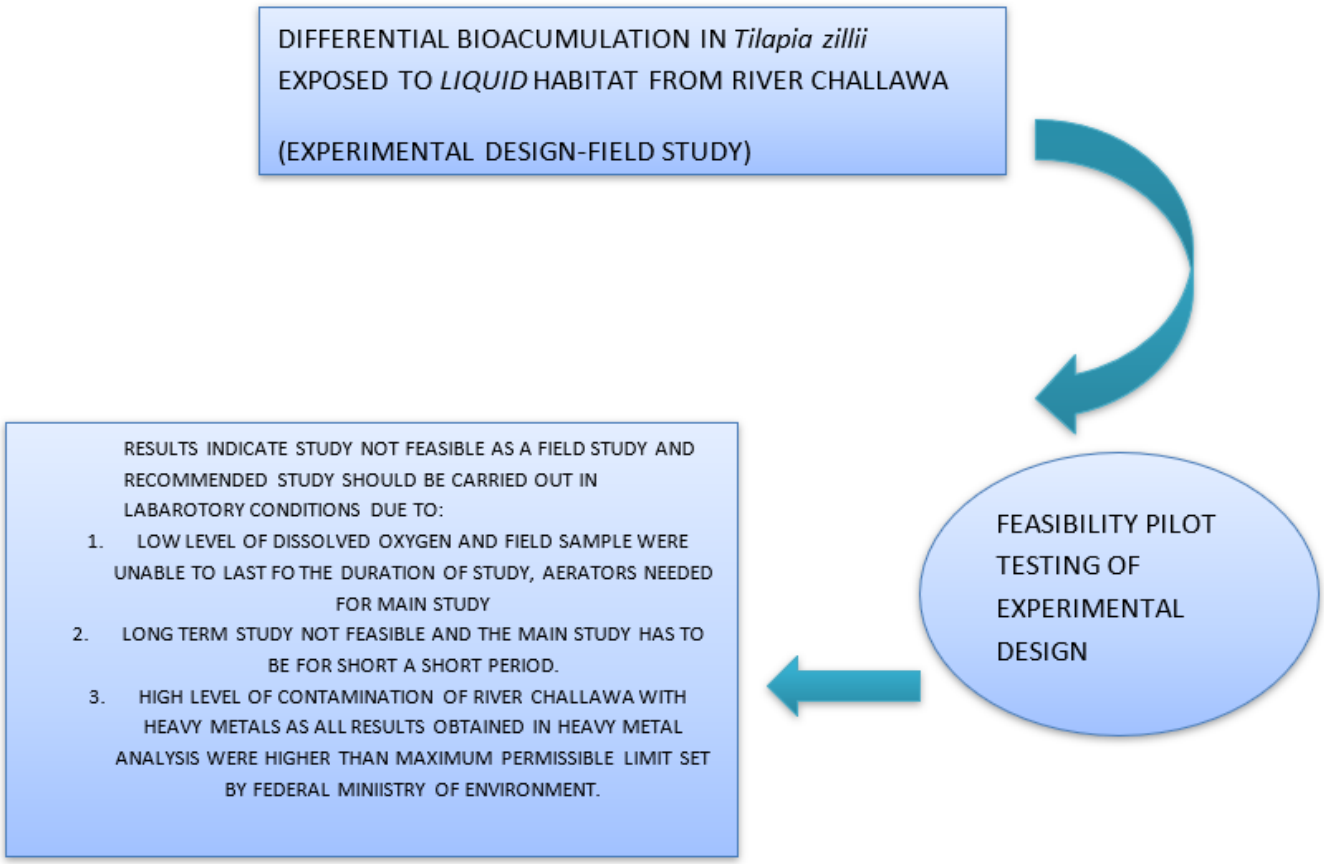
Abstract:- Pilot testing was undertaken prior to a bioassay to be carried out using Tilapia Zillii as the test model which would be exposed to liquid habitats from River Challawa, Kano State, to access the differential bioaccumulation of heavy metals (Pb, Cr, Cd, Al, Zn, Cu and Fe) to liver and skeletal muscles. A pilot study is a reflection of the procedure of the main (large scale) study and is used to validate the feasibility of every part of the study protocol. From the physicochemical and heavy metals of the test liquid habitat and the control, all the parameters assayed for where above the maximum permissible limit set by Federal ministry of Environment for surface water, dissolved oxygen however was below 6.0 mg/L which is required for optimal metabolism of Tilapia zillii and subsequently, aerators were introduced to increase their chances of survival throughout the period of the bioassay. After the exposure for 24 hours to the test liquid habitat and control, liver and skeletal muscle tissues were analyzed for heavy metals and iron had the highest in both tissues and across the liquid habitats, with the concentration of 4.138 to 9.276 mg/kg for liver tissues across the liquid habitats and 2.812 to 6.857 mg/kg for skeletal muscle across the liquid habitats,. Finding of the pilot testing, indicate that the feasibility of the main (large scale) study is achievable with alteration in few areas such as: introduction of aerators and the duration.

Keywords:- *Pilot Testing, Bioaccumulation, Feasibility, Heavy Metals.*



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I. INTRODUCION

Accumulation of heavy metals in the aquatic environment has direct consequences for human and the ecosystem, the most significant problem associated with heavy metals in the environment include accumulation through the food chain and persistence in nature with their toxicity (King and Jonathan, 2003;Dimari et al.,2008). The contamination of fresh water body with a wide range of pollutant has become a matter of great concern over the last decades (Vutukuru, 2005; Dirilgen, 2001), the natural aquatic system may extensively be contaminated with heavy metals released from domestic, industrial and other man-made activities (Velez and Montero, 1998).

Fishes, being major component of most aquatic habitats have been recognized as good bioaccumulators of organic and in-organic pollutants (King and Jonathan. 2003); the extent to which fish binds and accumulate heavy metals can be a bioindicator of the extent of potential toxicity arising from the ingestion of the fish (Jizierska and Witerska,2006).

Bioaccumulation occurs when an organism absorbs a potentially toxic substance at a rate faster than that, at which it is lost by catabolism or excretion. The degree of contamination is determined by the biochemical process of heavy metals binding to tissues, the binding is a function of both bioavailability and affinity of the metals in the tissue (Jizierska and Witerska,2006).the bioaccumulation of heavy metals in fish is an important factor in the degree of contamination of fish and by extension the risk level to man, accurate assessment of both the bioaccumulation of heavy metals and their bioaffinity for different tissues would shed light to the key question of the extent of the risk posed to human consumption of fish(Ovye *et al.*,2019).The accumulation of metal in fish in sub lethal exposure is time dependent. Usually, in the initial period of exposure metal is absorbed and accumulated at a high rate, and then the level stabilizes when equilibrium of metal uptake and excretion rate is attained. Metal distribution in various organs is also time-related. Accumulation of metals in the organs of fish is a function of uptake and elimination rates, and metal concentrations in various organs may change during and after exposure, according to various patterns. The effect of time on metal distribution within the organism is a complex issue due to different affinity of various metals to the tissues of various fish species (Jezierska and Witeska, 2001).

Most exposure studies (bioassay) of heavy metals carried out using fish species had made use of chemical additives comprising of few reference heavy metals of known concentration under laboratory condition (Machino *et al*.,2014), since field samples often contain unidentified component, appropriate referencing of all toxicant may not be practical, thus whole sample analysis with a bioindicator animal (such as fish) was proposed by United State Environmental Protection Agency to circumvent this limitation (Wieber, 1993). By exposing test organisms directly to field samples, the toxicity of any chemical including those not on the standard test list can be revealed in a single test, in addition bioavailability of heavy metals influenced by its affinities to the tissue of the organism as well as other unfavorable factor could be uncovered(Chu and Chow,2002). Contamination of freshwater bodies with a wide variety of pollutants especially heavy metals has been a matter of concern over the decade, not only because of the threat to public water supply but also the damage to aquatic life and their ecosystem, humans consume mostly the muscle of fish and the liver a representative level of contamination of the organism due to the fact that it is the main organ of detoxification. Bioaccumulation studies at the cellular level could be a critical factor in determining the extent of contamination of tissues and also to the degree of hazard that could result from the consumption of these fish by end consumers.

Prior to undertaking these bioassay using Tilapia zillii as a test sample exposed to liquid habitat from River Challawa to assess the differential bioaccumulation of some heavy metals to muscles and liver tissues of the fish, the main study covers obtaining live Tilapia zillii from Tiga Dam and subsequent exposure of the live samples to liquid habitats from two sampling stations using a modified enclosure in the form of the Malian trap, a pilot study was undertaken to determine the feasibility of the study. A pilot is a reflection of the procedure of the main study and is used to validate the feasibility of every part of the study protocol (Junyong, 2017). Analyzing its feasibility prior to performing the main study (also known as the full study or large-scale main trial) can be very beneficial for this purpose. A pilot study is the first step of the entire research protocol and is often a smaller-sized study assisting in planning and modification of the main study (Arnold et al., 2009). Historically, pilot and feasibility studies were not usually reported, and nor were they topics of much discussion in the research literature. While to some extent this continues to be the case in educational research, pilot and feasibility studies have recently become the focus of extensive debate in the health related literature. It would be beneficial if similar attention were given to pilot and feasibility studies in the broader research context (Fraser et al., 2018).

II. MATERIALS AND METHOD

- *Materials*

analytical grade chemicals were used without further purification in this study, Hydrochloric acid (99.9%), Nitric acid (90,00%) obtained from Sigma-Aldrich was used for the wet –aqua regia digestion, digital conductivity meter (Ddb-303a) was used to measured electrical conductivity , Double beam atomic absorption spectrophotometer (Dw-AA320NR) was used to measure the heavy metals, 752UV-Vis spectrophotometer was used to measure nitrate content of the liquid habitats, PH820 pH meter was used to measure the pH , Tilapia zillii was obtained from Tiga dam with the assistance of local fishermen using a cast net.

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➢ *Study Area*

River Challawa is the second largest River system in Kano state After River Kano, it is about 50km in length flowing eastward to finally join River Hadejia (Hussain and Ibrahim, 2017).it has a confluence with River Kano at Tamburawa that is located 20km south of Kano metropolis, the River represent major source of water for agricultural, industrial and domestic activities in Kano metropolis and environs (Kawo and Daneji, 2011). A major industrial layout Challawa industrial estate is sited near the River and majority of the effluent from the estate often end up drained into the River (Mustapha et al., 2019). On the other hand, Tiga dam was built between 1971 to 1974 by the administration of Governor Audu Bako in an attempt to improve food security through irrigation, the dam covers an area of 178 square kilometers and has a maximum capacity 2,000,000 (two million) cubic metres (Edwards, 2002). Irrigation and fishing are the major activities that go on in the dam and informed the use of the dam as a control for this study. Two sampling stations located in the River Challawa namely: station 1 and station 2, station 1 is location upstream before the point where the effluent enter the River and station 2 downstream after the point of entry point of effluent as shown in figure 1.

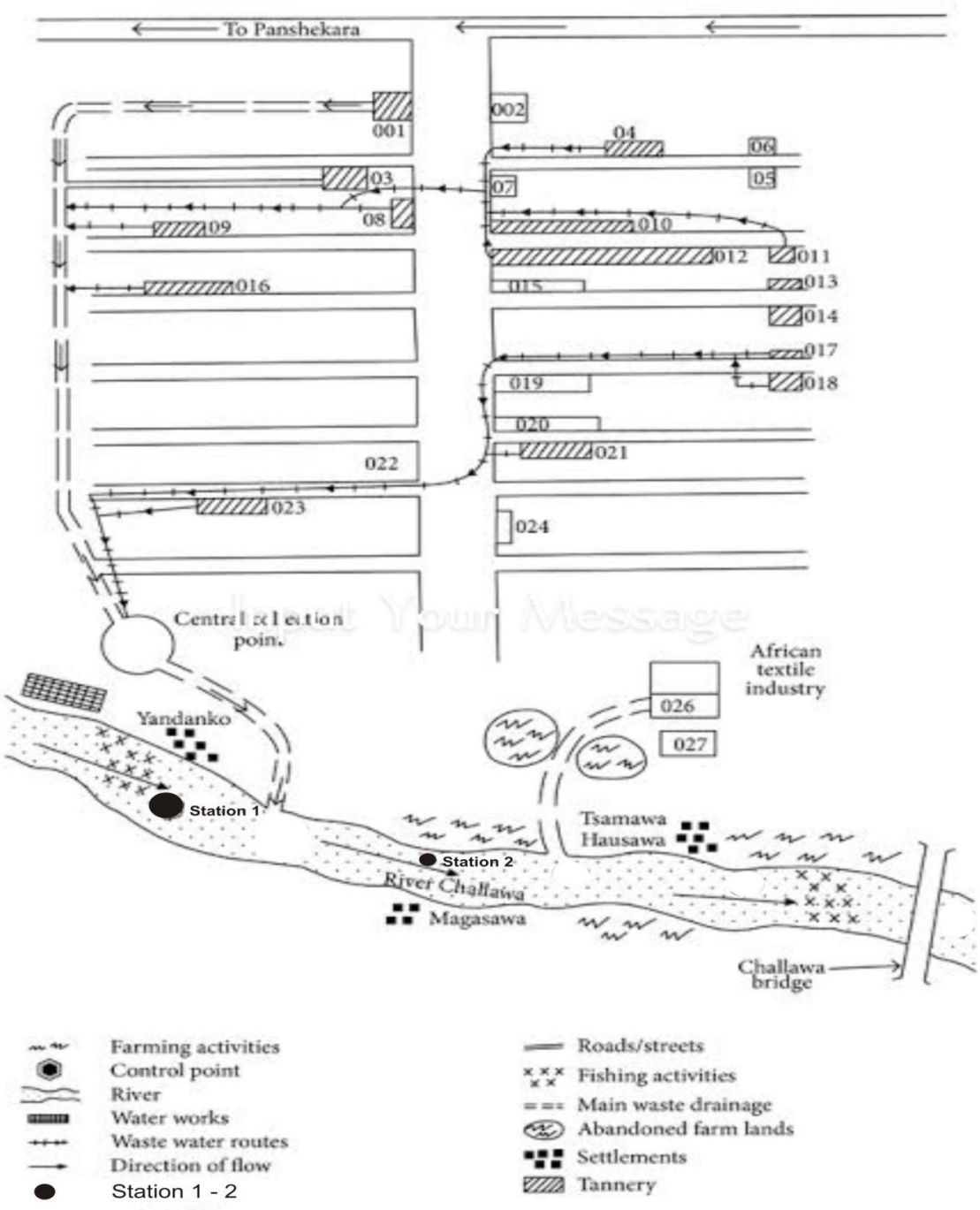

Fig 1:- A Graphical Sketch of Challawa Industrial and River Challawa Showing the Sample Stations.

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➢ *Collection of Test Sample*

For the purpose of these pilot study 90 fishes (tilapia zillii) were obtained from Tiga dam in Kano state, weighing about 60-100gwith the assistance of local fishermen, the samples were move to a large plastic container containing water and transported to the site of the study, after which they were administered anti-stressant (Bio-elite Anti –stress ®),the fish were divided into three groups namely; station 1(30 fishes), station 2 (30 fishes) and control (30 fishes). and where kept in the enclosure (modified Malian trap), the enclosure is cylindrical in shape and has a frame made up of cane covered with polyamide synthetic netting with stretched mesh size of 25.4nm, there was no entry point in the enclosure as no fish was intended to be caught, the enclosure had a length of 2.0 metres and breadth of 1.4 metres and were. The control group was treated to liquid habitat from where the fish were obtained (Tiga dam) and were kept in a polyvinyl chloride (PVC) tank for the same period as other groups. PVC materials were used due to the stability of materials used and reduced effect of leaching of materials. All grouped samples were fed with commercial feed (fish meal: protein content 57.7%, fat 1.8% conversion ratio 2:1)

➢ *Sample Collection, Preparation and Heavy Metal Analysis.*

Before the period of exposure the different liquid habitats were collected and some heavy metal concentration (Pb, Cr, Cd, Al, Zn, Cu and Fe) of the liquid habitat and physiochemical parameters. Heavy metals analysis were determined using atomic absorption spectrophotometer; while. After the period of exposure (24 hours), 2 fishes were withdrawn from each station with the aid of a gill net. After obtaining the sample, samples from the various group were dissected to obtain the tissues namely; the liver and muscle tissue. The muscle tissue was collected from the left side of the fish, above the lateral line and between the dorsal fin and the caudal fin; this is to prevent contamination by the content of the abdominal cavity (POPs kits, 2012). The liver was subsequently obtained with clean equipment and a new latex glove was use for each fish. Samples of liquid habitat of the experimental animals and their control were collected and analysed. And to assess the rate of bioaccumulation with time, 2 fishes were withdrawn whole tissues of the test sample was obtained from the two test station after varied period of exposure namely; 1st hour, 2nd hour,3rd hour, 4th hour,6th hour,10th hour,14th hour,22nd hour and 24th hour.

Acid digestion were followed by atomic absorption spectrophotometry, the fish samples organs (liver and muscle) was collected fresh and then dried separately for 24 hours to constant weight in an oven at 105 °C. An aqua regia wet method of digestion as described by Ang and Lee (2005) was used. Briefly, to 1 g of ground samples, 18 ml of a fresh mixture of hydrochloric acid and nitric acid in the ratio of 3:2 was added, the mixture was boiled over a water bath (95 °C). After complete digestion, the residue was made up to 50 ml with distilled water. Digested sample was stored in pre-cleaned polyethylene bottles until analysis using atomic absorption spectrophotometer.

➢ *Bioaccumulation Factor*

Bioaccumulation refers to the process by which a chemical substance is absorbed in an organism by all routes of exposure in natural environment, that is, dietary and ambient environmental sources (Arnot *et al*., 2003). It could also be referred to as the increase in the level of xenobiotic in biological organism overtime especially when compared to the level of the xenobiotic in the environment (Gupta *et al*., 2013). 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 (eqn 1) below.

$$BAF = \frac{concentration\ of\ chemical\ in\ organism\ or\ tissue}{concentration\ of\ chemical\ in\ the\ environment} \quad -----eqn\ 1$$

Source: Jezierska and witesta, 2001

Bioaccumulation factor is one of the methods of assessing bioaccumulation in an organism and is important for evaluating the risks a chemical possess to humans and the environment (Arnot *et al*., 2003). It indicates how persistent a xenobiotic is in the biological organism especially to biotransformation and subsequently to excretion.

➢ *Physicochemical Properties*

Physicochemical analyses of the different liquid habitats were carried out according to methods described by America Public Health Association (APHA) 1999. The parameters analysed include: pH, temperature, electrical conductivity, dissolved oxygen, sulphate, total alkalinity, total suspended solids, total dissolved solids, chloride, and nitrate.

The presence of chloride in natural water are mainly attributed to dissolution of salts deposit in the form of (Cl) ions in alkaline or neutral solution, potassium chloride indicate the end point of titration of silver nitrate and chloride, yielding silver chloride.

Fifty (50) ml of filtered sample is placed into a conical flask and 0.5 ml of potassium chromate indicator is added and titrated against standard silver nitrate till silver dichromate (AgCrO4) starts precipitating. Chloride is then determined by the expression in eqn 8.

Chloride= (A-B(N)(35.45))/(sample taken in ml)--------Eqn 2
A= volume of silver nitrate consumed by the sample
B= volume of silver nitrate consumed by the blank
N= normality of silver nitrate

Nitrates react with phenol disulphonic acid and produce a nitrate derivative which in alkaline solution develops yellow colour due to rearrangement of its structure. The colour is directly proportional to the concentration of nitrate present in the sample.

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50 ml of sample was pippeted into a porcelain dish and evaporated to dryness in a hot water bath, 2 ml of phenol disulphonic acid was added to concentrated ammonium hydroxide and distilled water was added with stirring to make it alkaline. The mixture was filtered into a beaker and made up to 50 ml with distilled water and absorbance read at 410 nm using a spectrophotometer after the development of colour concentration of nitrate is given in eqn 3 below.

Nitrate (mg/l) = (Absorbance of sample ×concentration o standard ×100)/(Absorbance of standard ×volume of samples)---Eqn 3

Total alkalinity is the total concentration of bases in water expressed as parts per million, these bases are usually Bicarbonates (HCO_3) and Carbonate (CO_3)and they act as a buffer system that prevents drastic changes in PH. It is measured by a titrimetric method with Hcl used as the acid for titration. The expression for total alkalinity is given by Eqn 4 below.

Total Alkalinity (mg/l) = (ml of Hcl with Phenohalein and methyl orange × normality of Hcl ×1000 ×50)/(ml of sample)-Eqn 4

Dissolved solids are solids that are in a dissolved state in solution. Water with high dissolved solids severally can induce unfavorable physiological reaction in the transient consumer. The difference in the weight of total solid and the total suspended solid expressed in mg/l (Eqn 5). 50 ml of rigorously shaken sample is filtered into a pre weighted fibre disk fitted into a suction pump and washed successively with distilled water. The filtrate is now heated in evaporating dish of known weight at 103 ºC in an oven until dryness is achieved.

TDS (mg/l)=(〖W_(f-) W〗 _i× 1000× 1000)/(volume of sample)----------Eqn 5
Wi = Initial weight of evaporated dish
Wf = final weight of evaporated dish.

Total suspended solids (TSS) are those solids which are retained by the filters of 1 micrometer pores as they are called non-filterable solids. Their quantity can be determined by passing a known volume of water sample (usually 50 ml) through a glass fiber filter apparatus and the filter is carefully removed from the filtration apparatus and dried for an hour at 103 °C in an oven, cooled in an dessicator and weighed for construct weigh. TSS is given by the expression (Eqn 6).

TSS (mg/l)=W_(1-) W_2×1000----------Eqn 6

for dissolved oxygen, dilution was prepared appropriate, for the samples to be tested and the diluted samples are transferred to corresponding glass stoppered bottles,heated to 200C and then dissolved oxygen of the sample was measured in mg/L using dissolved oxygen meter with electrode.

W1= weight of dried glass fibre filter and residue
W2= weight of glass fibre filter disk before filtering.

III. RESULTS AND DISCUSSION

➢ *Preliminary Report*

From the pilot study it was observed that the feasibility of the research as a field study was not achievable, this was due to the inability of the test model to survive for a long duration, as they lasted for barely only 6 hours on introduction to the liquid habitats.

To overcome these challenges faced during the pilot study the following measures were taken:

- The research was reverted from a field study to a laboratory study, and the fish sampled was kept in large PVC containers containing the various different liquid habitats.
- Aerators were introduced to increase oxygen level and also improve the chances of survival of the Tilapia zillii.

➢ *Result Of Pilot Study*

The pilot study which was undertaken by introducing *Tilapia zillii* to the different liquid habitat from the control and the two stations and exposing them for a period of 24 hours, samples were collected after 1, 2, 4, 6, 10, 14, 22 and 24 hours from the two test liquid habitats. From the results obtained there were increase in the level of these heavy metals after different duration of exposure as presented in figure 2 and figure 3. There was general increase in the level of all heavy metals after exposure for 1 and 2 hours in both test liquid habitat, the increase in the heavy metals was not continuous, as the levels of some heavy metals reduced with respect to preceding period after increased period of exposure. Also from the pilot study it was observed that the feasibility of the research for the period proposed was in doubt unless certain steps were taken, this was due to the inability of the test model to survive for a long duration, as they lasted for barely only 6 hours on introduction to the liquid habitats. To investigate the reasons behind this observation, a physicochemical analysis of the different test liquid habitat was carried out.

Analysis of physicochemical parameters of the sampled liquid habitat is presented in table 1. It shows that electrical conductivity had values ranging from 990 µs/ms to 3270 µs/ms , TDS has values ranging from 155 mg/l to a maximum of 1568 mg/l, TSS had its low values of 55.48 mg/l and its highest value of 55.48 mg/l. all parameters were above safety limits set by the Federal ministry of environment for surface water bodies with the exception of sulphate which had its highest recorded concentration as 235.79 mg/l, being below the maximum permissible limit of 500 mg/l. Dissolved oxygen was never up to the required standard of minimum of 6 mg/l, DO is very essential in fish as it is use for aerobic metabolic for energy production. To overcome these challenge aerators were introduced to increase oxygen level and also improve the chances of survival of the *Tilapia zillii*. Physicochemical analysis of the three liquid habitats presented a poor condition of the habitats, with parameters such as electrical conductivity; TDS and TSS were above limits set by the federal ministry of environment for surface water. DO which was not up to the required minimum of 6.0

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mg/l, DO which is very essential in fish for aerobic metabolism leading to energy production. *Tilapia* has been reported to breed effectively with a DO of 4.0 mg/l and optimally at 6.5 mg/l (Broaders *et al.*, 2005).

The levels of TDS and TSS were indicative of the high level of organic matter in the various habitats especially the test liquid habitat, which serve as a suitable medium for microorganism which compete with fish and other aquatic animals for the limited available oxygen, these findings agree with that of (Putshaka *et al.*, 2015). In the course of these pilot studies the fish samples exposed to the two test liquid habitats (upstream and downstream) display abnormal responses including: high water surface frequency with mouth and opercula opening erratic swimming which agrees with report by (Dahunsi *et al.*, 2012).

Increase in temperature increases the rate of accumulation of heavy metals by increasing the solubility of heavy metals, tissue permeability and metabolic rate as reported by (Perlman, 2013). Physicochemical factors such as: temperature, pH, total acidity, total hardness and salinity affects the uptake and accumulation of heavy metals in tissues of fishes, as increase in any of this factors will directly or indirectly affect the rate of accumulation of heavy metals in fish (Jizierska and Witeska, 2006).

Parameters	Control	Station 1	Station 2	MPL by Fed. Min. of Envir.
Conductivity (µs/ms)	990 ±6.255	2530 ±12.345	3270 ±20.456	1000
TDS (Mg/l)	155 ±4.774	1007 ±7.667	1568 ±11.222	500
TSS (Mg/l)	58.78 ±2.667.	53.68 ±3.456	55.48 ±6.778	30
Chloride (Mg/l)	179.250 ±11.223	214.750 ±9.897	397.010 ±8.342	350
Total alkalinity (Mg/l)	23.20 ±2.345	41.20 ±2.348	48.80 ±3.412	250
Sulphate (Mg/l)	53.98 ±2.346	177.74 ±8.726	235.79 ±12.663	500
Phosphate (Mg/l)	6.15 ±0567	5.25 ±0.456	9.93 ±1.332	3.5
pH	6.50 ±0.78	6.80 ±0.679	8.10 ±1.876	6.5-8.5
DO (Mg/l)	3.80 ±0.568	1.08 ±0.334	0.86 ±0.115	Minimum of 6

Table 1:- Physicochemical analysis of Pilot Study Liquid Habitats and maximum permissible limit set by Federal Ministry of environment for Surface water

Legend: MPL= Maximum Permissible limit; TDS= Total dissolved solute; TSS= Total suspended Solute; DO= Dissolved Oxygen

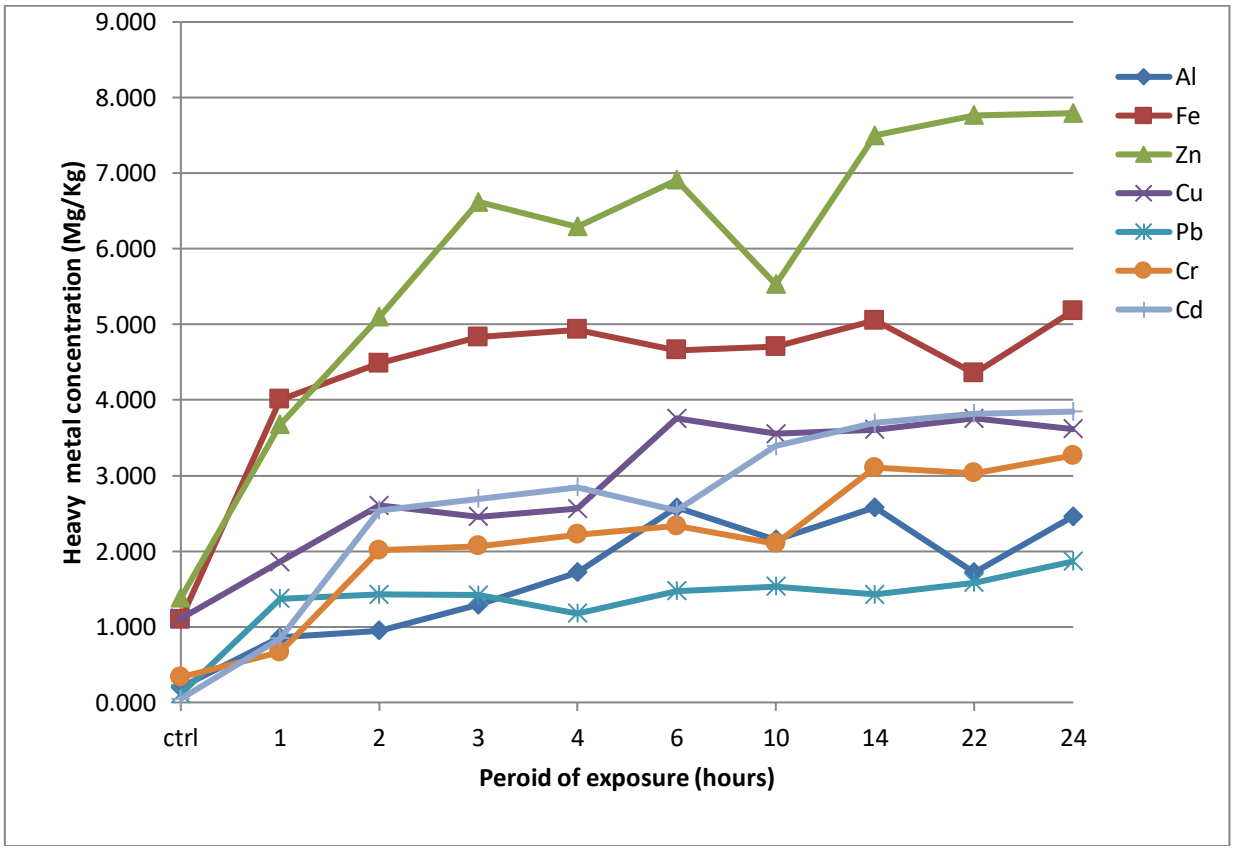

Fig 2:- Showing a graph of concentration of heavy metals in whole tissues of *Tilapia zillii* against time of exposure to liquid habitat from station 1 upstream of River Challawa.

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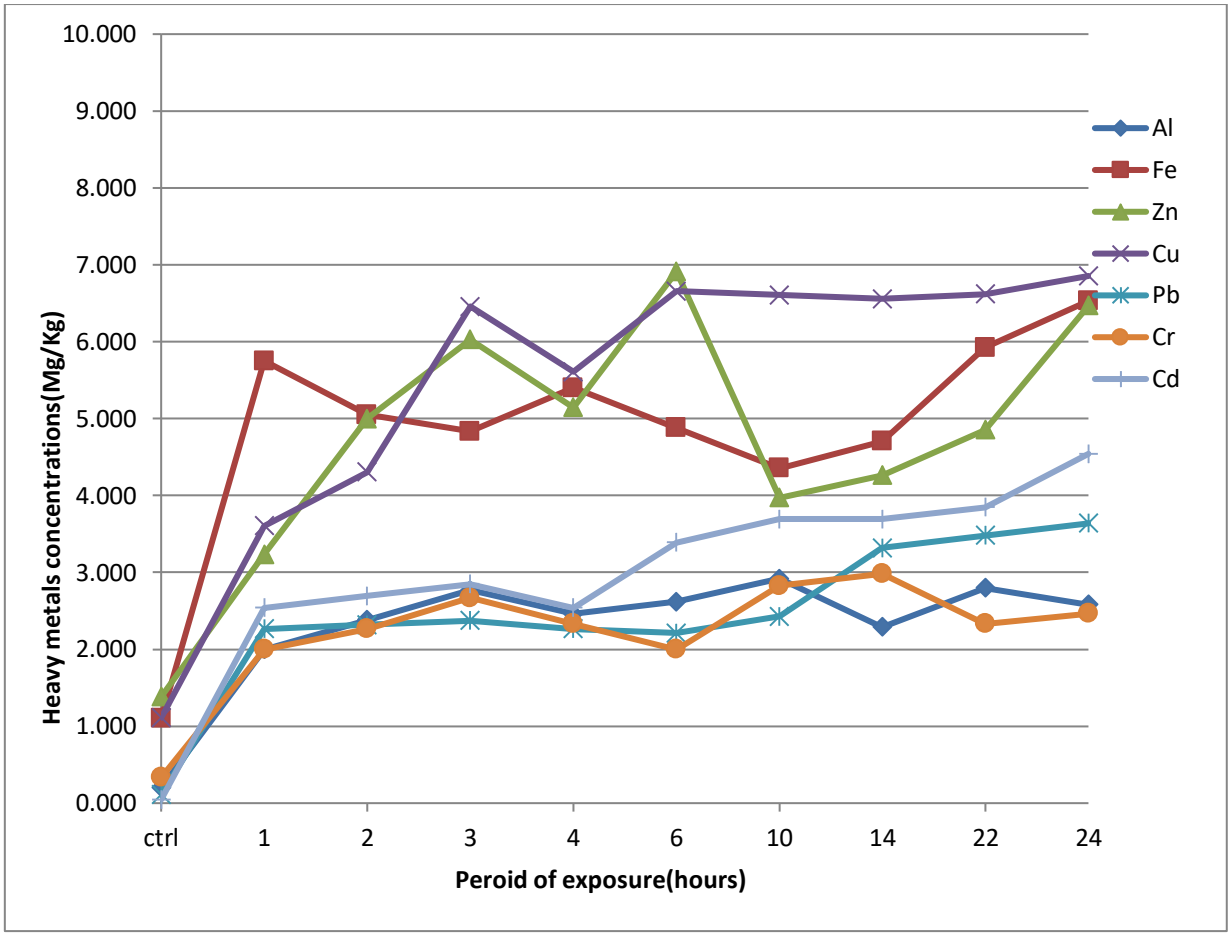

Fig 3:- Showing a graph of concentration of heavy metals in whole tissue of *Tilapia zillii* against time of exposure to liquid habitats from station 2 downstream of River Challawa.

Also the heavy metal levels of the liquid habitat was assessed and results are as presented in table 2. From the results presented in table 2, analysis of heavy metals shows that iron which had the highest concentration with values ranging from 5.750 ppm to as high as 12.822 ppm, while zinc had a concentration ranging from 4.142 ppm to as high as 8.720 ppm, and lead which had the lowest concentration had values ranging from 0.217 ppm to as high as 1.851 ppm**.** The levels of all the heavy metals of the test habitats are deleterious, unsafe and were all above the safety limit set by the Federal Ministry of Environment for surface water bodies. Results from heavy metals analysis of the test liquid habitats shows that the heavy metals examined were all above safe limits stipulated by the federal ministry of Environment and are considered deleterious, unsafe and poses a threat to the survival of aquatic life, this results agrees with findings of (Putshaka *et al*., 2015). *Tilapia zillii* accumulate heavy metals in their tissue after exposure to these heavy metals in the liquid habitats, zinc was the most abundant metal in the whole tissue of *Tilapia zillii* after exposure for 24 hours this finding is in agreement with work by Orata and Birgen, (2016) which studied three different fish species and found zinc to be the most abundant in all the tissues examined.

Metal	Control	Station 1	Station 2	MPL by Fed. Min. of Envr.
Cadmium	0.012 ±0.06	1.632 ±0.45	4.021 ±0.80	0.010
Chromium	0.083 ±0.02	1.002 ±0.33	4.133±0.25	0.500
Lead	0.217 ±0.56	0.824 ±0.72	1.851 ±0.15	0.100
Aluminium	0.403 ±0.80	1.411 ±0.63	3.420 ±0.42	0.900
Iron	5.750 ±0.65	8.530 ±0.33	12.822 ±0.13	0.500
Zinc	4.142 ±0.32	6.861 ±0.51	8.720 ±0.19	0.200
Copper	1.327 ±0.19	3.850 ±0.030	4.215 ±0.28	0.010

Table 2:- Mean Heavy metal concentration ± Standard Deviation in Control, Station 1, Station 2 and maximum permissible limit set by Federal Ministry of Environment for surface water.
Legend: MPL= maximum permissible limits by Federal Ministry of Environment. (Federal Ministry of Environment, 2011)

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	Cu(mg/kg)	Cd(mg/kg)	Cr(mg/kg)	Zn(mg/kg)	Al(mg/kg)	Pb(mg/kg)	Fe(mg/kg)
L-C	0.421±0.01	0.008±0.01	0.002±0.01	2.457±0.06	0.014±0.01	0.028±0.01	4.138±0.09
SM-C	0.209±0.03	0.010±0.01	0.001±0.01	0.467±0.01	0.006±0.01	0.016±0.01	2.812±0.04
L- ST 1	2.441±0.01	0.948±0.00	0.411±0.02	5.282±0.07	0.255±0.02	0.327±0.03	6.859±0.06
SM- ST 1	1.146±0.02	0.446±0.02	0.206±0.01	4.212±0.05	0.128±0.01	0.116±0.03	4.894±0.02
L- ST 2	3.441±0.03	1.948±0.04	2.148±0.05	7.488±0.08	2.416±0.02	0.672±0.05	9.276±0.15
SM-ST 2	2.164±0.05	0.981±0.01	0.914±0.01	5.921±0.04	1.211±0.03	0.247±0.03	6.857±0.04

Table 3:- Mean Concentration ± standard deviation of heavy metals (mg/kg) in liver and muscle tissues of Tilapia zillii exposed to three test liquid habitats

Legend: LC = Liver control; SMC = skeletal Muscle Control; LS1 = Liver station 1; SMS1 = Skeletal Muscle station 1; LS2 = Liver station 2; SMS2 = Skeletal Muscle station 2

After exposure of *Tilapia zillii* the different liquid habitats for 24 hours, heavy metals analysis was carried out for body tissues (liver and skeletal muscle tissues) and liquid habitats to obtain the bioaccumulation factor for liver tissues and skeletal muscle tissues (figure 4). Results of heavy metals analysis in the two tissues of liver and skeletal tissues of *Tilapia zillii* as presented in table 3 shows that iron had the highest concentration in the tissues with concentration of 4.138 mg/kg (liver), 2.812 mg/kg (skeletal muscle) for control liquid habitat, while 6.859 (liver) 4.894 (skeletal muscles) for the liquid habitat upstream and 9.276 (liver) and 6.857 (skeletal muscles) for liquid habitats downstream. Lead had the least observed concentration with the liver having a concentration ranging from 0.028 mg/kg to 0.672 mg/kg and skeletal muscles having a concentration that ranged between 0.006 mg/kg to 0.247 mg/kg across all the three test liquid habitats. 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 limit set. The concentration of heavy metal in the different habitat were in this order Fe > Zn >Cu > Cd > Al > Cr > Pb. 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.

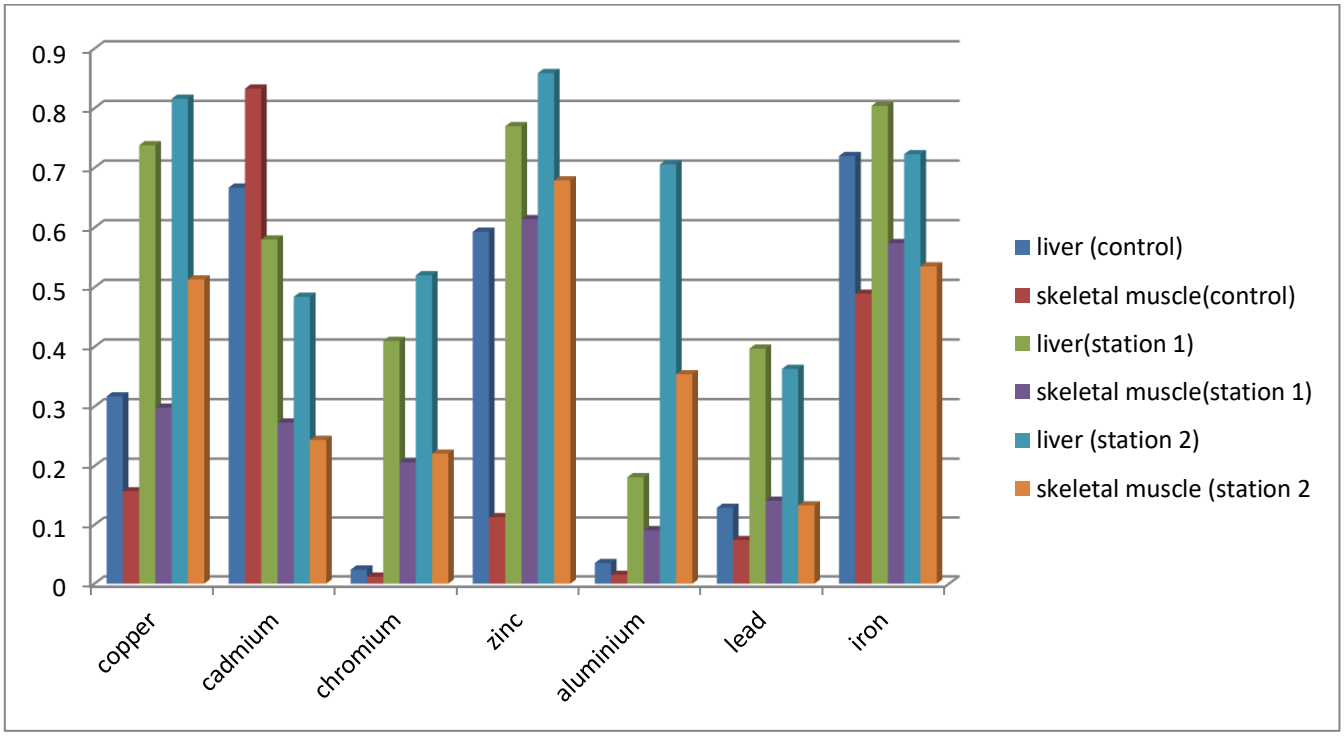

Fig 4:- Bioaccumulation factor for liver and skeletal muscles tissues of *Tilapia zillii* exposed to the test liquid habitats for a duration of 24 hours.

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 in a number of studies (Rashed, 2001; Dural *et al*., 2006; Ploetz *et al*., 2007; Agah *et al*., 2009).

Bioaccumulation factor calculated for liver tissues and skeletal muscles tissues of *Tilapia zillii* exposed to the three liquid habitats, of these liquid habitats. The downstream habitat had higher bioaccumulation factors with the exception of iron where the control had high values; these patterns were also maintained in the bioaccumulation factors of heavy metals in the tissues of the liver and skeletal muscles of *Tilapia zillii*. This observed pattern is elucidated by the assertion by (Jizierska and Witeska, 2006) that the more the concentration in the environment the more they can be taken up and accumulated in fish tissue.

The liver bioaccumulated more heavy metals than the skeletal muscles as contained in the results of the bioaccumulation factor of heavy metals for the two tissues across the three test habitats, Bervoets and Blust (2003) and Uysal *et al*., (2009) in their separate submissions had reported that muscle tissue had a weak accumulating potentials and accumulate lower level of heavy metals when compare to the liver. Conversely, the liver is considered to have a high accumulating ability due to the presence and activity of metal binding proteins such as MTs, which can bind with heavy metals and thus reducing their toxicity and allowing the liver to accumulate high concentration of these toxic heavy metals (Wu *et al*., 2006; Ploetz *et al*., 2007; Uysal *et al*., 2009).

IV. CONCLUSION

From the findings of this pilot study, it is concluded that there was a high level of heavy metals in River Challawa which bioaccumulates in liver and skeletal muscle tissues of *Tilapia zillii.* The feasibility of large scale study is achievable with certain alteration like introduction of aerators to enhance the chances of survival of the test sample. Long termed bioassay studies with the liquid test habitats are not achievable with *Tilapia zillii* as they have a very slim chance of survival in the long run. Although the skeletal muscle tissues of *Tilapia zillii* accumulated lower level of heavy metals when compare to the liver tissue, consumption of fish could pose serious threat to humans and other predators of fish.

- *Credit Authorship Contribution Statement*

Ovye, A.; conceptualization, methodology, resources, writing of original draft, writing –review and editing, Kabiru B.A; methodology, writing-review and editing. Oyekunle, O.A.: methodology, writing-review and editing. Agbara, S.A.; data curation, writing –review and editing. Faiza, G.H; writing –review and editing

- *Declaration of Competing Interest*

The authors declare that they have no known competing financial interest or personal relationship that could have appeared to influence the work reported in this paper

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