International Journal of Scientific Research in Chemical Sciences
Vol.6, Issue.2, pp.1-7, Apr (2019)

Research Paper

E-ISSN: 2455-3174

Detection and Quantification of Heavy metals Concentrations in Water and Muscle Tissue of Tilapia Zilli: A Case Study of Gwale Pond, Kano, Kano state Nigeria

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Available online at: www.isroset.org

Received: 09/Apr/2019, Accepted: 14/Apr/2019, Online: 30/Apr/2019

Abstract- Fresh water system has been a natural irreplaceable resource ever due to its potential to providing a continuous natural source of water for domestic and industrial purposes as well as a favorable breeding atmosphere for the growth of aquatic species. However, human activities such as illegal dumping of refuse in water render the safety of such water highly unbearable for drinking, domestic as well as economic activities such as irrigation and fishing. In the present study, heavy metals such as Ni, Cu, Zn and Pb were detected and quantitatively analyzed using atomic absorption spectrophotometer (AAS) in both water and muscle tissues of T. Zilli of Gwale Pond in Gwale Local Government Area of Kano State, Nigeria. The results of the one-way ANOVA test for both heavy metals distributions in the pond water as well as muscle tissue of T. Zilli were found to be highly significant ($P < 0.001$). Subsequent post-hoc test (Bonferroni's procedure) suggests that all the possible 4 pairs of mean scores are significantly different. The mean concentrations of the detected heavy metals in the pond water were found to be 2.75±1.37, 4.55± 1.81, 12.59±2.15 and 3.32±0.86 mg/L whereas the mean concentrations of the detected heavy metals in the muscle tissue of T. Zilli were found to be 4.50±0.69, 4.19±0.72, 13.48±2.31 and 5.40 ±01.92 mg/Kg corresponding to Ni, Cu, Zn and Pb respectively. The mean metals concentration values of Ni and Pb in both pond water and muscle tissue of T. Zilli were found to exceed the guidelines for the drinking water quality maximum permissible level of (Ni = 0.07 mg/L and Pb = 0.01/2 mg/L) as reported by FAO/WHO whereas Cu and Zn mean metals concentration values of (Cu =30 mg/L: Zn = 40 mg/L) in both pond water and muscle tissue of T. Zilli were found to have occurred within the range of the maximum permissible level of the standard water quality criteria recorded by FAO. The relatively higher values recorded for the heavy metals concentrations in the present study is an indication of water contamination which is well correlated with the relative higher values obtained for the pond water physicochemical parameters.

***Keywords*:** Detection, quantification, physicochemical parameters, concentrations of heavy metals, muscle tissue, tilapia zilli.

I. INTRODUCTION

Environmental pollution is a major threat to flora and fauna in the present contemporary world. Both air, land and water pollutions are considered the major sources of environmental pollution worldwide. Particulate matter, heavy metals and gaseous contaminants can lead to disastrous effect in both terrestrial and aquatic species provided human activities did not cease to exist. Water in particular, is the only natural source where any aquatic organism particularly fish can survive. Clean and healthy water supports a healthy growth of variety of fish species. Although, a variety of natural phenomena, such as; landslide, acid rain, flooding and human activities, such as domestic and industrial waste containing substances that could endanger the life of aquatic organisms, may also affect the healthy growth and safety of fish species in water. Water bodies such as rivers, lakes, streams, ponds, pools, tanks and dams have been reported as the most efficient mining and life sustaining media for fish species [1–3]. Tilapia fish species are among the most copious tropical fish species native of Africa that belongs to the family cichlid. They feed notably on the floating aquatic organisms such as the blue algae, insects and decayed food particles [4]. Despite the fact that they provide their consumers with a tremendous source of food and proteins, they can also serve as a good source of foreign exchange, employment opportunities as well as a reliable source of income for any nation that embarks on their farming. Tilapia fishes are widely explored across different regions in Nigeria due to their wide range of availability as well as their complex detoxification mechanisms through their body systems despite their vulnerability to certain environmentally harmful substances [5]. Examples of environmentally harmful substances include antibiotics, pesticides, herbicides, heavy metals as well as polycyclic aromatic compounds (PCACs) among others. Limbu et *al.*(2018) reported that chronic exposure to low environmental concentrations of legal doses of antibiotics could damage several physiological functions, nutritional metabolism, and subsequently, compromises fish immune system [6]. Heavy metals, which are classes of metals that have large atomic weight in the periodic table and are needed in a relatively trace amount are reported to have been present in water and are therefore absorbed by fishes and other aquatic species through a mechanism known as bioaccumulation [7–10]. Their presence in high amount may lead to some adverse effects, which could lead to certain health issues to fish consumers or even death to the fish species, thus, the need to detect, quantify and monitor the degree of their presence in a given water body. Their concentrations in several specimens such as water, soil, lettuce, spinach, onion, meat and meat products have been successfully assayed [10–12]. Heavy metals concentrations in fish species such as Cr, Ni, Cu, Zn, Pb, Fe, Cd and As have been qualitatively and quantitatively investigated [13–15]. Several tissues of tilapia species such as gills, livers, muscles and fins also have been exploited for heavy metals content by researchers [15-16]. Though it was argued by Yi et *al.* (2012) that heavy metals concentration variations could exist among fishes with different sizes due to the evolution of distinct ecological needs, swimming behaviors and metabolic activities [3]. A good practice of getting rid of such toxic heavy metals from water could be achieved by employing a versatile approach that is chemically feasible and economically viable. One of the established methods for the removal of heavy metal ions from aqueous solutions is through adsorption processes by means of activated carbon Nano composite [17]. Other processes include application of modified poly-acrylo-nitrile membrane and metal ion trap via incorporation of ethylene diamine tetra acetic acid into a robust metal-organic framework as current technologies are strategically becoming metal ions specific [18-19]. Hence, there is a need for effective monitoring strategy to reduce the risk associated to heavy metals concentrations in water. Rajesh Kumar et *al.* (2018) proposed analysis of heavy metals biomarkers in fish across seasonal variations as one of the crucial strategy to access bio-monitoring of pollution evolution in water [20]. Recently, an optical sensor has been fabricated for monitoring the extent of the deterioration of tilapia fish products as a function of decrease or increase in mesophilic bacteria counts with respect to time of exposure, thus suggesting a sudden competition of bacteria for nutrients, generating oscillations in the population density. This optical sensor is highly nondestructive and since fish and fish products are very much perishable and prone to microbial growth, which is always not easily detected by organoleptic evaluation. Therefore, the analysis of the headspace of fish specimens through gas sensing is an interesting approach to monitor fish freshness to ascertain safe consumption [21]. Because of the gravity of the presence of heavy metals in the living systems of humans, investigating their concentrations in the muscle tissues through detection and quantification became unavoidably crucial. Other factors that contribute greatly to the well-being of the fish species in a respective water body include physical water quality parameters such as turbidity, salinity, odor, color, temperature and chemical water quality parameters which include pH, heavy metals, phosphorus, nitrate and sulphate ions concentrations in a given water source [22]. In the present study, the physico-chemical parameters such as; temperature, pH, conductivity, total dissolve solids (TDS), phosphate and nitrate ions of the water as well as heavy metals (Ni, Cu, Zn and Pb) concentrations are detected and quantified in the muscle tissues of tilapia zilli in Gwale Pond, in the Gwale Local Government Area Council of Kano State, Nigeria.

II. EXPERIMENTAL DETAILS

II.1 Study area

Kano is located at the Sahel Climatic Zone having the annual rainfall period ranging from May to September with the annual rainfall distribution of about 450 to 1050 mm [23]. Details about the study area could be found in our previously reported literature [13].

II.2 Sampling and sample preparation

A total of 70 fresh samples of T. Zilli were analyzed throughout the study. Precisely 10 samples were collected monthly for a period of 7 months (January – July, 2018) in a clean polythene bags and brought into the laboratory for dissection and extraction of muscle tissues. Extracted tissues were dried under a preheated oven at 110^oC and then pulverized into powders.

II.3 Standard solutions

For preparation of standard solutions, the following reagents were used; Pure compounds of anhydrous nickel (ii) chloride [MF; $NiCl_2$, MW; 129.60 g/mol], hydrated copper (ii) nitrate [$(Cu (NO3)_2.3H_2O$, MW : 241.60 g/mol], zinc nitrate hexa-hydrate [MF; $(Zn (NO_3)_2. 6H_2O$, MW : 297.49 g/mol] lead nitrate [MF; $(Pb (NO_3)_2$, MW: 331.20 g/mol], HPLC grade nitric acid [MW; HNO_3, MW : 63.01 g/mol], perchloric acid [MF; $HClO_4$, MW: 100.45 g/mol] and deionized water [H_2O; MW: 18.02 g/mol]. Specific amount of either of the above salts was dissolved in a 1L volumetric flask and appropriately diluted with a deionized water to make 1000 mg/L standard solution. Lower concentrations were made from the standard solution through dilution method.

II.4 Acid digestion of samples

The present study adopted the method reported by Alturiqi and Albedair [12]. Acid mixture (10 mL of 65 % HNO_3 and 30 % $HClO_4$, 4:1 v/v) was added to the beaker containing 1 g of dried sample. The mixture was then digested at 80°C until a transparent solution was achieved. Solution was allowed to cool and subsequently filtered. The filtrate was diluted to 50 mL with deionized water. Analyses of the heavy metals concentrations (Ni, Cu, Zn, and Pb) with the corresponding prepared samples of T. Zilli were done by atomic absorption spectrophotometer (Model; Perkin Elmer 306).

II.5 Determination of physico-chemical parameters

The pond water temperature was measured by immersing a digital thermometer (model: Jenway 100) into the water surface for about 15 – 20 seconds until the temperature reading was stabilized. The total dissolved solid (TDS) and conductivity were also measured using a digital TDS and conductivity meter, HANNA (model; HI 96301) by dipping the probes into the water until the screen shows a stable reading as described by the manufacturers. Reading was expressed in mg/L and µS/cm for TDS and conductivity respectively. The dissolved oxygen (DO) was measured *in- situ* using a portable DO meter (model; HI 9146) in which the probe was inserted into the water until DO reading in (mg/L) was recorded as described by the manufacturers. The nitrate and phosphate ions were determined based on the method adopted by Nafiu et *al*. [7].

II.6 Statistical Analysis

Descriptive statistics were carried out to show mean and standard deviation (SD). The data was subjected to one-way analysis of variance (ANOVA) to determine mean differences between and within groups. Significance was set at two-tailed $p<0.05$ and all statistical analyses were carried out using IBM|SPSS Version 22.0.

III. RESULTS AND DISCUSSION

III.1 Results

Table 1. Means and standard deviations of the physico-chemical values obtained from the Gwale Pond Water across the analysis period (Jan – July).

Parameters	Mean	Std. Deviation
pH	7.57	.65
Temperature (°C)	27.09	2.60
DO (mg/L)	1.97	.94
TDS (mg/L)	1200.00	13.58
Conductivity µS/cm	1600.00	8.53
Nitrate (mg/L)	11.95	.72
Phosphate (mg/L)	2.50	1.12

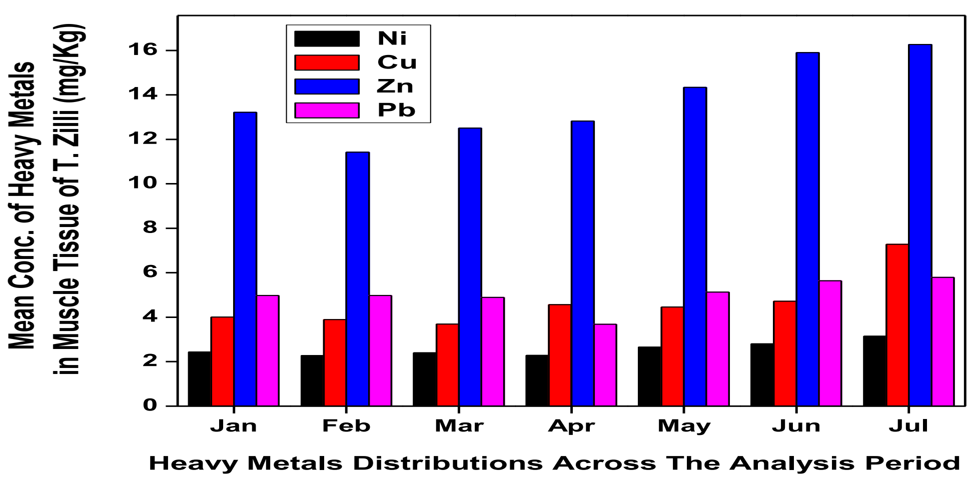

Fig. 1. Heavy metals distributions across the analysis period (Jan – July)

Table 2: Comparing mean concentrations of the four detected heavy metals in the pond water and the muscle tissue of the T. Zilli.

Parameters	**Heavy metals**	**Mean**	**Std. Deviation**	**P value[a]**
Conc. of Detected Heavy Metals in Pond Water (mg/L)	Ni	2.75	1.37	
	Cu	4.55	1.81	
	Zn	12.59	2.15	<0.001[b]
	Pb	3.32	.86	
Conc. of Detected Heavy Metals in Muscle Tissue of T. Zilli (mg/Kg)	Ni	4.50	.69	
	Cu	4.19	.72	
	Zn	13.48	2.31	<0.001[b]
	Pb	5.40	1.92	

[a]One-way ANOVA test

[b]All possible 4 pairs of mean scores are significantly different by post-hoc test (Bonferroni's procedure)

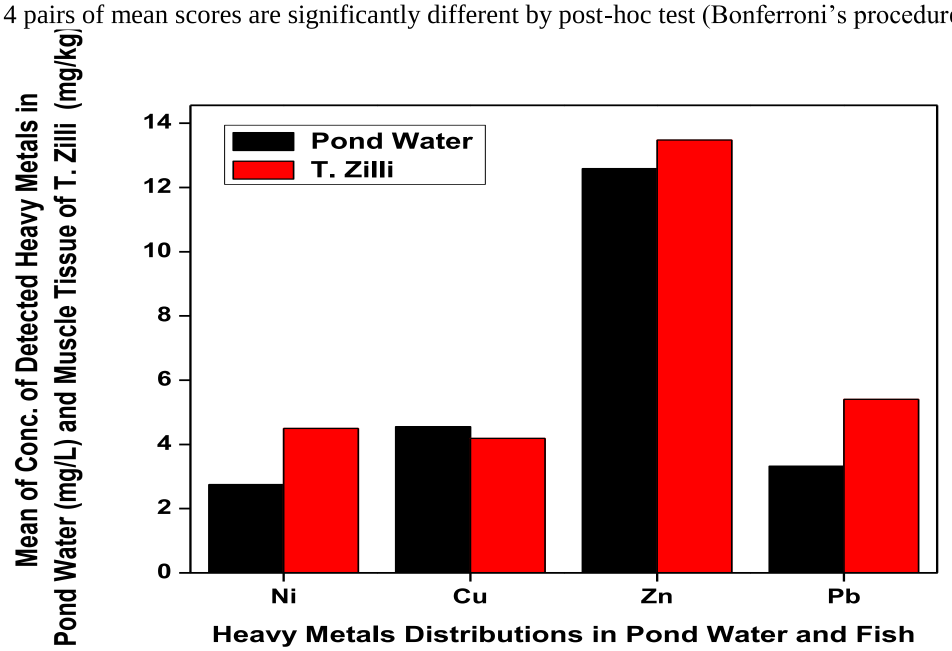

Fig. 2. Mean metal concentrations of detected heavy metals in the pond water and the muscle tissue of T. Zilli

III.2 Discussion

The detection and quantification of the concentrations of heavy metals such as Ni, Cu, Zn and Pb in both water and muscle tissue of T. Zilli of Gwale Pond located in the Gwale Local Government Area of Kano State Nigeria is presented. The present study also investigated some other physico-chemical parameters such as; pH, temperature, dissolved oxygen (DO), total dissolved solids (TDS), conductivity, nitrate (NO_3^-) as well as phosphate (PO_4^{2-}) ions compositions of the water as parts of biomarkers or as means of determining the level of the pond water quality which could eventually facilitate the evaluation of mean concentration of heavy metals in both the water matrix and the muscle tissue of the T. Zilli. The means and standard deviations of the physico-chemical parameters of the pond water across the analyses period (Jan – July) are presented in **Table 1**. The mean of the chemical parameters (DO, TDS, conductivity, NO_3^- and PO_4^{2-} ions) obtained in the present study across the pond water are relatively high which could be due to the effects of the seasonal variations, characteristic of Nigeria's climate [24]. These values appeared to be statistically highly significant ($P<0.01$) and were also found to be sufficiently high, which implies excessive pond water contamination according to the explanation of Nafiu et *al*. during their study on heavy metals determination as biomarkers in Kafinchiri Reservoir in Kano, State, Nigeria [7]. The mean concentrations of the heavy metals (Ni, Cu, Zn and Pb) across the analysis period (Jan – July) are shown in **Fig. 1**. The heavy metals distribution across the analysis period is relatively different due to the inevitable difference associated to the annual seasonal (dry and wet) variations in the Federal Republic of Nigeria. The distribution of the heavy metals is higher in the wet season than the dry season due to the effects of erosion as many foreign substances both edible and non-edible are unavoidably flooded into the water bodies. The highest and lowest mean metal concentrations of the analyzed heavy metals (Ni, Cu, Zn and Pb) in the pond water occurred at the month of July, whereas the lowest values are distributed across some of the months in the dry seasons, February, March and April as shown in **Fig. 1**. The mean metal concentrations of the detected heavy metals in the pond water and the muscle tissue of T. Zilli has been presented in **Table 2**. It could also be observed that both the mean, standard deviations as well as the significance level of the detected and quantified heavy metals are also illustrated. The results of the ANOVA test for both heavy metals distributions in the pond as well as T. Zilli were found to be highly significant ($P < 0.001$). Subsequent post-hoc test (Bonferroni's procedure) suggests that all the possible 4 pairs of mean scores are significantly different. Therefore, mean concentration of heavy metals in the pond water and the muscle tissue of T. Zilli are significantly different as illustrated in **Table 2**.

The mean concentrations of the detected heavy metals in the pond water were found to be 2.75±1.37, 4.55± 1.81, 12.59±2.15 and 3.32±0.86 mg/L corresponding to the Ni, Cu, Zn and Pb respectively. Similarly, the mean concentrations of the detected heavy metals in the muscle tissue of T. Zilli were found to be 4.50±0.69, 4.19±0.72, 13.48±2.31 and 5.40 ±01.92 mg/Kg also corresponding to Ni, Cu, Zn and Pb respectively. Some these obtained mean values in both the pond water and the muscle tissue of the T. Zilli were found to significantly exceed the maximum permissible level of the heavy metals concentrations in both water and fish as reported by the food and agriculture organization (FAO) of the united nations except in the case of Cu and Zn that has a mean metal concentration below maximum permissible level [25]. These proposed range of those maximum permissible level in fish and fish products on a fresh weight basis according to FAO are Cu (30 mg/L), Zn (40 mg/L) and Pb (2 mg/L). On the other hand, the maximum permissible level for Ni in water and fish was found to be 0.07 mg/L, according to the World Health Organization Standard [26]. Some of the heavy metals have significant physiological benefits to humans at certain level, whereas, some are not. It is therefore, imperative for water and fish quality regulations to be reviewed systematically since human activities affect ecosystem globally.

Although an updated guidelines for the drinking water quality may sometimes affect several experimental values of heavy metals obtained in the same water body at different sampling period due to the dynamism of humans anatomy and physiology which also varies from one individuals to another, hence some of the guidelines given by the regulatory bodies may be associated with the recommendation given by the experts based on diagnosis employed to a specific heavy metal contamination in a living system. To further analyze the range of Cu metal acceptance level in water, it was demonstrated that the maximum permissible level assigned for the concentration of Cu metal according to WHO, was given as 2 mg/L [26]. The toxicity of Cu according depends upon the hardness and pH of the water and for that reason, it is more intense in water with low alkalinity [27]. Such argument is in conformity with the range of pH values obtained in the present study. Zn is also an essential element that is commonly found in food and drinking water. As recommended by WHO, that a daily recommendation of Zn in an adult is between 15 – 20 mg/L. However, for a safer drinking water, it is recommended for Zn concentration not to exceed 3 mg/L [26]**.** Zn metal is highly essential to both animals and humans and hence serves as a shield to the toxicity of Cd and Pb [28]. Similarly, Malik et *al.* (2010) described a Pb metal as an element without a well-established biological function and causes a severe carcinogenic effect on humans and aquatic ecosystem [28]. The permissible level of Pb in the drinking water was reported to be 0.01 mg/L [26]**.**

According to recent WHO review on guidelines for the drinking water quality standard, the maximum permissible level for Pb and Cu concentrations in water were given as 0.01 and 1.3 mg/L respectively [29]. This current review is indeed crucial to the prevailing health risk effects associated to the degree of water and fish quality. The high obtained values of the mean metal concentration of the heavy metals in both pond water and the muscle tissue of the T. Zilli were all correlated with the high values obtained for the physico-chemical parameters as shown in **Table 1**. Reasons for high metals concentration may be attributed to the illegal waste disposals ranging from edible, non-edible, biodegradable and non-biodegradable resources, resulting to eutrophication (hypertrophication) which could subsequently leads to excessive oxygen depletion [27].

In comparison with other existing literatures of the investigated the levels of Zn and Pb in the surrounding water and gills of T. Zilli in Farfazai Pond located in the Kano metropolis, Nigeria high values of Zn (9.11 mg/L) and Pb (3.39 mg/L) metal concentrations in the gills of T. Zilli. As narrated in the paper, the reason of the occurrence of such high values was attributed to the illegal waste deposal by some part of the general public into the pond water [22]. Similarly, lower concentrations of heavy metals such as Cu, Zn and Pb were reported by Malik et. *al.* [28]**.** According to their study, the detected concentrations of Cu, Zn and Pb were found to be 0.398, 1.88 and 1.32 μg/g respectively.
Similar values of Zn and Pb were also determined in the muscle tissues of some tilapia species in River Jakara and Kusalla Dam in Kano [2]. From the study, the mean concentrations of heavy metals found in River Jakara were found to be (0.46±0.14,

15.83±5.05 and 0.57±0.20 mg/kg) whereas that of Kusalla Dam were found to be (0.38±0.31, 12.04±2.99 and 0.54±0.29 mg/kg) corresponding to Cu, Zn and Pb respectively.

The fluctuations of heavy metals in the system of fish species is quite unavoidable due to the ability of the heavy metals to co-exist in different compound's forms ranging from carbonates, oxides, phosphates and nitrates concentrations [30]. In summary, mean metal concentrations of heavy metals in both water and muscle tissues of T. Zilli were found to be significantly high, which were much, correlated with the relatively high-recorded values of the physico-chemical parameters. These high relative values obtained for the physico-chemical parameters could also serve as biomarkers to proper evaluation of standard water quality in various water bodies [7]. Water contamination was very much likely to have taken place in the present study, due to several factors consisting of poor drainage system, illegal domestic and industrial waste disposal in dry season, as well as flooding of eroded substances and rock minerals from the nearby environments during wet season.

IV. CONCLUSIONS

The detection and quantification of the concentrations of heavy metals such as Ni, Cu, Zn and Pb in both water and muscle tissue of T. Zilli of Gwale Pond located in the Gwale Local Government Area of Kano State Nigeria have been successful using atomic absorption spectrophotometer. The results of the one-way ANOVA test for both heavy metals distributions in the pond as well as T.Zilli were found to be highly significant ($P < 0.001$). Subsequent post-hoc test (Bonferroni's procedure) suggests that all the possible 4 pairs of mean scores are significantly different. The mean concentrations of the detected heavy metals in the pond water were found to be 2.75±1.37, 4.55± 1.81, 12.59±2.15 and 3.32±0.86 mg/L whereas the mean concentrations of the detected heavy metals in the muscle tissue of T. Zilli were found to be 4.50±0.69, 4.19±0.72, 13.48±2.31 and 5.40 ±01.92 mg/Kg corresponding to Ni, Cu, Zn and Pb respectively. The mean metals concentration values of Ni and Pb in both pond water and muscle tissue of T. Zilli were found to exceed the maximum permissible level of the standard water and fish quality of (Ni = 0.07 mg/L: Pb = 0.01/2 mg/L) as reported by FAO/WHO whereas Cu and Zn mean metals concentration values of (Cu =30 mg/L: Zn = 40 mg/L) in both pond water and muscle tissue of T. Zilli were found to occur within the permissible level of the standard water quality criteria as recorded by FAO. However, according to the updated versions of water quality guidelines as reported by WHO, the maximum permissible values for heavy metals concentrations such as Cu and Pb in water were found to be 1.3 and 0.01 mg/L respectively. Healthy maximum permissible level of Zn metal concentration in water in some perspective was also found to be 3 mg/L. The relatively higher values recorded for the heavy metals in the present study is an indication of water contamination which is well correlated with the relative higher values obtained for the physicochemical parameters. This study will go a long way to paving a good foundation of a novel research in exploring the amount of pollutants in the tissues of aquatic species as well as investigation of a number of parameters in any fresh water system in Nigeria and the globe at large.

V. RECOMMENDATIONS

- Government should enact and implement strict regulations on illegal disposal of waste in the pond water and any other fresh water body that benefits the public.
- Enlightenment campaigns to the public through different media platforms on the dangers attributed to the illegal dumping of refuse unto fresh water systems should be encouraged.
- Government should collaborate with private companies and provide a good mechanism that should ensure proper installation and monitoring of adequate trash bins across districts, streets or any point of strategic importance to the masses so as to reduce the risk of waste disposal into a fresh water system.
- The government and stakeholders should embark on effective routine monitoring programs in order to ascertain the degree of water quality as well as pollutant contamination at all level of fresh water systems.

VI. ACKNOWLEDGEMENT

The authors would like to thank the Tertiary Education Trust fund of the Federal Republic of Nigeria (TETFUND) for providing the financial support throughout the study.

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