

PRELIMINARY ASSESSMENT OF HEAVY METAL POLLUTION OF OPA RESERVOIR, ILE- IFE, SOUTHWEST NIGERIA USING *MORMYRUS RUME* AND *TILAPIA ZILLII*

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ABSTRACT

In this study, the concentrations of five selected heavy metals: Lead (Pb), Nickel (Ni), Mercury (Hg), Cadmium (Cd) and Selenium (Se) were determined in water and tissues of two fish species from Opa reservoir in Obafemi Awolowo University (OAU), Ile-Ife, Nigeria, with a view to assessing its pollution level. Water samples from the reservoir, and the liver, gills and fillet of six specimens of *Tilapia zillii* (Gervais, 1848) and six specimens of *Mormyrus rume* (Cuvier and Valenciennes, 1846) were analyzed using Flame Atomic Absorption Spectrophotometer (FAAS) for the selected metals. The mass order of the metals in the samples was: Pb>Ni>Se³Cd>Hg for water and Ni>Pb>Se>Cd³Hg for the fillet of both *T. zillii* and *M. rume* fillet. Ni concentration ranges in *T. zillii* fillet and *M. rume* fillet were as follows 2.60-3.00 µg/g and 2.20-2.80 µg/g, with the mean concentrations of 2.77±0.151 µg/g and 2.57±0.23. Se ranged in *T. zillii* fillet between 0.80 and 1.20 µg/g with mean concentration of 0.97±0.061 µg/g and in *M. rume* fillet, it ranged between 0.60 and 0.80 µg/g with mean concentration of 0.73±0.103 µg/g respectively. The two metals (Ni and Se) were higher in *T. zillii* fillet at 95% confidence interval than *M. rume*. Nickel and Cadmium were significantly higher at 95% level in *T. zillii* gill than *M. rume* gill. Ni in *T. zillii* gill ranged from 2.28-3.20 µg/g with mean concentration of 3.20±0.360 µg/g while Cd ranged from 0.60-0.80 µg/g with mean concentration of 0.73±0.103 µg/g. The concentrations of Pb, Ni and Hg in Opa reservoir water were higher than the permissible levels for EPA, 2002; WHO, 1983; WPCL, 2004 and SON, 2007 while nickel was much higher in the fillet than the FAO, 1976 and FAO 1983 recommended limits of heavy metals in fish food. The study concluded that the Opa reservoir was heavy metal polluted.

Keywords: Opa Reservoir, Heavy Metals, *Mormyrus rume* and *Tilapia zillii*

INTRODUCTION

Fish are widely used for assessing the health condition of aquatic ecosystems because they are at the end of most aquatic food chains, and as such, the concentration of pollutants in fish reflects the risk to humans who depend on them as a major source of animal protein. As the concentration of heavy metals in the environment increases, the metals inevitably enter the biogeochemical cycle (Riget *et al.*, 2004). Heavy metals are not biodegradable, but are assimilated and deposited in fish tissues with water and sediments being major sources as aquatic animals are nourished through them (Linnik and Zubenko, 2000). Heavy metals, when bio-accumulated and biomagnified via the food chain finally get to humans through consumption and ultimately resulting in health risks (Agah *et al.*, 2009).

Fish may concentrate large amounts of metals from the polluted water which may lead to adverse effects such as death and extinction of fish species.

Metal accumulation in fish tissues depends on abundance and availability of metals in aquatic environment, and may differ for various fish species living in the same water body (Adeyeye *et al.*, 1996; Kucuksegin *et al.*, 2006; Barbara and Malgorzata, 2006) depending on a number of factors. These differences result from different affinity of metals to fish tissues, different uptake, deposition and excretion rates. Factors such as temperature, pH and acidity have been responsible for metal accumulation in fish tissues with water temperature affecting metal deposition in various organs. High temperature promotes accumulation of cadmium especially in the most burdened organs such as kidney and liver. Temperature rise in the aquatic ecosystem helps to increase the metabolic rate, metal mobility and binding of metals to various tissues (Yang and Chen, 1996). Studies have shown that acidification of aquatic environment increases the concentration of Pb and Cd (Haines and Brumbaugh, 1994; Horwitz *et al.*, 1995). Acidity may enhance the solubility of

the metals in the water body and hence allow free movement of the ions in solution. Competitive uptake of H⁺ ions may hinder the absorption of the metals into the fish or it could damage or weaken the epithelia and enhance easy passage of metals into the fish tissues (Barbara and Malgorzata, 2006).

Parasad and Oberleas (1979) considered heavy metal determination as a useful test in the survey of environmental pollution. Thus, the determination of metals in fishes and water bodies can create environmental awareness on the risk in the consumption of the fishes (Adefemi *et al.*, 2008). In urban areas, careless disposal of industrial/municipal effluents and other wastes into river and lakes contribute greatly to the poor

quality of river water (Furtado *et al.*, 1998; Chindah *et al.*, 2004; Ugochukwu, 2004 and Emongore *et al.*, 2005). In Ile-Ife, the study area, municipal wastes are often disposed into Opa river which might have contributed greatly to the pollution level of the reservoir. There is therefore the need to assess the concentrations of some heavy metals in the water and fish samples from the Opa reservoir.

MATERIALS AND METHODS

Study Area

The map of the study area is shown in Figure 1. The Opa Reservoir, which lies between Latitudes 07°29' N and 07°30' N and Longitudes of 004°31'E and 004°32'E, is situated within the campus of OAU, Ile-Ife, Southwest Nigeria (Figure 1). The reservoir was created in 1979 by

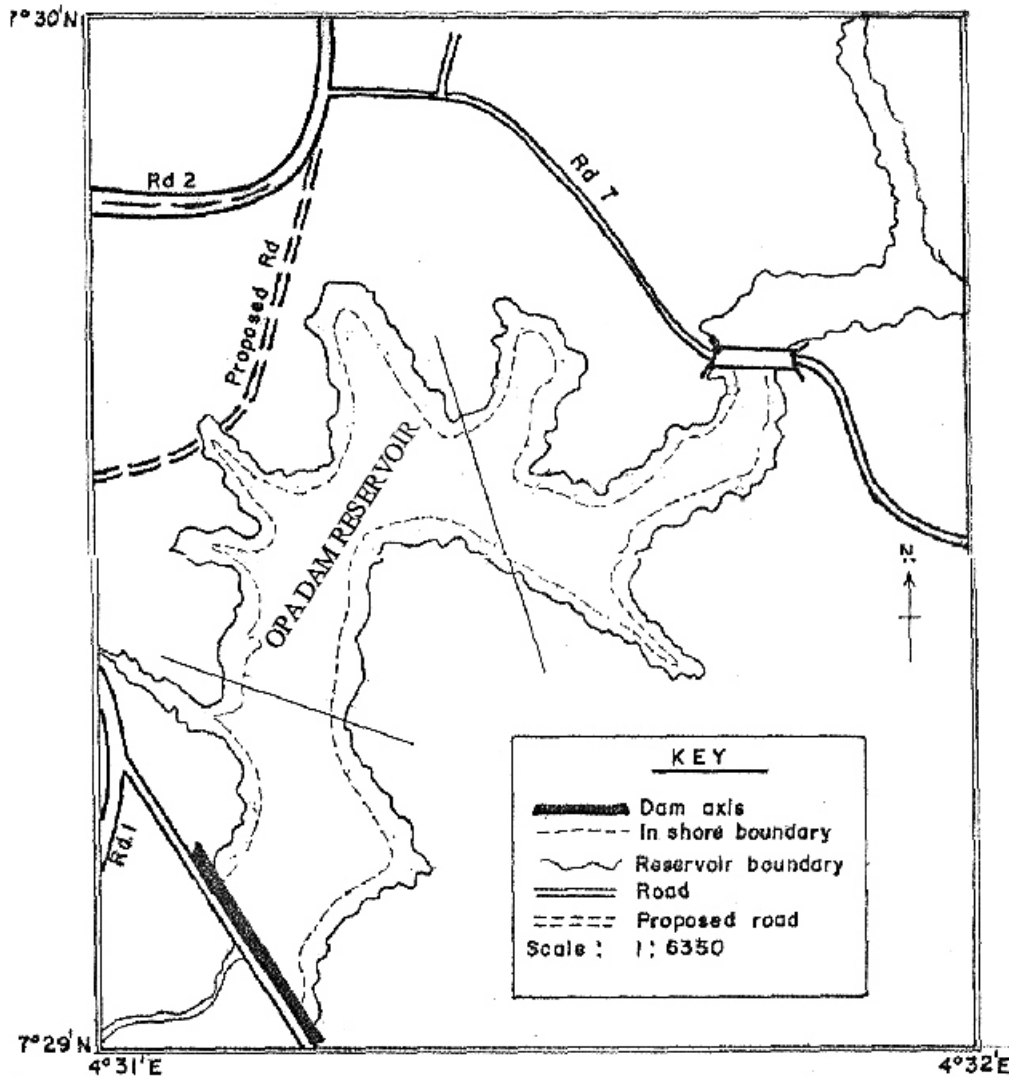


Figure 1: Location Map of Opa Dam and Reservoir, OAU, Ile-Ife
Source: (Komolafe, 2008).

impounding the Opa River for potable water supply and freshwater fisheries research. The climate of the area is characterized by two distinct seasons, dry and wet seasons. The wet season extends April to October and is marked by high rainfall, while the dry season on the other hand extends from November to March (Komolafe, 2008).

Sterilization of Apparatus, Reagents used and their Sources

The medical dissecting set, beakers, volumetric flasks, sample bottles, measuring cylinders, pipettes used, were first washed with liquid soap and rinsed with distilled water. Then, 10% HNO₃ was prepared and the apparatus washed in it, rinsed with distilled water and left to dry in the oven at 105°C. All the reagents used were of analytical grade. They included: Nitric acid from Riedel-de Haën, Germany, and Hydrogen Peroxide from British Drug House (BDH) Chemicals Ltd, Poole England.

Fish Collection and Pretreatment of Samples

Water samples at different points of the reservoir were collected at 10 - 15 cm depth in separate pre-conditioned polyethylene bottles from the reservoir and the pH determined *in situ* with portable pH meter (HI 9126) after calibration with buffer solutions (pH 4 and 9). The collected samples were filtered (with Whatman filter paper No 42) and the filtrate acidified with 2 mL concentrated HNO₃ per litre of filtered water to minimize precipitation and adsorption to container's wall. Six (6) specimens each of two common freshwater species of fish found at the peak of dry season (*Mormyrus rume*, family: Mormyridae, commonly called Trunk snout mormyrid and *Tilapia zillii*, family: Cichlidae, locally called Tilapia) were used for the study. The specimens labeled A - F and A₂ - F₂ respectively for *T. zillii* and *M. rume* were collected with the help of the fishermen. *T. zillii*, a hardy macrophytic feeder and *M. rume* which is a bottom deposit feeder were found to be ideal species for the assessment study on the effects of heavy metal contamination (Komolafe, 2008; Odejide and Fagbenro, 2010).

The fish specimens were transported to the laboratory in an ice cubes contained in a chest. They were thoroughly washed in the laboratory

with distilled water and each sample weighed on a Metler Balance Model 1210, the standard and the total lengths were taken and recorded. The specimens were dissected to remove the liver, gills and fillet for digestion and were analyzed separately.

Sample Drying and Dissolution

The dissected fish body parts (fillet, liver and gill) were oven-dried at a temperature of 60°C until a constant weight was achieved. The dried samples were ground in agate mortar and pestle for homogeneity.

A known amount (0.2 g) of the liver and 0.5 g each of gills and fillet were weighed separately and transferred to Teflon beakers with 10 ml of 1:1 HNO₃/H₂O₂ added to each beaker. The beaker was heated on a thermostatically controlled hot plate (Gallenkamp Magnetic Stirrer Regulator Hotplate) at 90°C for 20 minutes. The digested sample was transferred to a 100 ml volumetric flask and the flask made up to mark with deionized water. The whole content of the flask was then transferred to a 120 ml capacity Teflon bottle and stored at a temperature below 5°C prior to analysis. The water from Opa reservoir was also digested for analysis, 5 ml of the water was measured and digested through the same process and then made up to 100 ml by dilution with deionized water. This was done in order to know the level of heavy metal concentration in the water, and to ascertain the contamination source of the fish samples. The worked-up samples above were analyzed for heavy metal concentration using Flame Atomic Absorption Spectrophotometer (FAAS) Buck Model 205.

Quantification of Heavy Metals in Samples

The actual heavy metal concentration values in the samples were evaluated using the equation:

$$[M]_x = \frac{M_i \times F_v}{M_t}$$

Where: [M]_x = actual heavy metal concentration in sample;

M_i = Instrumental concentration obtained

M_t = Mass of fish part (liver, gill or fillet taken)

F_v = Final volume of digested samples

The transfer ratio, TF =

$$\frac{\text{Metal concentration in Fish part}}{\text{Metal concentration in water}}$$

Quality Control Measures

Analytical quality control measure was carried out by the preparation of blank and standard metal concentrations. Triplicates analysis of the samples were carried out and the mean values were used. The calibration of the FAAS used was done to evaluate its response with respect to known quantities of the standard solutions of the heavy metals of interest. Standard solutions of 20, 10, 6, 4, 2 and 1µg/g values were prepared by serial dilution for the determination of heavy metals in fish and water samples. These solutions were run to obtain the working calibration graph.

The reliability of all the analytical procedures adopted in this study was in terms of sensitivity, precision and accuracy. The elements Lead (Pb), Nickel (Ni), Mercury (Hg), Cadmium (Cd) and Selenium (Se) were analyzed at the most sensitive wavelength viz; Pb (279 Nm), Ni (233 Nm), Hg (253.7 Nm), Cd (228.9 Nm) and Se(196 Nm). The standard calibration curves (of optical density versus metal concentration) obtained showed high linearity level with r² values between 0.999 and 1.000, which are adjudged acceptable.

Statistical Analysis

Statistical analysis was carried out using Statistical Package for the Social Sciences (SPSS, version 16.0, Inc., Chicago, USA). The significant differences between groups were compared using two tailed analysis of independent t-Test at probability level of 95%. The data were displayed as mean ± standard deviation.

RESULTS AND DISCUSSION

Variation of Heavy Metals in Fish Species

The pH of Opa reservoir water was in the range of 7.4 -7.5 with a mean value of 7.44±0.55. This showed that the water was slightly alkaline. The mean concentrations of the analyzed heavy metals in the Opa reservoir water was in the order of Pb > Ni > Se > Cd > Hg (Fig. 2). The concentrations of Pb, Ni and Hg in the reservoir were higher than the permissible limits of the respective metals as recommended by EPA (2002), WHO (1985), WPCL (2004) and SON (2007) as shown (Table 1). Selenium was within the recommended limit of USEPA (1986) while Cd was above the limit. The reservoir water can therefore be said to be polluted with the assayed metals. Heavy metals in fresh water are usually available to fish in the soluble forms which may be labile and non-labile fractions. The labile fractions which are readily released are available for uptake by aquatic organisms including fish. However, the amount assimilated largely depends on the environmental condition of the water (Kock *et al.*, 1996; Barbara Malgorzata, 2006).

Table 1: Permissible Level of Heavy Metal in Raw Water/Drinking Water (mg/L)

Guidelines	Pb	Ni	Hg	Cd	Se
EPA (2002)	0.05	-	0.002	0.01	-
SON (2007)	0.01	0.02	0.001	0.05	-
WPCL (2004)	0.05	-	-	0.003	-
WHO (1985, 2003)	0.01	0.02	0.001	0.01	-
USEPA (1986)	-	-	0.002	0.005	0.05
This study	0.07	0.06	0.01	0.02	0.02

Table 2: Length and Weight Measurement for Investigated *T. zilli* and *M. rume*

Meristic Value	Statistics	<i>Tilapia Zilli</i>	<i>Mormyrus rume</i>
Weight(g)	Range	337-208	639.00-335.00
	X± SD	293.17 ± 46.52	476.83± 132.82
Standard Length (cm)	Range	20-17	40.00-30.00
	X± SD	18.60 ± 0.93	34.75 ± 3.72
Total Length (cm)	Range	25-21	45.10-34.00
	X± SD	23.37 ± 1.42	38.85 ± 4.00

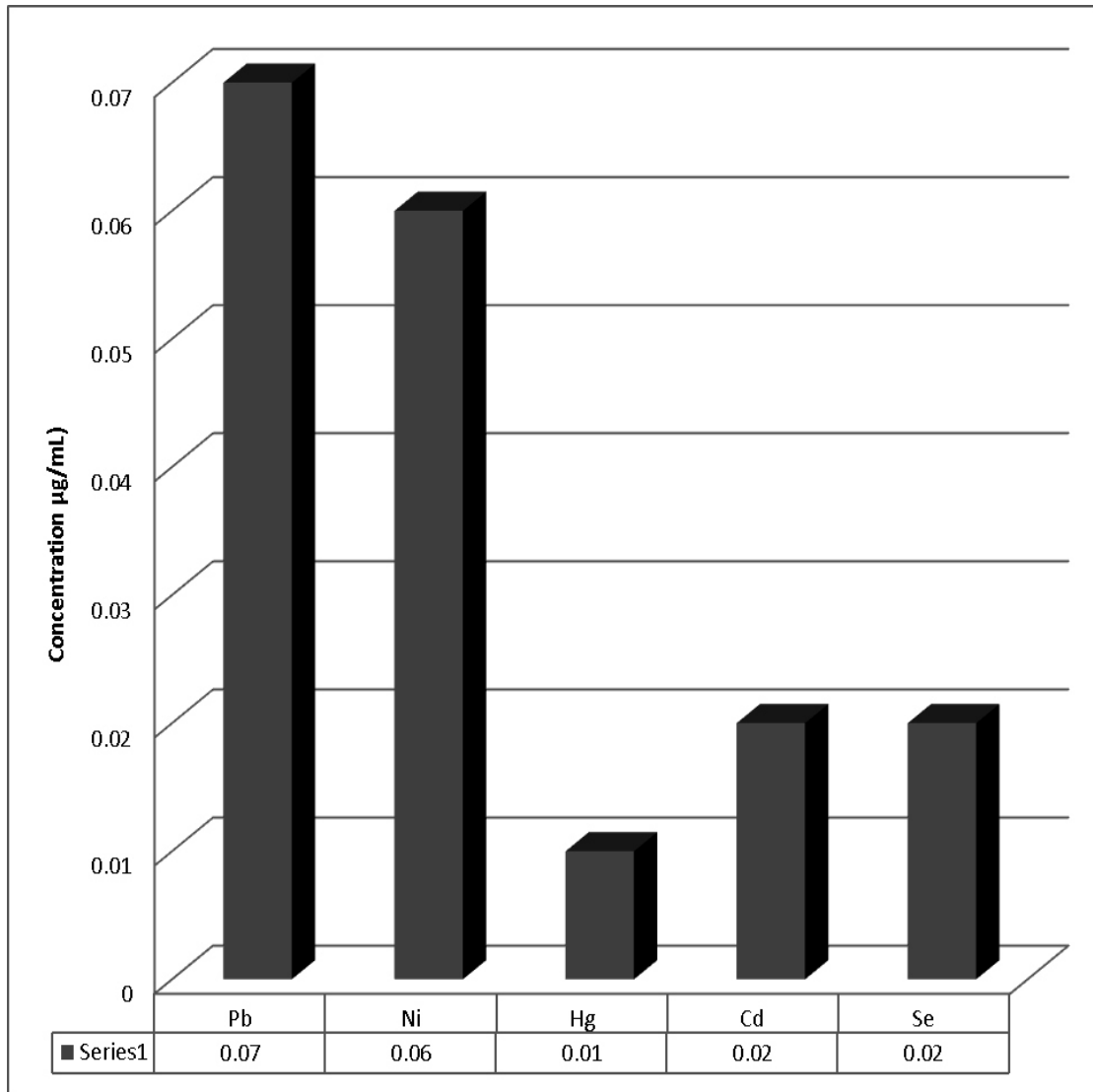


Figure 2: Mean Concentrations of Heavy Metals in Opa Reservoir Water

Table 2 shows the meristic characteristics of the fish specimens used in this study. The *Momyrus rume* specimens had higher standard and total lengths and weight than *Tilapia zillii*. However, weight and length of the fish did not reflect in the concentrations of the metals in *Momyrus rume* specimens. The differences recorded in the concentrations of the metals in the two species could be related to the species different modes of feeding. Insects, crustaceans, detritus and plant parts are widely consumed by the small, medium and large *M. rume* individuals. Insect were the prominent food item for large and medium specimens while crustaceans were the prominent food items for small fishes, as the fish size increased the preference for insects increased and the preference for protozoan, rotifers and diatoms decreased. Detritus and plant materials occur in

stomach of *M. rume* with detritus and rotifers more prominent among the smaller ones (Odedeji and Fagbenro, 2010). *Tilapia zillii* are omnivorous (the juveniles being more carnivorous) consuming a wide range of different food items mostly at the bottom, because they are bottom dwellers.

Generally, the order of accumulation of metals in the fish tissues irrespective of weight, length and species in this study was: liver>Gill> fillet. The result obtained was similar to other studies as reported by Zyadah, (1999), Farombi *et al.* (2007), Dimari *et al.* (2008), Akan *et al.* (2009); Abdel-Baki *et al.* (2011) and Adeyeye and Ayoola, (2012). In the studies, the metals concentration in the liver and gills were higher than in the muscles. Since liver and gills are good target organs of bioaccumulation of heavy metals, they could

serve as vital organs for the monitoring of water pollution. The levels of heavy metals in the gills of the fishes probably reflects the concentrations of metals in the waters, where the fish live, while the concentrations of metals in liver represented storage of metals in the fish body (Roméo *et al.*,

1999; Yilmaz, 2009). Fish muscles which is not an active organ like gills and liver, had low concentrations of metals as supported by the work of Canli and Kalay (1998), Yilmaz (2009) and Adeyeye and Ayoola (2012).

Table 3: Descriptive Statistics of the Concentration of Metals (µg/g) in *T. zillii* and *M. rume*

Fish specie	Tissue Sample	Pb		Ni		Hg		Cd		Se	
		Range	...	Range	X ± SD	Range	X ± SD	Range	X ± SD	Range	X ± SD
<i>Tilapia zillii</i>	Fillet	1.80-2.20	2.00±0.178	2.60-3.00	2.77±0.151	0.60-0.80	0.67±0.103	0.60-0.80	0.70±0.110	0.80-1.20	0.97±0.061
	Gill	2.20-2.80	2.43±0.234	2.28-3.20	3.02±0.360	0.60-1.00	0.83±0.151	0.60-0.80	0.73±0.103	0.80-1.20	0.97±0.151
	Liver	0.00-7.00	4.83±2.563	0.00-9.00	6.50±3.507	0.00-3.00	1.75±0.987	0.00-3.00	2.25±1.255	0.00-3.00	1.67±1.033
<i>Mormyrus rume</i>	Fillet	1.80-2.40	2.05±0.234	2.20-2.80	2.57±0.23	0.40-0.80	0.57±0.15	0.60-0.80	0.70±0.110	0.60-0.80	0.73±0.103
	Gill	2.40-3.00	2.65±0.27	1.50-2.40	2.05±0.31	0.60-1.00	0.83±0.154	0.80-1.20	1.08±0.170	0.60-0.80	0.72±0.099
	Liver	2.20-3.50	2.75±0.46	3.00-4.50	3.58±0.56	0.50-1.00	0.80±0.19	1.00-1.50	1.08±0.130	0.70-1.00	0.83±0.140
FAO (1976)/ FEPA (2003)/ FAO (1983) Limit in fish Food.			2.00		0.5-0.6		1.00		2.00		

Table 4 : Comparison of Mean Values of Heavy Metals in *T. zillii* and *M. rume* by Student's t-Test

Statistics	Tissue	Pb	Ni	Hg	Cd	Se
t-calculated/statistics	Fillet	1.00	-3.873	-1.168	0.00	-3.796
	Gill	1.117	-4.325	-0.159	3.971	-2.538
	Liver	-1.940	-2.070	-2.375	-2.078	-2.107
Probability (P) Sig value(2 tailed)	Fillet	0.363	0.012*	0.296	1.000	0.013*
	Gill	0.315	0.008**	0.880	0.011 *	0.052
	Liver	0.110	0.093	0.064	0.092	0.089

*= P 0.05 ** = P 0.01

Table 3 shows the mean and standard deviation values for the five metals assayed in the two fish species. Nickel had the highest concentrations in all the three tissues (liver, gills and fillet) in the two species. However, the concentration of the metals was significantly higher (p<0.05) in the fillet and gill of *Tilapia zillii* than in *Mormyrus rume* suggesting that *M. rume* fillet and gill had less affinity for Ni. Selenium was also significantly higher in *T. zillii* (P < 0.05) than in *M. rume* (Table 4). All the metals were more concentrated in *Tilapia zillii* organs than in *Mormyrus rume* (Table 3). The order of heavy metal accumulation in *Tilapia zillii* was Ni > Pb > Cd Se Hg (liver), Ni > Pb > Se > Hg > Cd (gill) and Ni > Pb > Se > Cd Hg (fillet). The order of heavy metal accumulation in the liver, gills and fillet of *Mormyrus rume* was Ni > Pb > Cd > Se > Hg, Pb > Ni > Cd > Hg > Se and Ni > Pb > Se > Cd Hg respectively. The fact that the metals were more concentrated in the liver than other organs in this study was in agreement with the reports of Hogstrand and Haux (1991);

Ambedkar and Muniyan (2012) that the liver stores and transport most substances in the body. Studies have shown that liver accumulates more metals than other organs in fish because of the high coordination of metallothionein protein in the liver with metals. It also detoxifies, transports and stores most substances in the body. It is an active site of pathological activities for contamination of tissues, since it sends/transports all the toxic substances to other parts of the body (Hogstrand and Haux, 1991, Ambedkar and Muniyan, 2012).

The results obtained in this study showed that the concentrations of the investigated metals in fish tissues were higher than the concentration in the reservoir, probably reflected bioaccumulation over a period of time. Aquatic animals usually show a higher level of heavy metals accumulation than their habitat. This suggests that Opa reservoir was the main source of the five metals in the fish tissues. *Tilapia zillii* in Opa reservoir is a microphytic feeder (Komolafe, 2008), adults being

especially herbivorous, consuming mainly aquatic plants, algae and other phytoplankton. Studies on the feeding habits of *T. zillii* in Lake Kinneret (Israel), showed that the main source of food was *Chironomida* pupae (Diptera) in the spring and winter and zooplankton in the summer and autumn with algae supplementing the diet throughout the year (Spataru, 1978). Thus, the feeding habit of *Tilapia zillii* might have made the metal accumulation to be more than in *M. rume* (as observed in Odedeji and Fagbenro, 2010).

Nickel being generally higher in fillet (the commonly eaten part of fish) than the permissible limits of FAO, 1976 and FAO (1983) needs to be monitored. The transfer factor of Ni from water to the fillet in *M. rume* ranged from 40.00 to 46.67 with a mean value of 43.88, while that of *T. zillii* ranged from 43.33 to 50.00 with mean value of 46.11. Lead transfer factor in *M. rume* ranged from 25.71 to 34.28, mean concentration of 29.05 and that of *T. zillii* ranged from 25.71 to 31.42 with mean value of 28.57. The transfer factor of Ni and Pb in the two species were almost the same. The concentrations of Lead and Cadmium were above the maximum permissible concentration which poses risk upon continuous consumption.

Anthropogenic activities such as farming and in flow of run-offs with all manner of environmental contaminants which settle as sediments in water bodies might have led to the high concentrations of the investigated metals in Opa reservoir and eventually in fish tissues. These contaminants act as repository for heavy metals. The time of sampling (dry season) could have contributed to the increase in the concentration of the metals in both the water and the fishes investigated because reduction in the volume of water will lead to higher concentration. Nickel had high concentrations both in the water and the fish species. This could lead to dermatitis, pulmonary fibrosis, oedema and neurological effects (ATSDR, 1997). The level of Pb and its accumulation over time may cause serious damage to the nervous system, decreased sperm production and cause renal dysfunction (ATSDR, 2007) over time by continuous consumption of the fish and the water (untreated).

CONCLUSION

The study revealed that Opa reservoir was contaminated with Pb, Ni and Hg with concentration levels well above regulatory permissible levels for consumption. The water might have been contaminated due to agricultural wastes, run offs, solid metal waste such as tins, sewage drainage, reduction in the water level at the time of sampling and other contamination from unknown sources. The two fish species investigated (*Mormyrus rume* and *Tilapia zillii*) from the reservoir were also contaminated mainly with Ni and Pb. Ni and Se were significantly higher ($P < 0.05$) in fillet of *T. zillii* fillet while Ni and Cd were higher ($P < 0.05$) in *T. zillii* gills than *M. rume* specimens. Regardless of weight and length, *T. zillii* accumulated more metal than *M. rume*.

Since heavy metal pollution effects on man and ecosystem are extensive and intensive, it is therefore recommended that regular monitoring of the reservoir for heavy metal levels be put in place to ensure the safety of human and reservoir ecosystem in general.

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