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Heavy Metals in *Heterobranchus Longifilis* (Cuvier And Valenciennes, 1840) Cultured in Earthen Ponds in Selected Communities in Warri Metropolis, Nigeria

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Abstract. The concentrations of Cadmium (Cd), Copper (Cu), Lead (Pb) and Zinc (Zn) in *Heterobranchus longifilis* (Mean total length= 40.52 cm; mean weight=563.25 g) cultured in earthen ponds in Warri, Nigeria, were determined by Atomic Absorption Spectrometric technique in order to provide baseline data and to ascertain the suitability of such fish for consumption. The mean concentrations of metals in *H. longifilis*, ranged from 0.13 mg/kg for Cd in January to 72.51 mg/kg for Cu in June with significant differences ($p<0.05$) observed in the mean concentrations of Cu, Pb and Zn in fish between months. The mean concentrations of metals in *H. longifilis*, ranged from 0.12 mg/kg for Cd at Jeddo to 69.27 mg/kg for Cu at Ubeji with significant differences ($p<0.05$) observed in the mean concentration of Zn in fish between stations. The bioaccumulation quotient (BQ) values ranged from 0.50 for Cd at Jeddo to 37.24 for Cu at Oboroke while the hazard quotient (HQ) ranged from 0.39 for Zn to 3.43 for Pb. The maximum acceptable risk (MAR) values ranged from 0.02 for Pb at Ekpan to 6.29 for Zn at Ubeji while the estimated average daily intake (EADI) of heavy metals ranged from 0.09 mg/person/day for Cd to 36.13 mg/person/day for Cu. It was concluded that Cd, Cu and Pb were the metals that presented a potential risk to the consuming public and that the heavy metal content in earthen ponds should be routinely monitored in order to keep metal levels within safe limits.

Keywords: Heavy metals, Hazard quotient, *Heterobranchus longifilis*.

INTRODUCTION

Heavy metals have gained attention from public and scientific communities globally owing to their toxicity to aquatic organisms and ultimate effects on humans (Jayaprabha *et al.*, 2014). The presence of heavy metals in aquatic ecosystems in excess of natural background levels has also become worrisome around the world (Akoma and Uhunmwango, 2017). The factors which contribute chiefly to the deleterious effects of metals are their non-biodegradability and their ability to bioaccumulate and biomagnify in aquatic media (Olowu *et al.*, 2015). Ponds in close proximity to urban areas are inevitably subjected to urban runoff especially during the wet season (Reuben *et al.*, 2018).). Frimpong *et al.*, (2014), observed that aquaculture in sub-saharan Africa is conducted mainly in earthen ponds which are relatively less intensive compared to the same method of food production in Europe and America. The choice of earthen ponds to culture fish in Nigeria could be linked to its fast production owing to the vast availability of natural food which supplements feed given to fish (Omitoyin, 2007). Growth of natural food in ponds is largely stimulated by

dissolved metals in form of minerals. Heavy metals associated with urban runoff are largely the same as those associated with municipal wastewater. According to the United Nations Educational Scientific and Cultural Organization/World Health Organization/United Nations Environment Programme (UNESCO/WHO/UNEP), water quality problems particularly associated with urban runoff are high levels of oil products and Lead (Pb), both arising from the use of automobiles, as well as a variety of other metals and xenobiotics associated with local industrial activity (UNESCO/WHO/UNEP, 1996). In addition, heavy metals can blend with runoff from tires, car exhaust, road asphalt, fuel combustion, parking dust and recreational land (Reddy, 2014). It is on this premise that earthen ponds need to be investigated and scrutinized for heavy metal content as they are largely prone to runoff owing to their peculiar way of construction. There is paucity of data regarding the heavy metal concentrations in fish specifically raised in earthen ponds in Warri, along the Niger Delta belt of Nigeria, which has warranted this research, against the backdrop that the town is widely known for its crude oil exploration activities which may conceivably impact on such ponds owing to their exposed nature. Igbagara *et al.,* (2016), Bluwey *et al.,* (2018) and Olawuyi (2018) have recognized a litany of environmental impacts associated with crude oil extractive operations and activities in the Niger Delta, of which Warri is an integral part, which affect surface water. Such crude oil extraction operations can also impact on soil and air which in turn can influence community health (Johnston *et al.,* 2019). A variety of heavy metals have been reported to be associated with crude oil extraction operations (Calamari and Naeve, 1994). Furthermore, earthen ponds are prone to both non-point and point sources of contamination especially the former. The fish species used in this study was *Heterobranchus longifilis* (Cuvier and Valenciennes, 1840). It was purposely selected for this research because it is the principal fish species cultured by farmers who operate earthen ponds in Warri. This is an important catfish species highly valued for food in Nigeria (Olaosebikan and Raji, 2013). Similarly, the heavy metals of interest namely: Lead (Pb), Cadmium (Cd), Zinc (Zn) and Copper (Cu) are typically found in urban runoff along with Nickel and Chromium (Jang *et al.*, 2005; Nabizadeh *et al.,* 2005). Particularly Zinc and Copper are essential in trace quantities for the maintenance of cellular processes in man (Food Safety Authority of Ireland, 2009) while non-essential elements such as Pb and Cd do not play metabolic functions and can be toxic even at low concentrations to recipients (Rajeshkumar and Li, 2018). The working hypothesis of the study is that it is possible for cultured fish to accumulate heavy metals just like fish in the wild. Information arising from the study is expected to guide consumers of cultured fish particularly *H. longifilis.*

The heavy metal levels in *H. longifilis* have been referenced in line with the thresholds for heavy metals in fish published by the Food and Agriculture Organization of the United Nations (1983), Commission Regulation (2008) and CODEX Alimentarius (2015). For emphasis and clarity the stipulated thresholds for Pb, Cd, Zn and Cu in fish are 0.30 mg/kg, 0.10 mg/kg, 40 mg/kg and 30 mg/kg respectively.

MATERIALS AND METHODS

Study Area

Warri town is located in Delta state, Nigeria and has geographic co-ordinates of Latitude 5° 31' N and Longitude 5° 45 'E. (Figure 1).

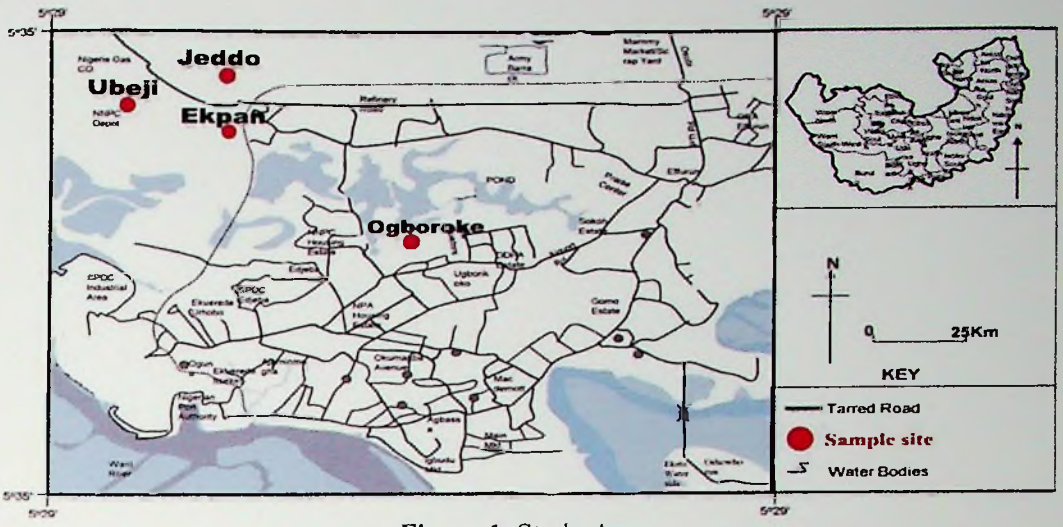


Figure 1. Study Area

It is a renowned hub for crude oil refining activities. Extensive details of the study area have previously been reported by Wangboje and Oghenesode (2017). The town has two distinct climatic seasons, namely, wet season (April- October) and dry season (November-March). Four communities namely, Oboroke, Ekpan, Ubeji and Jeddo, were purposely chosen for the study based on the availability of earthen ponds in these areas and levels of anthropogenic activities. Oboroke plays host to elementary schools, fishing activities, an oil servicing company and markets. Ekpan hosts the Warri refining and petrochemical company, a wood market, sawmill, Motor Park, elementary schools, markets, hotels and a general hospital. Ubeji and Jeddo both have markets, hospitals, elementary schools and petroleum tank farms. One pond from each of the aforesaid communities was selected for this pilot study based on available resources. Characteristics of the sampled earthen ponds are presented in Table 1.

Table 1. Characteristics of sampled earthen ponds

Station	Name of cultured species	Number sampled	Mean total length of fish (cm)	Mean weight of fish(g)	Earthen pond type	Stocking density (Fish/M^3)	Artificial feed
Oboroke	*H. longifilis*	12	38.52± 1.24	525.26± 0.78	Excavated	18	Coppens®
Ekpan	*H. longifilis*	12	42.61± 0.45	631.15±1.07	Excavated	16	Coppens®
Ubeji	*H. longifilis*	12	41.73± 0.57	552.27±0.98	Excavated	25	Coppens®
Jeddo	*H. longifilis*	12	39.25± 1.09	544.32±1.15	Excavated	17	Coppens®

Collection of Fish and Water Samples

Fish samples of seemingly similar size were netted from the earthen ponds after which they were washed with water from the pond to remove adhering debris and transported to the laboratory within 24 hours in a Thermolineo® ice chest. Surface water samples were collected in 1 Litre capacity polythene bottles with screw caps at approximately 30 cm depth. Water samples were acidified with 10% nitric acid to a pH of 1.5, in order to keep metals in solution and to prevent them from adhering to the walls of the bottles as a standard practice. Water samples were transported to the laboratory within 24 hours on ice. The sampling campaign was carried out between January and June, 2018. Collection of both fish and water samples over months was done in order to identify the possibility of temporal variations. Water samples were used for bioaccumulation studies.

Laboratory Procedures

In the laboratory, the identities of the fish species were confirmed using taxonomic keys (Idodo-Umeh, 2003; Adesulu and Sydenham, 2007) and a field guide (Olaosebikan and Raji, 2013). They were weighed whole to the nearest gram using a Scout Pro SPU402® electronic top loading scale while their total lengths were recorded to the nearest centimetre, using a measuring board. Myonematic tissues were excised with a stainless steel lancet from the flanks of fish specimens and oven dried at a temperature of 90°C for 48 hours in a Surgifield-Uniscope® (SM 9023 model) laboratory oven. Each dried sample was ground separately with a porcelain mortar and pestle and kept in labelled plastic vials with covers prior to digestion. Digestion was achieved following the method of Muiruri *et al.,* (2013). The digest was stored in a 100 ml plastic reagent bottle ready for Atomic absorption spectrophotometer (AAS) analysis. For the analysis of pond water, fifty (50) ml of water was measured into a 250 ml conical flask. Twenty (20) ml of concentrated HNO_3 was added and the mixture was heated (80 ° C) over medium flame under a hood till the solution was reduced to 10 ml. The digest was transferred into a 250 ml volumetric flask and made up to mark with distilled water. Each sample was stored in a sterile reagent bottle with a glass stopper prior to analysis. All digests were analysed for Zn, Pb, Fe, Cu and Cd by means of an Atomic Absorption Spectrophotometer (Unicam® 969 series) equipped with solar software using air acetylene flame as an oxidant. Concentrations of metals in fish and water were expressed in mg/kg (wet weight) and mg/L respectively, while Blanks, spiked samples, reference material analyses and duplicate analyses were performed for all analyses as part of the quality assurance procedures. All reagents used were of analytical grade (SIGMA, U.S.A.). Statistical software (GENSTAT® version 13.3 for Windows) was used for analysing generated data. Analysis of variance (ANOVA) was used to test for significant differences ($p<0.05$) between mean values of heavy metals while Duncan Multiple Range Test was used to separate significant means. Microsoft Excel (for Windows 2010) was used for all graphical presentations.

Bioaccumulation quotient (BQ)

The Bioaccumulation Quotient (BQ) or Bioaccumulation factor for heavy metals in fish was ascertained using the method by Eikenberry *et al.,* (2015).

$$\frac{Heavy\ metal\ level\ in\ fish}{Heavy\ metal\ level\ in\ water}$$

Hazard Quotient (HQ) for Heavy Metals

The hazard quotient (HQ) for chemical elements is a comparison of the measured concentration of site-related elements in

ecological matrices with specific health-based criteria (Newstead *et al.*, 2002).

$$HQ = \frac{\text{Concentration of heavy metal in fish sample}}{\text{Health based criteria}}$$

Maximum acceptable risk index (MAR) for heavy metals

The maximum acceptable risk index (MAR), is a simplified representation of bio magnifications in food webs (Reinhold *et al.*, 1999).

$$MAR = \frac{\text{Dietary no observed effect concentration of chemical element in man}}{\text{Bioaccumulation Quotient (BQ) for chemical element in fish}}$$

Where: MAR>1 = High MAR level and MAR<1= Low MAR level.

Estimated Average Daily Intake (EADI) of Heavy Metals by Man

The EADI was obtained by multiplying the mean heavy metal content in fish (mg/kg) by the per capita fish consumption of an area (0.04kg/person/day in the Niger Delta) and dividing the product by the typical human adult body weight, estimated to be 70 kg (Ezemonye *et al.*, 2017).

RESULTS

Concentrations of heavy metals in water

As shown in Table 2, the mean concentrations of heavy metals in water by month, ranged from 0.02 mg/L for Pb in March to 3.56 mg/L for Cu in May with significant differences ($p<0.05$) observed in the mean concentrations of metals in water between months. The mean concentrations of metals in water by station ranged from 0.07 mg/L for Pb at Ekpan to 3.47 mg/L for Cu at Ubeji with significant differences ($p<0.05$) observed in the mean concentrations of Cu, Pb and Zn in water between stations (Table 3).

Table 2. Mean concentrations (mg/L) of heavy metals in water by month

Month	Cd	Cu	Pb	Zn
January	0.23 ± 0.11^{b}	1.72 ± 0.28^{c}	1.27 ± 1.49^{a}	0.89 ± 0.65^{c}
February	0.29 ± 0.13^{a}	1.34 ± 0.38^{c}	0.21 ± 0.13^{b}	0.41 ± 0.22^{c}
March	0.21 ± 0.03^{b}	3.04 ± 2.33^{a}	0.02 ± 0.01^{c}	1.32 ± 1.04^{b}
April	0.26 ± 0.17^{a}	1.48 ± 0.39^{c}	0.06 ± 0.04^{c}	1.39 ± 1.10^{b}
May	0.19 ± 0.19^{b}	3.56 ± 3.95^{a}	0.09 ± 0.06^{c}	1.18 ± 1.23^{b}
June	0.42 ± 0.42^{a}	2.56 ± 0.64^{b}	0.17 ± 0.12^{b}	2.68 ± 2.18^{a}
SEM	0.08	1.34	0.72	0.87
*Pond Threshold	0.05	0.02	0.1	1.0

Columns with similar letters are not significantly different ($p>0.05$). * Schneider (1971)

Table 3. Mean concentrations (mg/L) of heavy metals in water by station

Station	Cd	Cu	Pb	Zn
Oboroke	0.22 ± 0.08^{a}	1.76 ± 0.74^{b}	0.91 ± 2.01^{a}	0.57 ± 0.51^{b}
Ekpan	0.27 ± 0.12^{a}	1.89 ± 1.89^{b}	0.07 ± 0.05^{b}	1.18 ± 1.38^{b}
Ubeji	0.31 ± 0.18^{a}	3.47 ± 3.37^{a}	0.08 ± 0.06^{b}	2.17 ± 1.62^{a}
Jeddo	0.24 ± 0.08^{a}	1.98 ± 1.24^{b}	0.17 ± 0.16^{b}	1.32 ± 1.21^{b}
SEM	0.08	1.08	0.58	0.72
Pond Threshold	0.05	0.02	0.1	1.0

Columns with similar letters are not significantly different ($p>0.05$)

Concentrations of Heavy Metals in *H. longifilis*

As presented in Table 4, the mean concentrations of metals in *H. longifilis* by month, ranged from 0.13 mg/kg for Cd in January to 72.51 mg/kg for Cu in June with significant differences ($p<0.05$) observed in the mean concentrations of Cu, Pb and Zn in fish between months. As shown in Table 5, the mean concentrations of metals in *H. longifilis* by station, ranged from 0.12 mg/kg for Cd at Jeddo to 69.27 mg/kg for Cu at Ubeji with significant differences ($p<0.05$) observed in the mean concentration of Zn in fish between stations.

Table 4. Mean concentrations (mg/kg) of heavy metals in *Heterobranchus longifilis* by month

Month	Cd	Cu	Pb	Zn
January	0.13 ± 0.05^a	52.08 ± 19.95^b	3.24 ± 2.13^a	57.82 ± 32.15^a
February	0.17 ± 0.08^a	55.03 ± 11.17^b	0.55 ± 0.26^b	7.51 ± 4.69^b
March	0.16 ± 0.05^a	65.75 ± 8.77^a	0.45 ± 0.29^b	6.25 ± 1.31^b
April	0.17 ± 0.08^a	62.55 ± 2.48^a	0.37 ± 0.23^b	6.85 ± 1.56^b
May	0.17 ± 0.15^a	71.58 ± 6.44^a	0.73 ± 0.34^b	7.71 ± 1.91^b
June	0.15 ± 0.14^a	72.51 ± 10.68^a	0.85 ± 0.71^b	8.53 ± 0.62^b
SEM	0.05	7.97	0.67	9.42
Threshold	0.10*	30**	0.30***	40**

Columns with similar letters are not significantly different ($p>0.05$) *Commission Regulation (2008) ** FAO (1983) *** CODEX Alimentarius (2015)

Table 5. Mean concentrations (mg/kg) of heavy metals in *Heterobranchus longifilis* by station

Station	Cd	Cu	Pb	Zn
Oboroke	0.15 ± 0.05^a	65.55 ± 12.42^a	0.66 ± 0.34^a	13.48 ± 11.15^b
Ekpan	0.16 ± 0.07^a	58.92 ± 9.47^a	1.18 ± 1.91^a	23.07 ± 40.34^a
Ubeji	0.18 ± 0.07^a	69.27 ± 12.29^a	1.36 ± 1.78^a	13.81 ± 16.96^b
Jeddo	0.12 ± 0.04^a	59.25 ± 15.93^a	0.93 ± 0.56^a	12.73 ± 14.43^b
SEM	0.03	7.35	0.81	132.71
Threshold	0.10	30	0.30	40

Columns with similar letters are not significantly different ($p>0.05$)

The Bioaccumulation quotient (BQ) values for metals in fish ranged from 0.50 for Cd at Jeddo to 37.24 for Cu at Oboroke (Figure 2) while the Hazard quotient (HQ) for metals ranged from 0.39 for Zn to 3.43 for Pb as shown in Figure 3. The Maximum acceptable risk (MAR) values for metals in fish ranged from 0.02 for Pb at Ekpan to 6.29 for Zn at Ubeji (Figure 4) while the Estimated average daily intake (EADI) of heavy metals ranged from 0.09 mg/person/day for Cd to 36.13 mg/person/day for Cu as presented in Figure 5. As presented in Figure 6, the total heavy metal load in *H. longifilis* by station ranged from 73.03 mg/kg at Jeddo to 84.62 mg/kg at Ubeji while the heavy metal quota peaked at 78.86 % and 59.53 % for Cu in fish and water respectively (Figure 7). The condition factor for fish ranged from 0.30 at Jeddo to 0.92 at Oboroke (Figure 8).

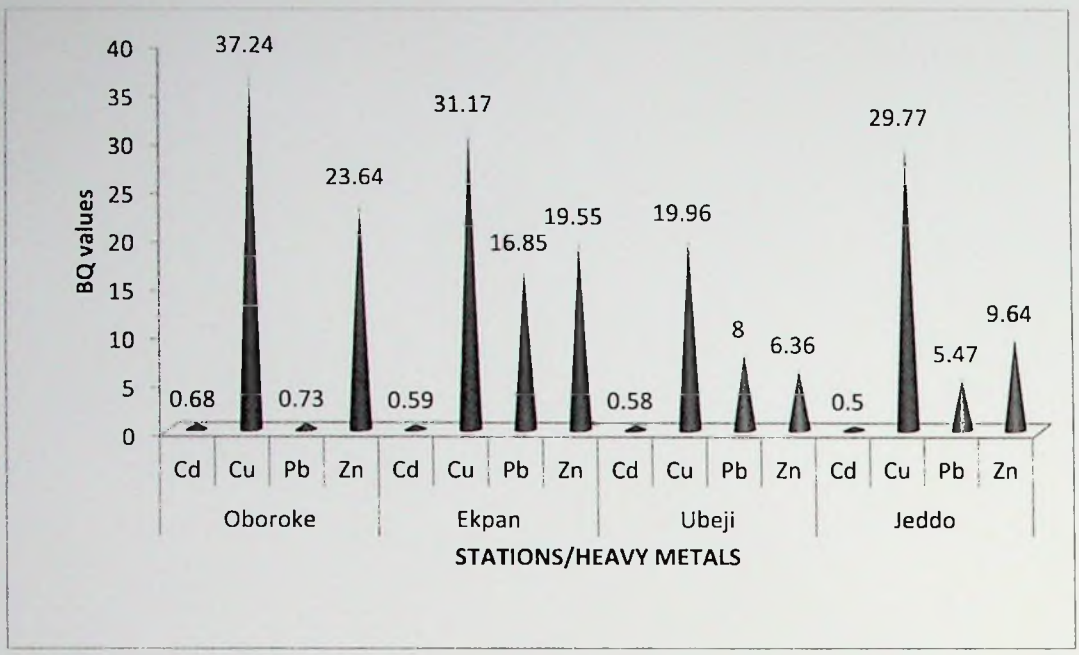


Figure 2. Bioaccumulation quotient (BQ) for heavy metals in *Heterobranchus longifilis*

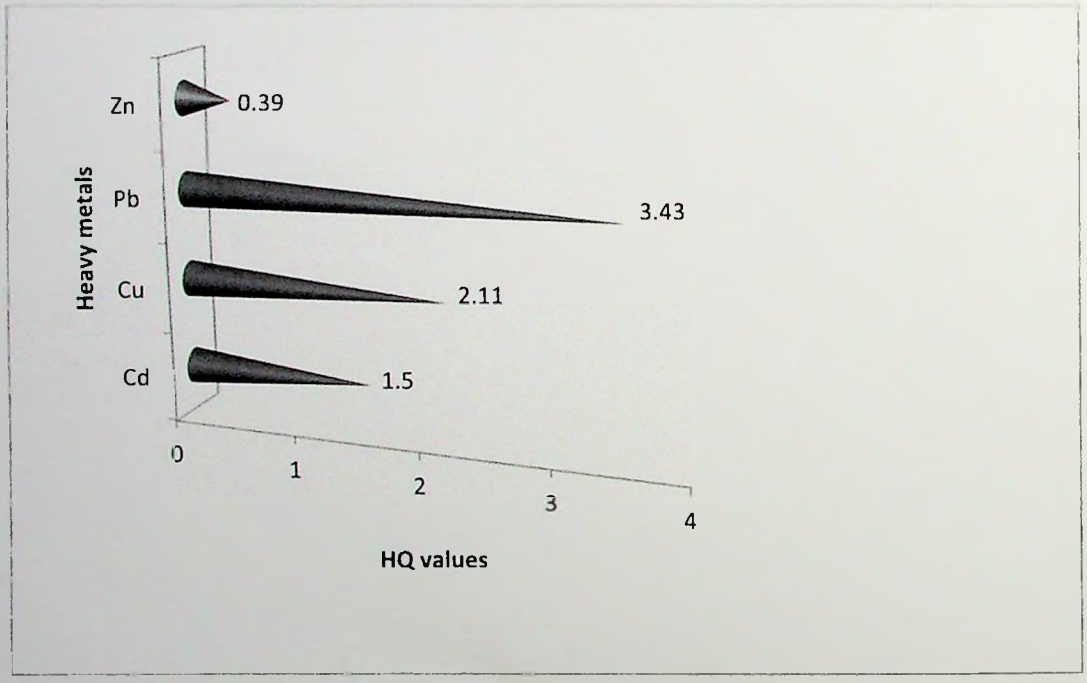


Figure 3. Hazard quotient (HQ) values for heavy metals in *Heterobranchus longifilis*

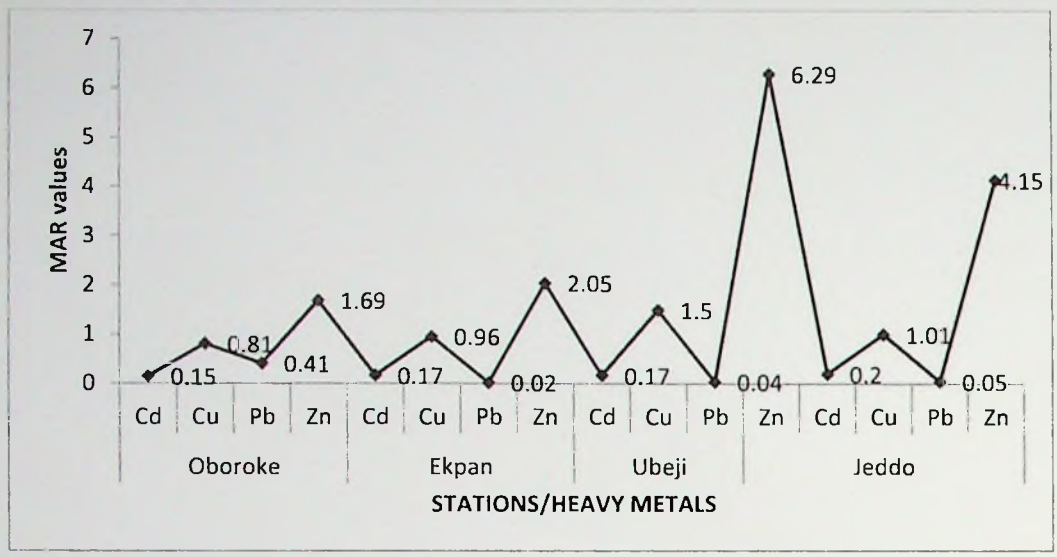


Figure 4. Maximum acceptable risk (MAR) values for heavy metals in *Heterobranchus longifilis*

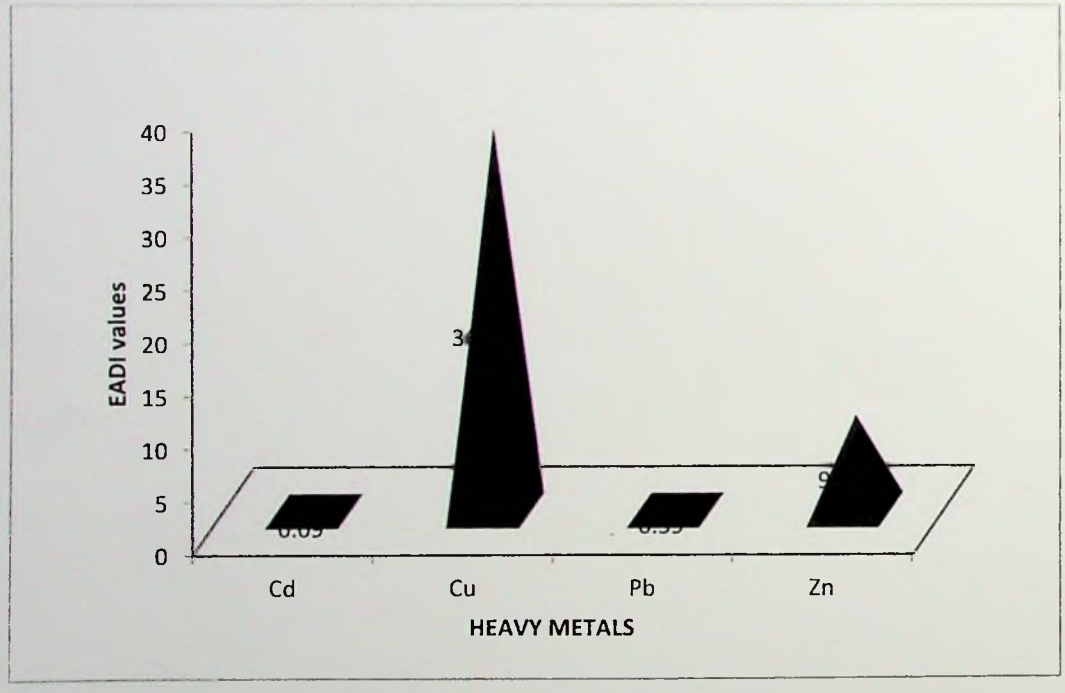


Figure 5. Estimated average daily intake (EADI) for heavy metals

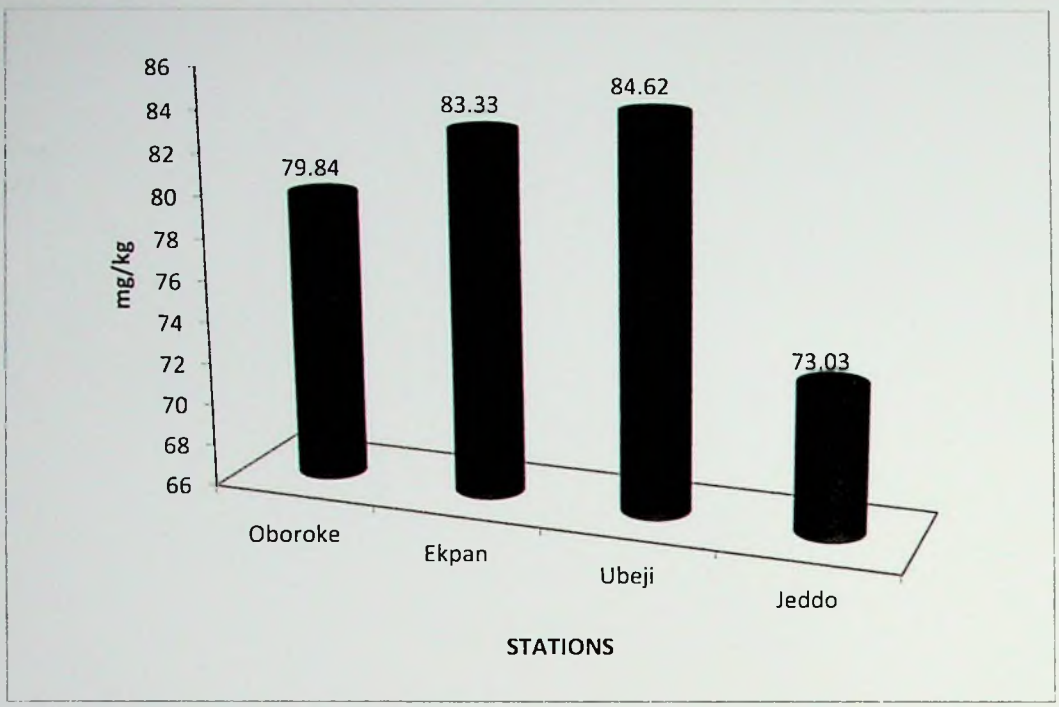


Figure 6. Total heavy metal load in *Heterobranchus longifilis* by station

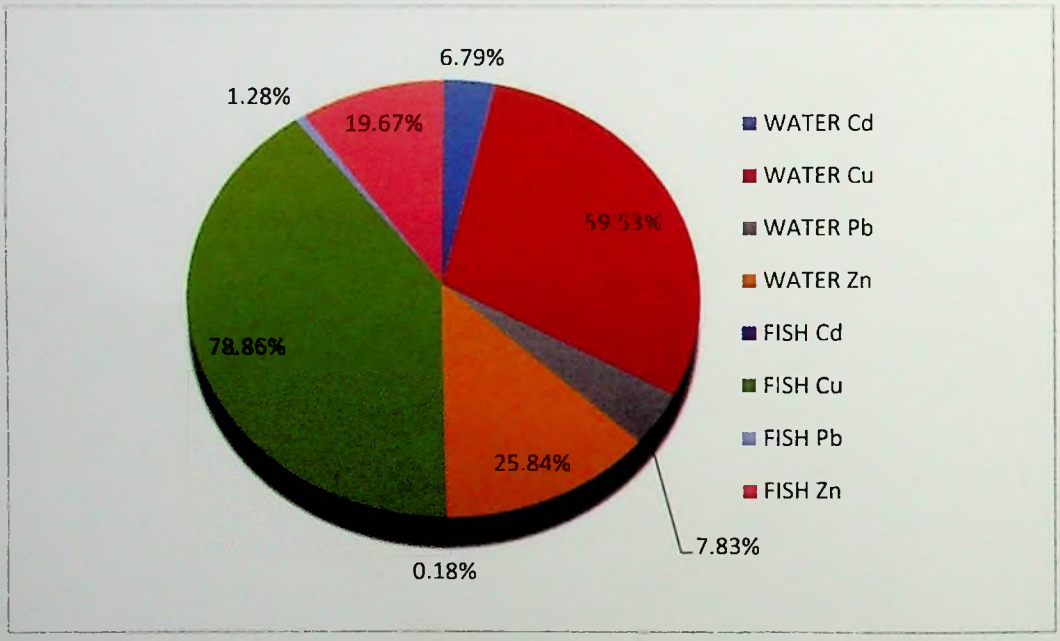


Figure 7. Heavy metal quota in water and fish for the study period

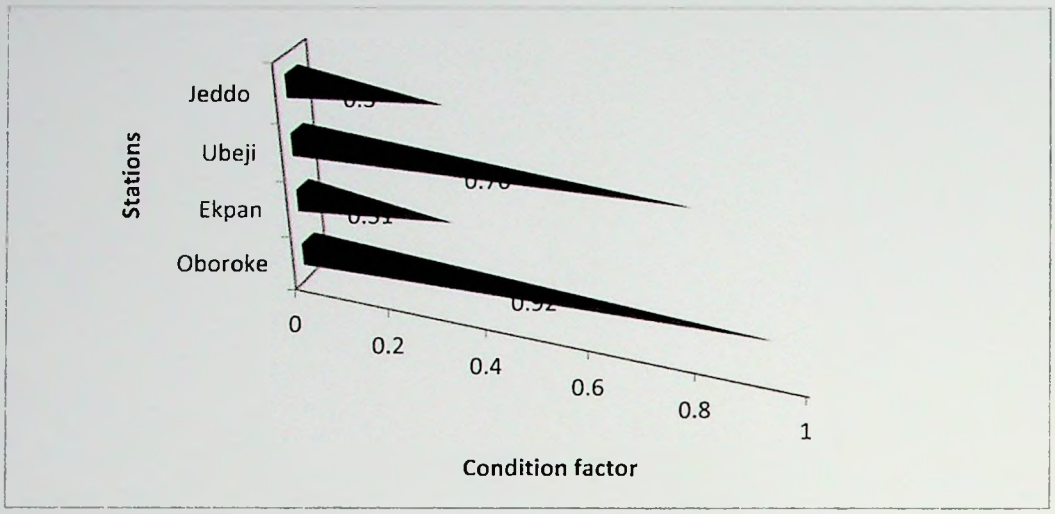


Figure 8. Condition factor for fish by station

DISCUSSION

It was observed that the mean concentrations of heavy metals in *H. longifilis* in the examined earthen ponds took the general order Cu>Zn>Pb>Cd , indicating that Cu was readily more bioavailable to the fish than the other metals while Cd was the least in terms of bioavailability. The heavy metal quota in water and consequently in fish, further gives a graphic representation of this observation. In addition, this observation was supported by the BQ values for metals in fish, which was the highest and lowest for Cu and Cd respectively. Generally, Cd was the only metal not bio accumulated by fish. This observation can be attributed to the fact that Cd had the least mean concentration in water compared to the other metals and therefore was not readily available to fish. It could also mean that the metal was speedily metabolized by fish so that bioaccumulation was nil. According to Aldoghachi *et al.*, (2016), heavy metals that are not metabolized in the body of fish become toxic and will accumulate in soft tissues. Levels of metals in fish are known to be influenced by growth rate, metabolism, feeding patterns and ecological requirements of individual fish species (Ibrahim *et al.*, 2018). According to the Australian and New Zealand guidelines for fresh and marine water quality, metal bioaccumulation by fish can be influenced by pH, kinetics of uptake, depuration time, redox status in the gut, concentration and chemical speciation of the metal as well as the ability of fish to internally regulate metal concentrations or inactivate effects of accumulated metals (ANZECC/ARMCANZ, 2000). Apart from Zn, there were no significant differences ($p>0.05$) in the mean concentrations of the metals in fish between stations, giving an indication of the relative steady or uniform status of these metals to fish during the duration of the study. It was observed that the mean concentrations of Cd, Cu and Pb in fish were highest at Ubeji station, clearly suggesting a higher dose of these metals at this station compared to the other stations while the mean concentrations of Cd and Zn in fish were lowest at Jeddo station. In addition, the total heavy metal load in fish was at a peak at Ubeji and was the least at Jeddo,

suggesting a conceivably higher impact of metals at the former station. It is logical to categorically state that runoff from the petroleum tank farm at Ubeji could be contributing to the high levels of metals in the earthen pond at this point. This assertion is made within the framework that runoff from the tank farm could conceivably reach the earthen pond, making the depot a potential point source of contamination. This assertion is buttressed by the fact that refined petroleum products typically contain heavy metals such as Pb, Cd, Cr, Cu, Ni, Fe and Zn (Oketola *et al.,* 2013; Akpoveta and Osakwe, 2014). Similarly, the highest mean concentration of Zn in fish was observed at Ekpan. The refining and petrochemical company at Ekpan could be linked to the high levels of Zn in fish. This deduction is made on the premise that refineries are known sources of heavy metals especially Ni, Zn, V, Pb, Fe and Mn (Calamari and Naeve, 1994). Generally adjoining roads, farms, landscapes and the atmosphere have been recognized as non-point sources of pond water contamination (Brown and Froemke, 2012). Significant differences ($p<0.05$) were observed in the mean concentrations of Cu, Pb and Zn in fish between months, giving an indication of seasonal variation in the dynamics of the metals in fish between months. For example, the peak concentrations of Cd, Pb and Zn in fish were recorded within the dry season while the peak concentration of Cu in fish was recorded in the wet season. Furthermore, the lowest concentrations of Cd, Cu, Pb and Zn in fish were recorded in the dry season. In order to fully understand the dynamics of heavy metals in the pond system, it may be necessary to analyse the pond soil or sediment, which can serve as a repository for metals. Water samples could also be collected from both upstream and downstream points while data could be collected over a longer period such as a year or two in order to make more profound assertions and deductions. Health-wise, the mean concentrations of Cd, Cu and Pb in fish exceeded established International thresholds of 0.10 mg/kg, 30 mg/kg and 0.30 mg/kg respectively for these metals in fish. For example, the mean concentration of Cd in fish was one and a half times over its limit while Cu and Pb were twice and thrice over their respective limits. This observation clearly indicates the unfitness of such fish for prolonged consumption by man. In this study, the applied thresholds for metals in fish were Food and Agricultural Organization of the United Nations (FAO, 1983), Commission Regulation (2008) and CODEX Alimentarius (2015). The mean concentrations of Cd (0.19 mg/kg), Pb (3.31 mg/kg) and Zn (21.71 mg/kg) in a similar catfish (*Pseudoplatystoma corruscans*) from the Paraopeba River in Brazil (Arantes *et al.,* 2016) were much higher than the corresponding metals in fish in this study. The workers apportioned the contamination of fish to the receipt of effluents from electroplating and steel industries in the vicinity. The extremely high mean value of Zn (60 mg/kg) recorded for *Mastacembelus armatus* by Ashraf *et al.,* (2012) from a pond in Bestari Jaya, Malaysia, quadrupled the mean concentration of Zn found in fish in this study. The workers attributed the high level of Zn and other detected metals to the influx of effluents from a former tin mining zone. The mean concentrations of Cu (0.54 mg/kg) and Cd (0.06 mg/kg) observed in *Clarias gariepinus* (African sharp tooth catfish) from Lake Njuwa, Adamawa state, Nigeria by Ibrahim *et al.,* (2018), were far less than the mean concentrations of the same metals in fish in this study, suggesting a much higher interaction between fish and these metals in this study. In water, the general rank profile of heavy metals was Cu>Zn>Pb>Cd, giving an indication of a dominant presence of Cu and its associated compounds in water. Station-wise, Cu had the highest

CONCLUSION

The bifurcated objectives of this research were successfully achieved. The study provided baseline data for some heavy metals in *H. longifilis* cultured in earthen ponds in Warri town, Nigeria, which could serve as a reference point for future studies. Furthermore, it was revealed that the mean concentrations of Cd, Cu and Pb in *H. longifilis* surpassed established International thresholds for these metals in fish indicating the unfitness of such fish for prolonged consumption by man. The aforesaid metals were also shown to be a potential threat to the health of *H. longifilis* in the earthen ponds. Following the outcome of the study, it becomes mandatory for the heavy metal content in these earthen ponds to be routinely monitored in order to keep metal levels within safe limits. Furthermore, it is proffered that heavy metals not included in this research be incorporated in future studies in order to extend the metal repository for the study area.

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