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Assessment of Heavy Metals (Zn, Pb, Cr and Cd) Levels in the Gill, Intestine and Flesh of *Oreocromis niloticus* and *Liza grandisquamis* obtained from Azuabie and Okujagu-Ama creeks in the Niger Delta, Nigeria.

M. Moslen*, I. K. E. Ekweozor and K. A. Ogbuehi
Department of Animal and Environmental Biology, Rivers State University of Science and Technology, Port Harcourt, Nigeria.
*Corresponding author: email – moslen4c@yahoo.com

Abstract
The Azuabie and Okujagu-Ama creeks, are prone to pollution due to the discharge of industrial, domestic and abattoir wastes into the creeks. The aim of this study was to assess the level of some heavy metals (Zn, Pb, Cr and Cd) in the gill, intestine and fleshy tissue of *Oreochromis niloticus* and *Liza grandisquamis* obtained from the creeks. Fish Samples were analysed following the method of ASTM and determined using Atomic Absorption Spectrophotometery. Results indicated fish samples examined generally had elevated concentrations of heavy metals. In the gill and intestine of *Orechromis niloticus*, it was observed that Zn>Cr>Pb>Cd but in the fleshy tissue Zn>Pb>Cd>Cr but generally, Zn had the highest concentration in the different parts of the fish examined. Also, in the tissues of *Liza grandisquamis,* it was observed that generally, Zn>Cr>Pb>Cd. Though, individual metals varied in concentrations, the gill, intestine and flesh of both species of fish samples did not show significant difference (p>0.05) in their metal levels. The t-Test of significance for each metal between the two creeks was also not significantly different (P>0.05), implying that metal concentrations in fish from the two creeks were almost the same. It was however, noticed that the concentration of metals in fish from the creeks generally exceeded limits in sea food set by WHO, FAO and FEPA. Conclusively, there was gradual bioaccumulation and possible biomagnefication of heavy metals in fish from Azuabie and Okujagu-Ama creeks and this could be a risk for consumers of sea food from such presumed polluted areas.

Keywords: Heavy metals, Fish, Azuabie and Okujagu-Ama creek, Niger Delta

1.0 **Introduction**

Rapid growth and expansion of industries in recent years has resulted in the substantial increase in effluents which, are normally discharged into open land or aquatic environment causing a number of environmental problems (Ramona *et al*., 2001, Chezhieon *et al*., 2010). Indiscriminate discharge of these industrial wastes have aggravated the problem of aquatic pollution and contamination thereby causing alterations in the natural condition of aquatic medium and consequently results in changes in the internal mechanism as well as morphological imbalance of aquatic organisms (Yadav *et al*., 2005). Human activities have often led to increased levels of heavy metals in the environment. Metal concentrations in the biota are generally low, except in the vicinity of metal pollution (Lindqvist and Block, 1994). The accumulation of heavy metals in an aquatic environment has direct consequences to man and to the ecosystem. Although metals such as Cu and Zn are generally regarded as essential trace metals in view of their valuable role in metabolic activities in organisms, other metals like Cd, Pb, Ni and Hg exhibit extreme toxicity even at trace levels (Merian, 1991; DWAF, 1996). However, it is of interest to note that most essential metals are toxic when supplied in concentrations in excess of the optimum levels. Tam and Wong (1995) stated that heavy metal contamination in aquatic environments is of critical concern due to the toxicity of metals and their accumulation in aquatic habitats. Among the aquatic fauna, fish is the most susceptible to heavy metal toxicants (Nwaedozie, 1998) and so, are more vulnerable to metal contamination than any other aquatic fauna. Heavy metals could enter the aquatic environment from both natural and anthropogenic sources. Natural sources include weathering of minerals and soils (Merian, 1991). Anthropogenic inputs are mainly from industrial effluents, domestic effluents, rural and urban storm water runoff and soil heaps (Agbozu and Ekweozor, 2001). Ibok, *et al*., (1989) reported elevated levels of Hg, Zn, Cu, Co, Sb, Cd, and Pb in fishes from some streams in Ikot Ekpene area of Nigeria. Fishes were considered as better specimens for use in the investigation of pollutant loads than the water samples because of the significant levels of metals they bioaccumulate. Significant heavy metal levels were recorded in fishes of Warri River (Atuma and Egborge, 1986); fish and Shellfish in the Niger Delta (Kakulu, *et al*., 1987). Data provided by Agada (1994) also showed evidence of selected heavy metal contamination of *Chrysicthys nigrodigitatus* (catfish) and *Pseudotohithus elongatus* (croaker) respectively. Despite the relatively low concentrations of trace metals in the surrounding medium, marine organisms take up and accumulate them in soft tissues to concentrations above ambient environmental levels that are capable of exerting biological effects. The body content of a trace metal in any organism results from the net balance between the processes of metal uptake and metal loss. Fishes used for this study were collected from Azuabie and Okujagu-Ama creeks in the Niger Delta area of Nigeria. The aim of this study was to assess the tissue concentrations of heavy metals (Pb, Zn, Cr and Cd) in *Oreochromis niloticus* and *Liza grandisquamis* and to compare their concentrations in the gill, intestine and fleshy tissue.

2.0 Materials and methods

2.1 Study site

Azuabie and Okujagu-Ama creeks are situated on the eastern part of the Bonny estuary in southern Nigeria (Fig. 1).

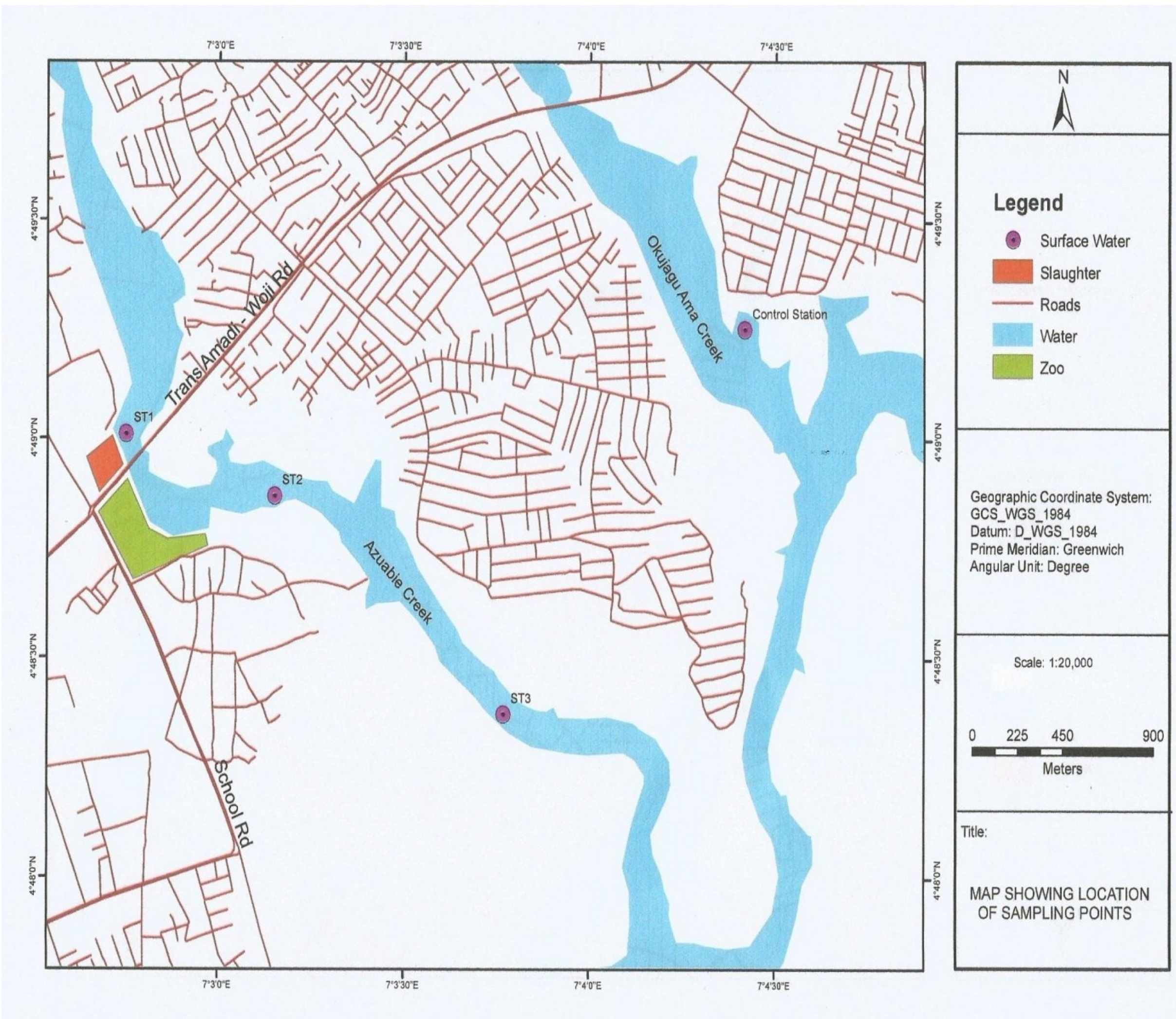

Fig.1. Map of study area showing locations of Azuabie and Okujagu-Ama creeks

The Azuabie creek and associated water systems (upper reaches of the Bonny Estuary) is one of the most important aquatic ecosystems in the Niger Delta exceptionally prone to pollution from numerous human activities within and outside the area. Among the various human activities, fishing, boating navigation, laundry, disposal of human bodily wastes, bathing and swimming go on in and around the area unregulated. This aquatic bodies also receives effluent discharges from many industries, residential buildings and the main Port Harcourt abattoir sited along the bank at Trans-Amadi industrial layout.

2.2 Analysis of Heavy metal in fish samples

Fish samples were obtained directly from fishermen on the Azuabie and Okujagu-Ama creeks and preserved in frozen conditions prior to analysis. The frozen samples were allowed to soften at room temperature and only the boneless tissue, (gills, intestine and the fleshy tissue) were taken for metal analysis. The organs were removed with a stainless steel knife, and samples were dried to constant weight at 90^{o}C for two days in clean acid washed petri-dishes. After drying, the samples were kept in a desiccator and allowed to cool. The samples were then crushed into fine powders using porcelain mortar and pestle, 1g of each sample was taken and digested by microwave digestion method. In this method of digestion, Nitric Acid (Analar grade) and hydrogen peroxide (Analar Grade) in the ratio of 3:1 were added to the samples. The mixtures were digested at 150 ^{o}C for 30 minutes in the microwave oven. Microwave digestion method was used in this work because it is a more accurate method for digestion of samples (especially for organic samples) than other methods such as dry ashing and wet digestion. The hydrogen peroxide added to the sample with nitric acid reduces nitrous vapour and speeds up digestion of organic substances by increasing the temperature of reaction in the digestion process. The digested samples were filtered with 20 ml of deionized water. The filtrate was collected with clean acid-washed and appropriately labeled 50 ml polyethylene sampling containers for analysis by Atomic Absorption Spectrometer (Model 210VGP Buck Scientific, USA).

2.3 Statistical Analysis

ANOVA was used to determine significant difference between the different parts (gills, intestine and flesh) of the fish examined and also the concentrations between the different heavy metals. The t-Test was then used for test of significance for heavy metal concentration between the two creeks examined. All statistical analysis and presentation of results was done using Microsoft excel.

3.0 Results

Distribution of heavy metal in gill, intestine and flesh of tilapia.

The variations in the mean concentration of heavy metal in gill, intestine and flesh of *Oreochromis niloticus* in Azuabie and Okujagu-Ama creeks are shown in Fig 2 while the summary of the ANOVA and t-Test output are presented in Tables 1 and 2.

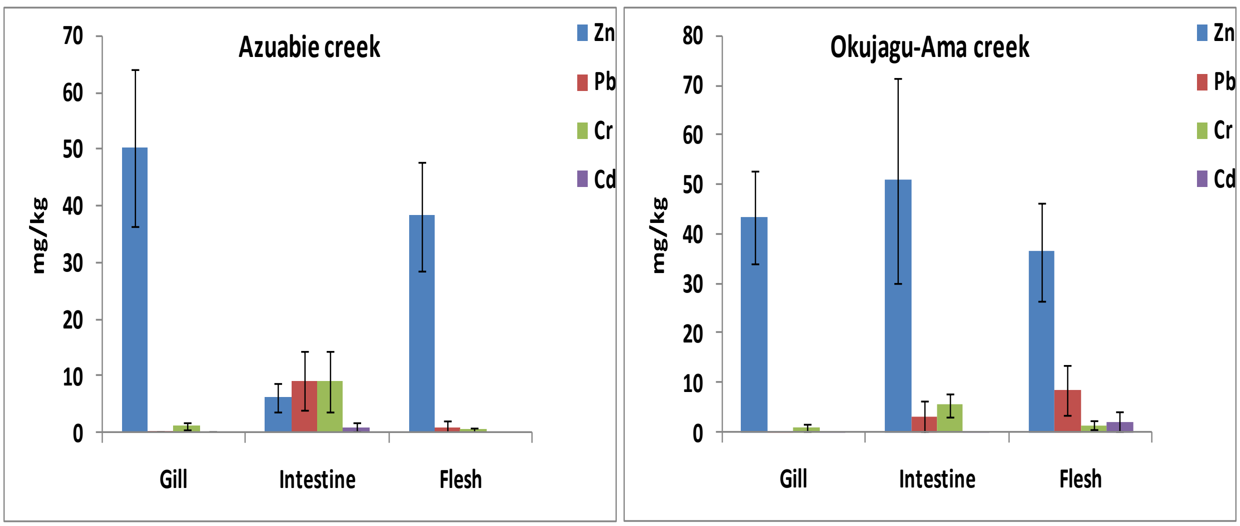

Fig.2: The concentration (mean ± SD) of metals in gills, intestine and flesh of *Oreochromis niloticus* from Azuabie and Okujagu-Ama Creeks.

Table 1: Summary of ANOVA output for different tissues of *Oreochromis niloticus*

Variables	MS	F-values
Gill, Intestine and Flesh of *Oreochromis niloticus* in Azuabie creek	244.2188	0.5678^{ns}
Gill, Intestine and Flesh of *Oreochromis niloticus* in Okujagu-Ama creek	113.9457	0.3045^{ns}

Key: ns = not significant

Table 2: Summary of t-Test of significance for heavy metals in *Oreochromis* between the two creeks

Heavy Metals	T-critical	P-value
Zn	4.3026	0.9478^{ns}
Pb	4.3026	0.5778^{ns}
Cr	4.3026	0.8744^{ns}
Cd	4.3026	0.1835^{ns}

Key: ns = not significant

The mean concentrations of Zinc in gill, intestine and flesh were 50.33 mgkg^{-1}, 6.26 mgkg^{-1} and 38.21 mgkg^{-1} in Azuabie creek while in Okujagu-Ama creek Zn values were 43.42 mgkg^{-1}, 50.77 mgkg^{-1} and 36.50 mgkg^{-1} respectively. The mean concentrations of lead in gill, intestine and flesh were 0.16 mgkg^{-1}, 9.23 mgkg^{-1} and 1.08 mgkg^{-1} respectively in Azuabie creek while Pb values in fish from Okujagu-Ama creek were 0.01mgkg^{-1}, 3.22 mgkg^{-1} and 8.63 mgkg^{-1} in gill, intestine and flesh respectively. The mean concentration of chromium in gill, intestine and flesh were 1.22 mgkg^{-1}, 9.12 mgkg^{-1} and 0.46 mgkg^{-1} respectively in fish from Azuabie creek while mean values of chromium in fish from Okujagu-Ama creek were 1.22 mgkg^{-1}, 5.65 mgkg^{-1}, 1.50 mgkg^{-1} in gill, intestine and flesh respectively. The mean concentration of cadmium in gill, intestine and flesh were 0.12 mgkg^{-1}, 1.06 mgkg^{-1}and 0.01 mgkg^{-1} respectively in Azuabie creek while mean cadmium concentration in fish obtained from Okujagu-Ama creek were 0.01 mgkg^{-1}, 0.01 mgkg^{-1} and 2.21 mgkg^{-1} in gill, intestine and flesh respectively. In Azuabie and Okujagu-Ama creeks, the concentrations between heavy metals in *Oreochrimis niloticus* varied significantly ($p<0.05$) but this was not significantly different ($p>0.05$) when compared between the different parts of the fish (gill, intestine and flesh) observed. None of the heavy metals in *Oreochromis niloticus* showed significant difference ($p>0.05$) when compared between the two creeks examined. In the gill and intestine of *Oreochromis niloticus*, it was observed that Zn>Cr>Pb>Cd but in the fleshy tissue Zn>Pb>Cd>Cr. In general, Zn had the highest concentration in the different parts of the fish examined.

The mean concentrations of heavy metals in gill, intestine and fleshy tissue of *Liza grandisquamis* obtained from Azuabie and Okujagu-Ama creeks are shown in Fig. 3 while the summary of the ANOVA and t-Test output are presented in Tables 3 and 4.

Table 3: Summary of ANOVA output for different tissues of *Oreochromis niloticus*

Variables	MS	F-values
Gill, Intestine and Flesh of *Liza grandisquamis* in Azuabie creek	42.8566	0.1297^{ns}
Gill, Intestine and Flesh of *Liza grandisquamis* in Okujagu-Ama creek	14.9595	0.0342^{ns}

Key: ns = not significant

Table 4: Summary of t-Test of significance for heavy metals in *Liza* between the two creeks

Heavy Metals	T-critical	P-value
Zn	4.3026	0.5403^{ns}
Pb	4.3026	0.9168^{ns}
Cr	4.3026	0.6127^{ns}
Cd	4.3026	0.7539^{ns}

Key: ns = not significant

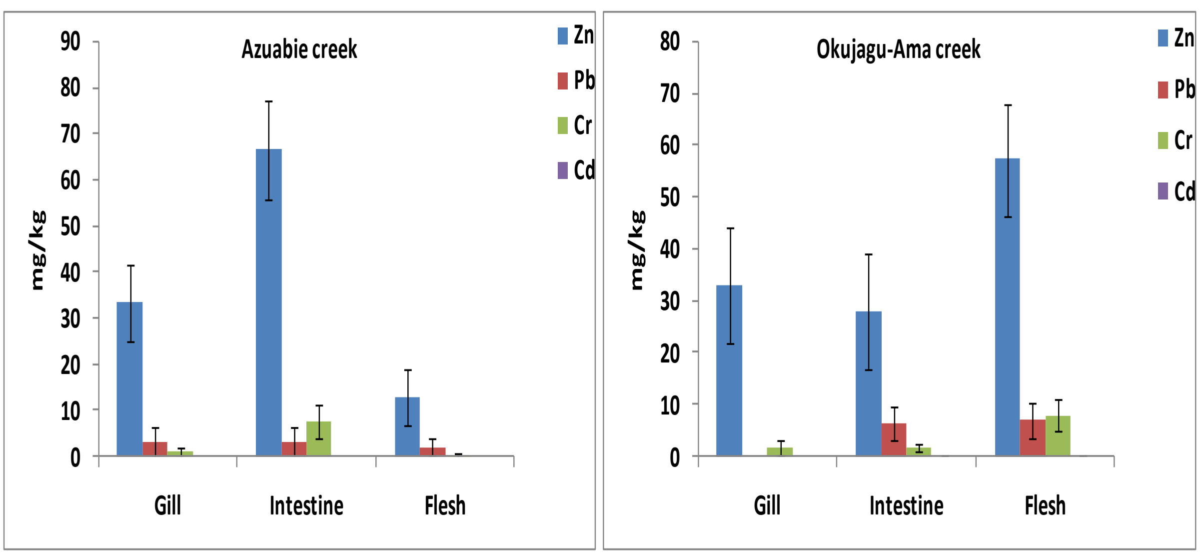

Fig. 3: Variations in the concentration of heavy metals (mean ± SD) in gills, intestine and fleshy tissue of *Liza grandisquamis* from Azuabie and Okujagu-Ama creeks.

The mean concentration of zinc in gill, intestine and fleshy tissues of *Liza grandisquamis* obtained from Azuabie creek were 33.38 $mgkg^{-1}$, 66.43$mgkg^{-1}$ and 13.07$mgkg^{-1}$ respectively while those obtained from Okujagu-Ama creek were 33.05 $mgkg^{-1}$, 27.90 $mgkg^{-1}$ and 57.22 $mgkg^{-1}$ in gill, intestine and flesh respectively. The mean concentration of lead in gill, intestine and flesh were 3.22 $mgkg^{-1}$, 3.22 $mgkg^{-1}$ and 2.16 $mgkg^{-1}$ respectively in Azuabie creek while mean Pb concentration in gill, intestine and flesh in *Liza* obtained from the Okujagu-Ama creek were 0.01 $mgkg^{-1}$, 6.42 $mgkg^{-1}$ and 7.04 $mgkg^{-1}$ respectively. The mean concentration of chromium in gill, intestine and flesh of *Liza grandisquamis* samples from Azuabie creek were 1.08 $mgkg^{-1}$, 7.71 $mgkg^{-1}$ and 0.46 $mgkg^{-1}$ respectively while fish samples obtained from Okujagu-Ama creek had mean chromium concentration of 1.70 $mgkg^{-1}$, 1.65 $mgkg^{-1}$ and 8.01 $mgkg^{-1}$ in gill, intestine and flesh respectively. The mean concentration of cadmium in gill, intestine and flesh of *Liza grandisquamis* samples collected from Azuabie creek were all 0.01 $mgkg^{-1}$ for the respective fish parts while mean cadmium concentration in samples from Okujagu-Ama creek were 0.01 $mgkg^{-1}$, 0.12 $mgkg^{-1}$, 0.12 $mgkg^{-1}$ in gill, intestine and flesh respectively. In samples of *Liza grandisquamis* obtained from Azuabie creek concentrations between heavy metals was significantly different ($p<0.05$) but the different parts (gill, intestine and fleshy tissue) of the fish compared did not show significant difference ($p>0.05$) in their heavy metal content. The different heavy metals in *Liza grandisquamis* did not give significant difference ($p>0.05$) when compared between the two creeks examined depicting equal level of contamination.

4.0 Discussion

Fishes are sensitive indicators of heavy metals pollution (Adeyemi *et al*., 1996). Heavy metals have been reported to exert negative effect on biological processes in general and may influence the nutritional and biological status of sea foods (Udosen *et al*., 2001). The Zn, Cd, Cr, Pb concentration in fish species collected from Azuabie creek and Okujagu-Ama creek were compared with national and international standards. Although, the safe limits for heavy metals in seafood vary from region to region (Ashraf, 2006). The results showed that the heavy metals in *Oreochromis niloticus* and *Liza gradisquamis* found in Azuabie and Okujagu-Ama creek generally exceeded FAO (1983), WHO (1985) and FEPA (2003) permissible limits of Cr (1.0ppm), Pb (2.0ppm) and Cd (2.0 ppm). This result is in consonance with the findings of Moslen and Miebaka (2016) who reported that the concentrations of Cr, Ni, Pb and Ag in fish (*Periophthalmus* sp) obtained from Azuabie creek were above the limits in sea food set by WHO (1985), FAO (1983) and FEPA (2003). The implication of this is that anthropogenic input of heavy metals into the creek is gradually being bioaccumulated by fish species in the area. The Zn, Cd, Cr, Pb concentration in gill, intestine and flesh of *Oreochromis niloticus* and *Liza grandisquamis* found in Azuabie creek and Okujagu-Ama creek were compared to determine the variations in organs ability to accumulate metals. It was observed that the ability of gill, intestine and flesh of fish species to accumulate metals did not follow any trend. This is contrary to the report of Elnabris *et al*. (2013), where muscle had the least accumulation of heavy metal. The ANOVA result also showed that there was no significant difference ($p>0.05$) in the accumulation of heavy metals between the different organs (gill, intestine and flesh) examined though concentrations between individual metals differed. Pollution of aquatic ecosystems by heavy metals is an important environmental problem, as heavy metals constitute some of the most dangerous toxicants that can be bioaccumulated in living tissues (Guo *et al.,* 1997; Omoregie *et al.,* 2002). In this study, metal concentrations in fish samples between the two creeks was not significantly different ($P>0.05$) suggesting the creeks may be equally polluted or inter migration of fish species between the two creeks was apparent considering the mobile nature of the fish samples observed. Daka *et al*. (2008) in a study of heavy metals in some fish species of the Azuabie creek had reported mean Cd and Pb concentrations of 0.055 $mgkg^{-1}$ and 0.186 $mgkg^{-1}$ in *Tilapia mariae* and 0.050 $mgkg^{-1}$ and 0.104 $mgkg^{-1}$ in *Liza falcipinnis*. These values were generally less than what was obtained in this study implying gradual accumulation of such metals in the study area over time. Daka *et al*. (2008) also reported that the concentrations of Pb (0.104 - 0.310 mg/kg^{-1}) were significantly higher in such fish as *Chrysichthys nigrodigitatus* and *Gobius niger* than other species examined. They concluded that the concentrations of cadmium and lead found in the fish from the upper Bonny estuary appear to indicate that they are suitable for human consumption. This study has shown that the report of Daka *et al*. (2008) may longer be tenable due the higher concentration of metals in fish from the Azuabie creeks which exceeds limits in sea food set by WHO (1985), FAO (1983) and FEPA (2003). In conclusion, there is gradual increase in metal concentration in different parts of fish (gill, intestine and flesh) obtained from Azuabie and Okujagu-Ama creeks over time. This implies bioaccumulation and possible magnification along the food chain. This potent health risk for consumers of seafood caught from such presumed polluted environment.

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