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Zn, Pb, Cr and Cd concentrations in fish, water and sediment from the Azuabie Creek, Port Harcourt.

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ABSTRACT:. Gills, intestine and muscle samples of *O* niloticus, water and surface sediments of Azuabie Creek and a central station were collected and analysed for Zn, Pb, Cr and Cd using AAS model 210VGP Buck Scientific USA. Heavy metal concentrations in fish muscle show high concentration especially Pb which is far above the 0.5 mg/kg permissible limit of FAO. The bioaccumulation factor, estimated daily intake and hazard quotient were estimated. An hazard index of 7.56 was recorded in Azuabie Creek. The concentrations of metals in the fish organs assessed varied significantly ($P < 0.05$) © JASEM
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Keywords: Heavy metals, Bioaccumulation factor, EDI, *Oreochromis niloticus*, Azuabie creek.

Heavy metal contamination in the aquatic environment most often has direct consequences to aquatic organisms and man, and fish species show inter and intra specific variations in their accumulation (El-Moselhy *et al.,* 2014). Some heavy metals are essential like Cu, Zn, Fe, Mn and Cr which may not be hazardous except when they exceed acceptable limits. Others like Pb, As, Hg and Cd are non essential and harmful to biota in minute concentrations. For this reason there has been a lot of interest in heavy metal concentration in local fish species from different parts of the world (El-Moselhy *et al*., 2014; Thomas and Mohaideen, 2015) including Nigeria (Ekpo *et al.,* 2008; Ugbomeh and Bakor, 2015; Wangboje and Ikhuabe, 2015; Ugbomeh and Akani, 2016). In most of these surveys, the heavy metal concentrations were above permissible limits, indicating a potential risk to humans and other aquatic organisms.

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

Recent advances in the assessment of aquatic metal pollution usually incorporates a study to evaluate risk to human health through Estimated Daily Intake (EDI), Target Hazard Quotient (THQ) and Carcinogenic Risk (CR) of heavy metals in the wild species of edible indigenous biota so as to effectively predict potential health consequences that can serve as scientific basis for decision-making and policy development in relation to human consumers. However, a study regarding the heavy metal concentration of water and sediment in Azuabie Creek in relation to an important edible tilapia alongside information on health risk associated with the levels of heavy metals are still limited. The aim of this study was to assess the tissue concentrations of these heavy metals (Pb, Zn, Cr and Cd) in the gill, intestine and muscle of *Oreochromis niloticus*, and estimate the EDI and the HI.

MATERIALS AND METHOD

Description of Study Area: The study site Azuabie Creek, is located between longitude $7^0 00^0$ and $7^0 15^0$ N and latitude 4^0 28^0E and $4^0 40^0$N. It is a tributary of the Upper Bonny Estuary in Port Harcourt. Characteristically, the area is a typical estuarine tidal water zone with little fresh water input but with extensive mangrove swamps and inter-tidal mud flats. It is strategically located within SouthWestern flanks of Port Harcourt and Okrika in Rivers State, Nigeria. The aquatic ecosystem is bounded by thick mangrove forest dominated with *Rhizophora* species interspersed by white mangrove (*Avicennia germinans*) and *Nypa fructicans*. This aquatic body receives effluents and discharges from many industrial, municipal and the main Port Harcourt abattoir sited along the bank.

A total of four stations (Fig.1) at least 500metres apart along the creek was selected and sampled monthly for a period of three (3) months (October, 2015 to December, 2015). Co-ordinates and visual notes of permanent and semi-permanent structures were used in marking the sampling locations. Sampling operation at the designated stations was

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carried out using an open motorized boat. Samples of water, sediment and fish were taken over the three month's period. The location and description of the sampling stations are summarized in Table1 .

Fig. 1: Map showing study stations in Azuabie Creek and Okujagu Creek

Table 1: Study Sites, co-ordinates and description in Azuabie Creek and the upper reaches of the Bonny River Estuary

Station	Locations	Coordinates	Description of Location
Station 1	Azuabie	N04^0,48'59.2 E007^{0}02'43.1	Abattoir, sited under the overhead bridge at the Port Harcourt main slaughter house.
Station 2	Azuabie	No4^0,48'47.7 E007 03'08.4	Port Harcourt ZOO . The stations has gravelly bottom due to hyper-current turbulence
Station 3	Azuabie	No4^0,48'17.3 E00703'42.1	About 500m down stream
Station 4	Okujagu Creek	No9^0,49'15.2 E00704'21.2	Okujagu Creek lies to the left, while very dense, luxuriant, and protected mangrove swamp.

Collection of Samples: Water samples were collected for physico-chemical and heavy metal analysis using appropriate sample bottles as described in APHA (2002). Sediment samples were collected from the top 2cm of sediment surface using a benthic grab, while fish samples (*Oreochromis niloticus)* were collected from artisanal fishermen seen fishing the area using cast nets. All samples were collected in replicates. Fish samples were stored in the refrigerator until analysis.

Laboratory analysis: The analysis of physicochemical parameters of water was done following standard methods (APHA, 2002).

In sediment analysis, small quantities of the sediment samples were air-dried; 1g of each was digested with Equia-Regia (mixture of HCL and HNO_3 in the ratio 3:1), filtered with 20ml of de-ionized water and the filtrates stored in clean acid-washed and appropriately labeled 30ml polyethylene sample container for analysis by Atomic Absorption spectrometric method using Atomic Absorption Spectrometer (Model 210VGP Buck Scientific, USA).

The fish samples were allowed to thaw, measured to the nearest 0.1cm and the muscle, gills and the intestine were used for the metal analysis after the method of APHA (2002) using the Atomic Absorption Spectrometric method with an Atomic Absorption Spectrometer (Model 210VGP Buck Scientific, USA).

Data was also subjected to analysis of variance (ANOVA) to determine relationships and levels of significance. Where ANOVA result showed a significant difference, Tukey test were performed for mean separation.
Bioaccumulation factor was estimated as the ratio of the concentration of heavy metal in animal tissue (fish species) to the concentration of heavy metals in the sediment (BAF = bio accumulation factor (Falusi and Olanipekun, 2007)).

$$BAF = \frac{\text{Heavy metal concentration animal}}{\text{Heavy metal concentration in sediment}}$$

The public health risk associated with consumption of edible biota (fish species collected from the Azuabie Creek was evaluated by using the Estimated Daily Intake (EDI) as in Ugbomeh and Jaja (2013) to determine the Target Hazard Quotient (THQ).

Target Hazard Quotient (THQ) is given by: **THQ = EDI / R_{FDO}**

Where EDI = Estimated Daily Intake

R_{FDO} = the reference oral dose of individual metal (mg/kg/day)

R_{FDO} of the investigated metals are as follows:
Cd = $1x10^{-3,}$ Pb = $4x10^{-3}$, Cr = 1.5 and Zn = $3x10^{-1}$

The estimated daily intake (EDI) of each heavy metal in this exposure pathway was determined by the equation

$$EDI = \frac{E_F \ x \ E_D \ x \ F_{IR} \ x \ C_F \ x \ C_M}{W_{AB} \ x \ T_A} \ x \ 10^{-3}$$

E_F = The exposure frequency 365 days/year
E_D = The exposure duration, equivalent to verge life time (65 years)
F_{IR} = The fresh food ingestion rate (g/person/day) which is considered to be 200g / person / day
C_F = The conversion factor = 0.208
C_M = The heavy metal concentration in food stuffs (mg/kg d-w)
W_{AB} = Average body weight (60 kg)
T_A = Is the average exposure time for non-carcinogens (it is equal to (E_F x E_D) as used by Wang *et al*., (2005).

To estimate the risk to human health through more than one heavy metal, the hazard index (HI) has been developed (USEPA, 1989). The hazard index is the sum of the hazard quotients for all heavy metals, which was calculated by the equation -
$HI = \sum HQ = HQ_{cr} + HQ_{zn} + HQ_{pb} + HQ_{cd}$ (Guerra *et al*, 2010).

RESULTS AND DISCUSSION

The physico chemical parameters in Table 2 show the water characteristics as at the time of sampling. It was acidic with low DO values of between 2.2 to 4.81 mg/l. The lowest DO was at station 1 which is at the abattoir with all the organic waste as run-off. Other than that the values were typical of an estuarine environment. Heavy metal concentration in water compared to those of sediments indicated that they were higher in sediment than in water (Table 3). The water parameters varied between stations and some were significant at $p < 0.05$.

Table 2: Physico-Chemical Propertie (Mean ± SD) of Azuabie and Okujagu-Ama Creek of Upper Bonny Estuary Surface

Parameters	Station 1	Station 2	Station 3	Station 4
Temp °C	[b]27.37±0.21	[a]27.93±0.31	[a] 28.33±28.33	[a] 28.7±0.26
pH	[a]6.38±0.07	[a]6.45±0.02	[a]6.53±6.53	[a]6.45±0.07
EC (µs/cm)	9.75±3.82	12.91±1.08	16.87±16.87	15.2±4.16
TDS mg/l	4.96±2.71	6.83±1.11	8.98±8.98	7.05±2.73
Salinity ppt	5.3±2.21	7.08±0.68	9.53±9.53	8.39±2.52
Turbidity (NTU)	7.24±1.01	3.98±3.83	5.74±5.74	7.45±0.99
DO mg/l	[c]2.2±0.19	[bc]2.61±0.16	[ab]3.84±3.84	[a]4.81±0.31
SO_4 mg/l	413.06±430.38	451.37±397.37	430.77±430.77	433.87±351.72
NO_4 mg/l	3.54±2.24	3.54±2.26	5.08±5.08	6.4±5.95
BOD mg/l	[a]4.76±0.41	[a]4.76±0.39	[b]1.47±1.47	[b]1.84±1.47

Means with different letters along the row are significantly diiferent ($p < 0.05$). SD = Standard deviation

The heavy metal concentration in fish muscle showed high concentration especially of Pb (3.49 and 3.95 mg/kg) which were far above the 0.5 mg/kg permissible limit of FAO (1989), 2 mg/kg of the WHO (1989) and 0.2 mg/kg of the EC (2005) as shown in Tables 3, 4 and 5. Cd concentrationwas high above the 0.5 mg/kg limit at Okujuagu-Ama but not at Azuabie. There was no bioaccumulation of Zn, Pb and Cr at Azuabie as shown by the BAF of < 1. Cd however showed some bioaccumulation with BAF of 1.33. At Okujuagu-Ama, zn and Cr showed some bioaccumulation but not for Cd. These are variations found within same species that depends on a lot of factors, like the age (El-Moselhy *et al*., 2014) migratory ability of fish and differential exposure, health conditions etc. In estimates of BAF it is important to estimate the Home range of the fish using the statistic

H = -2.91 + 3.14HAB + 1.65In L Where
H = Home range
HAB = 0 for rivers and 1 for lakes
L = Length of fish.

The home range is important as smaller value gives the greater likelihood of representing the study site than a larger value. This is because that a fish is caught in a place does not mean the heavy metals are from that site. The home range for *O.niloticus* in this study was $1.95m^2$.

The HQ (Table 3 and 4) were all < 1 except for Zn at both Creeks. Zn is a an essential heavy metal with a permissible limit in fish muscle of 40 mg/kg (FAO, 1989) or 100mg/kg (WHO,1989). The values obtained in this study therefore pose no threat, but requires continous monitoring. The HI at Azuabie Creek excluding Zn was 0.89 compared to 1.20 at Okujuagu-Ama. This implies a higher carcinogenic risk from consumption of *O. niloticus* from Okujuagu-ama Creek. Wangboje and Ikhuabe (2015) recorded HQ of 0.05 for Zn and 0.43 for Cd in *O. niloticus* and up to 10.74 for Zn in *Hemichromis fasciatus* from the River Niger. The Pb concentrations (2.02 mg/kg in *O. niloticus* to 7.64 in *Alestes baremose*) were above permissible limits as observed in this study. Another observation in this study is the higher concentration of all metals in Okujuagu-Ama creek suggesting that the abattoir on Azuabie Creek may not be the source of the heavy metal pollution.

Table 3. Concentrations of heavy metals in fish muscle, sediment, water and the estimated BAF, EDI, HQ and HI of *O. niloticus* from Azuabie Creek

Heavy metal	Fish muscle mg/kg	Sediment mg/kg	Water mg/l	BAF	EDI	HQ	HI= Σ HQ
Zn	31.6	178.8	0.02	0.18	0.02	6.67	
Pb	3.49	18.75	0.05	0.19	2.4×10^{-3}	0.61	
Cr	3.6	14.27	0.15	0.25	2.5×10^{-3}	1.7×10^{-3}	
Cd	0.40	0.30	0.21	1.33	2.8×10^{-4}	0.28	7.56

HI excluding Zn = 0.89

Table 4 Concentrations of heavy metals in fish muscle, sediment, water and the estimated BAF, EDI, HQ and HI of *O. niloticus* from Okuluagu-Ama Creek.

Heavy metal	Fish Muscle mg/kg	Sediment mg/kg	Water mg/l	BAF	EDI	HQ	HI = Σ HQ
Zn	43.56	27.57	0.05	1.58	0.03	10.07	
Pb	3.95	-	0.07	-	2.7×10^{-3}	0.69	
Cr	2.79	2.8	0.43	1.00	0.002	1.3×10^{-3}	
Cd	0.74	8.65	0.31	0.09	5.1×10^{-4}	0.51	11.27

HI excluding Zn = 1.20.

There were variations in the concentration of heavy metals in the organs assessed (Table 5). Cr and Pb were higher in the intestine implying food as the main source of entry in the fish, such that the food and feeding habits of the fish becomes an important factor. Zn was higher in the gills than in the intestine suggesting ionic absorption across the gill as a probable source of entry, so it can be suggested that Zn was accumulated from the water. The low concentration of Pb in the gills is not in agreement with Yarkwan and Apeh (2015) that recorded Pb concentrations of up to 12.15 mg/kg in the gills of *T. zilli* and 2.08 mg/kg in the muscles of *Clarias gariepinus*. Also the higher concentrations in the muscles in this study is not in agreement with El-Moselhy *et al*. (2014) and Ugbomeh and Akani (2016) that observed lower concentration of all metals in the muscles

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Table 5: The metal concentration in tissues of *O. niloticus* species (mean ± SD) Azuabie Creek and Okujuagu-Ama

Azuabie Creek Metals (mg/Kg)	Gill	Intestine	Muscle	FAO (1989) limit	WHO (1989) limit	EC (2005)limit
Zn	^{c}50.33±13.75	^{a}6.26±12.49	^{ab}31.6 ±9.63	40	100	-
Pb	^{a}0.16±0.25	^{c}9.23±5.27	^{b}3.49±1.86	0.5	2	0.2
Cr	^{a}1.22±0.70	^{c}9.12±5.25	^{b}3.6±0.45	0.5	1	0.05
Cd	^{a}0.12±0.11	^{b}1.06±0.76	^{ab}0.40±0.01	-	-	-

Okujuagu-Ama Creek Metals (mg/Kg)	Gill	Intestine	Muscle	FAO (1989) limit	WHO (1989) limit	EC (2005)limit
Zn	^{a}43.42±9.35	^{b}50.77±20.60	^{a}43.56±9.95	40	100	-
Pb	^{a}0.01±0.00	^{b}3.22±3.22	^{b}3.95±4.92	0.5	2	0.2
Cr	^{a}1.22±0.70	^{c}5.65±2.28	^{b}2.79±0.95	0.5	1	0.05
Cd	^{a}0.01±0.00	^{a}0.01±0.00	^{b}0.74±1.97	-	-	-

Means with different letters along the row are different at $p < 0.05$

An implication in this study is that Cd and Zn have the tendency to bio-magnify compared to Pb and Cr. Although the HQ values were below 1 (except Zn), the cummulative HI at both creeks > 1 indicating a toxicity hazard risk by these metals. This study highlights the concentration of Pb, Zn, Cd and Cr in a commonly available tilapia as heavily contaminated (especially for Pb and Cd) in both Azuabie and Okujuagu-Ama Creeks. It is feared that there is potential risk with the continous consumption of this fish. Some form of risk management should be introduced to reduce the intake of this fish

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