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Studies on some Physico-Chemical parameters and heavy metals concentration in the tissues of *Oreochromis niloticus* and *Clarias gariepinus* from Amansea River of Anambra state, Nigeria

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Abstract
The physico-chemical parameters of Amansea River and the bioaccumulation of heavy metals in two most prevalence fish species *Oreochromis nilotics* and *Clarias gariepinus* of the river, were studied from February to September. The fish specimens were procured monthly from artisanal fisher folks from three sampling stations I, II and III. The samples were taking to the laboratory, and were analysed for heavy metals using 240 Version Atomic Absorption Spectrophometer (AAS) method. There were presence of Arsenic (As), Lead (Pb), Mercury (Hg), Chromium (Cr) and Cadmium (Cd) presence in the two fish species tested. The heavy metal ranking in descending order in *O. niloticus* are As>Pb>Hg>Cr>Cd, with mean concentration mg/kg^{-1} values of 5.093±0.153, 1.039±0.059, 0.134±0.012, 0.084±0.003 and 0.053±0.007 respectively, while *C. gariepinus* heavy metal ranking in descending order are Cd<As<Hg<Pb<Cr, with heavy metal mean plus standard deviation (SD) concentration mg/kg^{-1} values of 2.805±0.008, 2.784±0.058, 0.652±0.003, 0.555±0.008 and 0.365±0.026 respectively. The heavy metal concentration in the tissues of both fish species were higher than permissible limit for fish by the WHO/FAO of 0.2/0.5, 0.30/0.30, 0.05/, 0.05/0.05 and 0.020/0.02 mg/kg^{-1}, rendering fishes from the river dangerous for human consumption. The physico-chemical parameters (T^{o}, pH, Salinity, DO, and Conductivity) of 28^{0}C, 6.60, 0.25ppt., 6.29mg/L^{-1} and 84.08us/cm^{-1} respectively were within the Federal Ministry of Environment standard for fish survival and growth. The high level of heavy metal concentration, calls for serious intervention, monitoring, control and management of activities going on along the river to prevent sicknesses associated with heavy metals bioaccumulation.

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Keywords: Amansea River, Heavy metals, Bioaccumulation, *Oreochromis niloticus*, *Clarias gariepinus*, Anthropogenic, Atomic Absorption Spectrophometer

Introduction
The relevance of fish in the nutritional diet status of the world population, can never be over emphasis in the face of declining meat production and wild fish capture, consequence of climate change (global warming), vagaries of nature (flooding, erosion, landslide, siltation, earth quake and tsunami), diseases, pollution (sewage, industrial effluents, chemicals, fertilizers, pesticides and herbicides), heavy metals, obnoxious fishing methods (poison and dynamites) and over fishing (use of under size mesh nets) (Yusef, *et al*., 2002 and Okeke *et al*., 2014) [26, 14]. This had prompted countries all over the world, to commence exploring avenues on how natural water bodies can be conserve to once again be relevant to fish production and fisheries conservation and sustainability of natural habitat.

Heavy metals have been reported to have negative effect on metabolic processes in general and may influence the nutritional and biological status of aquatic resources (WHO, 1985, FEPA, 2003 and Otitogu *et al*., 2003) [23, 9]. Heavy metals have the capacity of after protracted accumulation in the body to initiate the malfunction or damage of the vital organs (eyes, hearts, liver, kidney, reproductive organs and skin lesion) (Khanna and Khanna, 2011 and Satsananan, 2014). It has been pointed out as the leading cause of death and diseases to the tune of 14,000 people daily (Pink, 2006) [19]. The physico-chemical parameters have considerable bearings on the life of lotic organisms (Gonzale, 1979 and Arazu *et al*., 2015) [11, 5].

Amansea River situates between Anambra and Enugu State of Nigeria. The heavy metals status has not been ascertained despite being impacted by anthropogenic factors like sand dredging and tipping, agricultural activities, flooding, sewage, industrial effluents discharge, obnoxious fishing (dynamites and poison), over fishing and washing of articulated vehicle (oil tankers). These activities when continued uncontrolled, has some negative effects on physisco-chemical equillibrum of water development, growth and survival of aquatic fauna (fish). The study of heavy metals in Amansea river (fresh water), is more important in comparison to other pollutants, owing to their non-degradable nature, accumulative properties and long biological half-lives and it is difficult to remove them completely from the environment once they enter (Ademoriti, 1996). These inorganic compounds (metals) from natural and anthropogenic sources continuously enter the aquatic ecosystem where they could pose serious threat to the food chain. Consequently, had necessitated the conduct of this research, to investigate some physico-chemical parameters (Temperature (T°), pH, Salinity, Dissolved oxygen (DO) and Conductivity) of the water and the level of some heavy metals like Asenic (As), Cadmium (Cd), Lead (Pb), Mercury (Hg) and Chromium (Cr) in two most prevalent fish species *O. niloticus* and *C. gariepinus* from the river, in order to determine their bioaccumulation profile and suitability for human consumption and advice the government accordingly. The study of some physico-chemical parameters of the water and some heavy metals concentration in Amansea River and the muscle of the most prevalent fish species, is as a result of anthropogenic and degradable activities that goes on along the river like the cattle market, abattoir, sand dredging and tipping, sewage dumping, effluents discharge, washing of articulated vehicle (oil tankers), agricultural activities and serving as sink to all floods and water run-off from Awka capital and surrounding towns and the health and economic implications to fisher-folks and the consumers.

Materials and Methods

Study area

Fig 1: Map of Nigeria

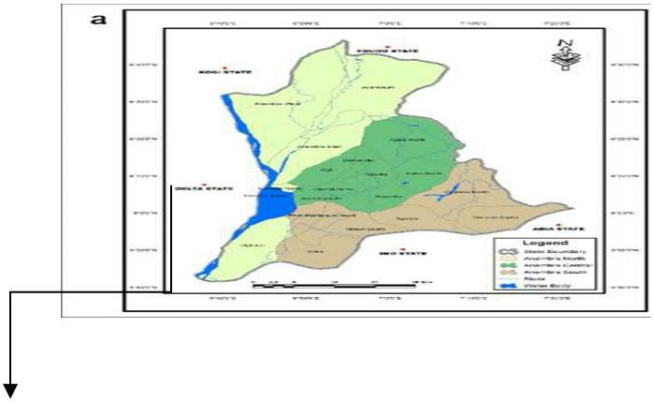

Fig 2: Map of Anambra State

Fig 3: Map showing the study area (Amansea River)

Fig 4: Pictorial view of sampling stations

Amansea River is a natural boundary of Anambra and Enugu States of Nigeria at Amansea town. It flow through three Local Government Areas; Awka North, Awka South and Orumba North of Anambra state before entering Anambra East Local Government Area (LGA) of Anambra State, where it empties into Omambala River as its tributaries, which in turn empties into to the River Niger as its tributaries at Onitsha North Local Government Area, and from there, it flows towards the southern part of Nigeria, from where it finally empties into the Atlantic ocean as shown in figure 1.

Research design

Sampling Stations (SS): Were chosen as sampling station I (SSI), sampling station II (SSII) and sampling station III (SSIII) with the geo-coordinates of Latitude $N6^0$ 14'16 and Longitude $E7^0$ 6'26, Latitude $N6^0$ 15'13 and Longitude $E7^0$ 8'35 and Latitude $N6^0$ 21'10 and Longitude $E7^0$ 14'28 respectively.

Sampling station I (SS.I): At this station of the river it is called Ajali river and it is located at Amansea and Ebenebe towns in Awka North L.G.A. Activities that goes on at this station includes; fishing, agriculture, sand dredging and tipping, domestic activities (washing of clothes and utensil with detergents), community market situates here and serves as sink for run-off and flood from around the surrounding towns.

Sampling station II (SS. II): This station is at Amanea town. The popularity of the river as Amansea River is because two major tarred road (Express and old roads) that link Awka the capital of Anambra State and Enugu the capital of Enugu State crossed the river at Amansea town. Activities that goes on at this station includes; illegal and obnoxious fishing, agriculture, dumping of human and cattle sewage as a result of the cattle market around, industrial effluents discharge, sand dredging and tipping, washing of articulated vehicles (oil tankers) and sink for run-off from the state capital and the environs.

Sampling station III (SSIII): This station is located at Omaku town in Oji-river Local Government Area of Enugu State. Activities here include; fishing, agriculture, domestic, sand dredging and tipping.

The study of some physico-chemical parameters of the water and some heavy metal concentration in the muscle of the most prevalent fish species, is as a result of anthropogenic and degradable activities that goes on along the river like the cattle market, abattoir, sand dredging and tipping, sewage dumping, effluents discharge, washing of articulated vehicle (oil tankers), agricultural activities and serving as a sink to all floods and run-off from Awka capital and surrounding towns and the health and economic implications to fisher-folks and the consumers. Also, the continuous dwindling the annual fish capture by fisher folks.

Collection of fish samples

The fish specimens of *Oreochromis niloticus* and *Clarias gariepinus* were caught by artisanal fisher-folks, with the help of nets, hooks and lines and other types of fishing gears at the various sampling stations. The specimens were kept in clean polyethylene bags, sealed, labelled appropriately and kept in an ice box before transporting them to Springboard laboratories, Plot 3, House 1, Udoka Housing Estate, Awka, Anambra State, for heavy metal analysis.

Heavy metals analysis

The heavy metals of As, Pb, Hg, Cd and Cr analysis were conducted using Varian AA240 Atomic Absorption Spectrophometer (AAS) according to APHA (1995) [4] method. AAS working principle is based on allowing the fish samples to thaw and the fish flesh removed using stainless surgical knife. The samples were oven dried at 80^0C for 24 hrs. After which they were separately ground to powdery form with the help of ceramic mortar and pestle. Two grams (2.0gm) of dried tissue samples were digested with 6ml. of concentrated Nitric acid (HNO_3) and 1ml. of 30% Hydrogen peroxide (H_2O_2). The digestion was carried out in a microwave digester. The completely digested samples were filtered using What-man filter paper and diluted in a volumetric flask with 25ml. distilled water. The resultant solutions were analysed for heavy metals using Varian AA 240 Atomic Absorption Spectrometer (AAS). Atomic absorption spectrophomeer working principle is based on the sample being aspirated into the flame and atomized when the AAS's light beam is directed through the flame into the monochromatic and onto the detector that measures the amount of light absorbed by the atomized element in the flame. Since metals have their own characteristic absorption wavelength, a source lamp composed of the element is used, which makes the method relatively free from spectral or radiation interferences. Therefore, the amount of energy of the characteristic wavelength absorbed in the flame is proportional to the concentration of the element in the sample.

Physico-chemical Parameters

The physico-chemical parameters of Temperature (T^o), pH, Salinity, Dissolved oxygen (DO) and conductivity, Turbidity and nitrate were measured monthly at sampling stations I, II and III throughout the duration of this research. The temperature, pH and conductivity, were measured in-situ at the site using a pen type pH meter (HANNA H198106 Model) made in the United Kingdom, with temperature display. This was done by dipping the probe inside the river to a depth of 0.3m, stirred and allowed to stabilize before taking readings. The dissolved oxygen (DO) of the water samples were obtained through redox titration using Winkler test method. The conductivity of the water was measured in-situ by using turbimeter. The probe was immersed inside the water at a depth of 0.3m and gently stirred and allowed to stabilize

Statistical analysis

All values obtained were expressed as mean ± SD and analyzed using SPSS, Window 20.0 software and subjected to one-way Analysis of Variance (ANOVA) at 0.05 level of significance, with a post hoc test using Duncan Multiple Range Test (DMRT), to determine if the mean concentration were significantly different for each sampling specimen from the sampling stations. The heavy metal contents were compared with the permissible limit recommended by the World Health Organization WHO (2001) and Food and Agricultural Organization (FAO) (2003) [8].

Results

Table 1 shows the mean heavy metal concentration in *O. niloticus* and *C. gariepinus* from the sampling stations within

the period under study (February-September) and International permissible limit of heavy metals. The mean values of heavy metal concentration of sampled fish species from all the sampling stations from February to September and the international permissible maximum limit. The mean metal (Pb, As, Hg, Cd and CR) concentration values in *O. niloticus* and *C. gariepinus* flesh are 1.047, 5.133, 0.135, 0.019 (mg/kg^{-1}) and 0.072 and 0.058, 2.804, 0.663, 2.807 and 0.343 (mg/kg^{-1}) respectively.

Table 1: Mean Heavy metal concentration in *O. niloticus* and *C. gariepinus* from Feb-September from Amansea River Sampling Station I

Species	***Oreochromis nilotcus***					***Clarias gariepinus***				
Metal / Month	**Pb**	**As**	**Hg**	**Cd**	**Cr**	**Pb**	**As**	**Hg**	**Cd**	**Cr**
Fedruary	1.O76	5.327	0.136	0.019	0.072	0.584	2.817	0.661	2.817	0.351
March	0.985	5.214	0.137	0.014	0.079	0.596	2.817	0.665	2.820	0.355
April	1.102	5.013	0.126	0.017	0.080	0.541	2.765	0.672	2.819	0.336
May	1.103	5.352	0.135	0.018	0.062	0.591	2.891	0.658	2.796	0.345
June	1.007	4.945	0.138	0.016	0.069	0.582	2.855	0.674	2.799	0.340
July	1.115	5.018	0.119	0.021	0.070	0.592	2.695	0.660	2.811	0.370
August	0.986	5.116	0.140	0.019	0.068	0.557	2.783	0.662	2.816	0.356
September	1.001	5.102	0.146	0.018	0.077	0.577	2.820	0.654	2.816	0.327
Mean ẍ	1.047	5.133	0.135	0.018	0.072	0.576	2.804	0.663	2.807	0.343
Standard Dev.	0.059	0.153	0.227	0.007	0.0001	0.008	0.058	0.008	0.617	0.0001
WHO/FAO STD.	0.30	5.00	0.05	0.20	0.05	5.00	5.00	0.20	0.05	0.05

Table 2 shows the mean heavy metal concentration values in the tissues of *O. niloticus* and *C. gariepinus.* The two fish species showed high concentration of As (5.040mg/kg^{-1}), Pb (1.047mg/kg^{-1}) and Cd (2.818mg/kg^{-1}) and As (2.707mg/kg^{-1}) respectively of heavy metals within the period under study respectively.

Table 2: The mean monthly Heavy metal concentration of *O. niloticus* and *C gariepinus* from Amansea River at Sampling Station II

Species	***Oreochromis niloticus***					***Clarias gariepinus***				
Metal/Months	**Pb**	**As**	**Hg (mg/kg^{-1})**	**Cd**	**Cr**	**Pb**	**As**	**Hg (mg/kg^{-1})**	**Cd**	**Cr**
February	0.985	5.012	0.105	0.021	0.072	0.584	2.710	0.609	2.828	0.332
March	0.006	4.904	0.138	0.011	0.081	0.596	2.721	0.605	2.822	0.345
April	1.001	5.277	0.132	0.019	0.082	0.582	2.715	0.615	2.813	0.312
May	1.105	5.006	0.140	0.017	0.081	0.555	2.698	0.605	2.801	0.305
June	1.010	5.110	0.138	0.017	0.075	0.580	2.715	0.607	2.825	0.295
July	1.124	4.897	0.129	0.019	0.071	0.577	2.725	0.618	2.830	0.289
August	0.993	5.001	0.135	0.021	0.083	0.583	2.670	0.629	2.817	0.301
September	0.986	5.211	0.141	0.019	0.078	0.574	2.713	0.614	2.811	0.316
Mean ẍ	1.047	5.040	0.132	0.016	0.078	0.576	2.707	0.612	2.818	0.362
SD	0.059	0.136	0.012	0.037	0.0001	0.003	0.015	0.006	0.010	0.001
WHO/FAO	0.30	5.00	0.05	0.02	09.05	0.30	5.00	0.05	0.02	0.05

Table 3 shows heavy metal concentration values in the tissues of *Oreochromis niloticus* and *Clarias gariepinus* species from Amansea River, at Sampling Station III.

Table 3: Mean monthly heavy metal concentration of *O. miloticus* and *C. gariepinus* From Amansea River at Sampling Station III

Species	***Oreochromis niloticus***						***Clarias gariepinus***			
Metals/Months	**Pb**	**As**	**Hg (mg/kg^{-1})**	**Cd**	**Cr**	**Pb**	**As**	**Hg (mg/kg^{-1})**	**Cd**	**Cr**
February	1.031	4.995	0.140	0.018	0.112	0.540	2.850	0.684	2.793	0.452
March	1.029	5.119	0.135	0.019	0.105	0.542	2.839	0.684	2.975	0.425
April	0.998	5.232	0.138	0.021	0.114	0.547	2.845	0.678	2.781	0.357
May	1.078	5.322	0.127	0.018	0.096	0.539	2.841	0.680	2.786	0.382
June	1.062	5.285	0.140	0.021	0.089	0.549	2.830	0.682	2.790	0.327
July	0.997	4.885	0.131	0.019	0.095	2.541	2.852	0.675	2.792	0.330
August	1.070	5.001	0.129	0.019	0.106	0.545	2.837	0.678	2.782	0.402
September	1.059	5.010	0.140	0.020	0.103	0.533	2.893	0.680	2.798	0.440
Mean ẍ	1.045	5.106	0.135	0.019	0.103	0.542	2.842	0.680	2.790	0.390
Standard dev.	0.004	0.198	0.005	0.20	0.0002	0.004	0.006	0.003	0.006	0.003
W.H.O./FAO	0.30	5.00	0.05	0.02	0.05	0.30	5.00	0.05	0.02	0.05

Table 4: The mean concentration values of *O. niloticuus* and *C. gariepinuus* from Amansea River from Sampling Stations I, II and III within the period of study

Species	***Oreochromis niloticus***					***Clarias gariepinus***				
Metal/Station	**Pb**	**As**	**Hg (Mg/kg^{-1})**	**C d**	**Cr**	**Pb**	**As**	**Hg (Mg/kg^{-1})**	**Cd**	**Cr**
SS 1	1.047	5.133	0.135	0.018	0.072	0.576	2.804	0.663	2.807	0.343
SS 11	1.025	5.040	0.132	0.016	0.078	0.576	2.707	0.614	2.819	0.362
SS 111	1.045	5.108	0.135	0.019	0.103	0.542	2.842	0.680	2.790	0.390

Mean ẍ	1.039	5.094	0.134	0.018	0.084	0.565	2.784	0.652	2.805	0.365
SD.	0.059	0.153	0.012	0.003	0.007	0.55	0.058	0.003	0.003	0.026
WHO/FAO.	0.30	5.00	0.05	0.02	0.05	0.30	5.00	0.05	0.02	0.05

Table 5: Mean and range of some physico-chemical parameters of Amansea River within the period under study

Station	SSI		SSII		SSIII		
Parameters	**Mean**	**Range**	**Mean**	**Range**	**Mean**	**Range**	**FME**
Temperature(^{0}C)	28.50	26.10-30.14	28.10	26.00-30.24	29.15	27-31	15 - 32
pH	6.71	6.02-7.40	6.50	5.47-7.13	6.55	4.85-8.25	6.5-8.00
Turbidity (cm)	14.05	12.58-14.20	15.29	14.73-15.46	13.75	13.65-13.81	30-80
Salinity (ppt)	0.50	0.35-0.55	0.55	0.20-0.50	0.55	0.35-0.55	0.5
DO (mg/L^{-1})	6.50	4.50-6.80	6.73	4.23-6.25	5.64	4.92-7.60	4.5-8
BOD (mg/L^{-1})	4.12	3.84-4.17	3.52	3.15-3.65	3.64	3.45-4.01	6.00
Conductivity (Ns/cm-1)	63	20-92	66	23-91	58	19-90	100
Nitrate (mg/L)	3.24	2.82-3.70	2.39	2.52-2.62	3.51	2.93-3.62	20

Discussion

Heavy metals are naturally occurring compounds of the earth's crust that are as a rule neither created nor destroyed, but are simply redistributed. These toxic metals could be distributed in aquatic ecosystem through anthropogenic processes, industrial and environmental pollution through effluent disposal, such that aquatic organisms are contaminated with continuous influx of these toxic effluents that contaminate the river and biota. These heavy metals are metals in their metallic form and their densities are greater than 4g/ce. Aquatic organisms accumulate these toxic metals from water, soil sediments, diet and contaminant residues may ultimately reach concentrations a hundred or a thousand of times above those measured in the water, soil sediment and food (Godwin, *et al.*, 2003) [10]. This research, shows that the mean concentration of heavy metals under investigation in *O. niloticus* and *C. garipinus* varied and followed a decreasing ranked profile of As > Pb > Hg >Cr > Cd of (5.093, 1.039, 0.0.134, 0.084, and 0.053)mg/kg, and Cd > As > Hg > Pb> Cr of (2.805, 2.784, 0.0.652, 0.555 and 0.365)mg/kg respectively. The heavy metals present in *O. niloticus* showed significance difference ($p < 0.05$) in *O. niloticus* and *C. gariepinus* fish samples throughout the duration of study and were above the international permissible limit by (WHO, 2012). Obasohan (2007) [13] reported accumulation of chromium in *Parachanna obscrura* of 0.40-5.61ppm, *Tilapia zilli* (29.8 – 31.6ppm and *Clarias gariepinus* 28.1-32.2 ppm and this was at variance with what was obtained in this study. Erdogrul and Erbilir (2007) [7] reported high cadmium concentration above WHO (2008) [25] permissible limit in *Cyprinus carpio* from Sir Dam lake, which concurred with this research. This studies, agrees with the findings of Anglin-Brown *et al*. (1995) [3] who pointed to run off from agricultural farms, which contain inorganic fertilizers, agrochemicals, pesticides and scrapes from refuse dump sites as causes of heavy metals accumulation in aquatic environment. There was no significance variance in the monthly heavy metals concentration in the sampled fish species during the dry months and wet months. This is at variance with the observations of Olatunji and Oladele (2012) [10], who observed high concentration of heavy metals during the dry season than in rainy season. These fishes bioaccumulate metals and are able to absorb them and their bodies, accommodate their presence and are stored in fatty tissues, and because they are not essential elements needed by the body, will bioaccumulate if further exposed to contamination (Straford, 2015). Wangboye *et al*. (2016) opined that bioaccumulation of heavy metals by fishes, exposes fish eating species including man to contaminations and associate health issues like organ failure (heart, kidney, liver) and carcinogenic aliments. Habba *et al.*, (2022) opine that aquatic system polluted by heavy metals are to due soil erosion caused by the rain through natural pollution and man-made contamination, and (90%) due to liquid flow from various industrial, urban and agricultural activities

High concentration of heavy metals in Amansea river can be attributed to the deposition of sewage, influx of chemicals from agricultural land, dredging and sand tipping activities, industrial effluents, livestock excretes and offals from abattoir, washing of vehicles (oil tankers), which Qui *et al*. (2000) [20] agreed as sources of heavy metals concentration in water bodies. Anderton (2001) [2], Divrikliel et *al*. (2003) and WHO (2004), all noted that heavy metals above the permissible limits are capable of damaging the organs of the body (brain, kidney, liver, heart, spleen, reproductive, blood, skin and the central nervous system).

Asenic (As) and Chromium (Cr) were detected in the tissues of *O. niloticus* and *C. gariepinus* in high concentration from the three sampling stations as shown in tables 1, 2,3 and 4 from Amansea River throughout the duration of this study. This research also shows that Asenic concentration in *Oreochromis niloticus* was lower than that of *Clarias gariepinus* as shown is all the tables.

The physico-chemical parameters of the river (Temperature, Dissolved oxygen, Salinity, pH, Electrical conductivity) were observed to be within the acceptable limit for survival and growth of aquatic organisms in the wild as shown in table 5. The turbidity varied from station to station in consonant to the level of anthropogenic activities (dredging and sand tipping, domestic, water runoff and soil type) going on there. There was no significant difference ($p < 0.05$) in temperature values of all the sampling stations, because they are typical values of the tropics with high temperature ranges. Similar temperatures were recorded by Moslen (2008) in Azuabie creek and Okeke *et al*. (2016) [15] in Ogbujilekwe/Idozu river in Anambra State. The mean hydrogen ion concentration values at SSI, SSII and SSIII, were 6.71, 6.50 and 6.55 respectively and are within the acceptable range for human and livestock by the World Health Organization. Osman and Kloas (2010) opined that naturally occurring fresh water has a pH range of 6.0 and 8.0. The dissolved oxygen is a vital element to all forms of aquatic life. The dissolved oxygen range from the three sampling stations were 6.50 -7.0mg/L, 6.10 -6.9mg/L and 5.60 -6.90mg/L and are within FME (2008) permissible range of 5.0 -7.0mg/L.

Conclusion

Consequence of high concentration of heavy metals in *O. niloticus* and *H. niloticus* from Amansea River, there is need for appropriate regulatory measures to be installed to checkmate the activities that are going on along and around the river, in order to reduce and prevent further deterioration in the river and danger it posses to aquatic biota (fish) and human consumers.

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