

The Biochemical Effects of Waters from Awba Dam and Rivers Around Ewekoro Cement Factory, Ogun State, Nigeria on Selected Organs of the African Catfish (*Clarias gariepinus*)

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Date Received: 07-06-2023

Date Accepted: 18-10-2023

DOI: <https://doi.org/10.48198/NJPAS/23.B06>

ABSTRACT

The environment is polluted by indiscriminate disposal of sewage, industrial waste, toxic heavy metals and human activities. The settlements around Ewekoro cement factory depend on surrounding rivers for their domestic purposes. Thus, the study was designed to evaluate the biochemical effects of waters from Awba dam (located in University of Ibadan, Nigeria), rivers (Itori, Alaguntan, Elebute), and groundwater around Ewekoro cement factory, Ogun state, Nigeria on selected tissues of African catfish (*Clarias gariepinus*). Sixty juvenile African catfish were randomly assigned into six groups of 10 fish and cultivated in waters from Awba dam, and Ewekoro area while tapwater served as control for 12 weeks. Results showed that the turbidity, taste and colour of water samples were objectionable according to the NESREA standard. The concentrations of heavy metals; Co, Mn, Ni, Pb, Fe and Cr in water samples from Itori, Alaguntan and Elebute rivers, groundwater and Awba dam exceeded the permissible NESREA and WHO standards. The heavy metals; Fe, Pb, Co and Mn accumulated in the gills, kidneys and brain of *C. gariepinus* grown in waters from Awba dam, Ewekoro area rivers and groundwater. The levels of urea, nitric oxide, micronuclei (MN) and lipid peroxidation as well as activities of myeloperoxidase and lactate dehydrogenase were significantly high in *C. gariepinus* cultivated in these waters. The levels of antioxidant parameters (SOD, CAT and GSH) were significantly low in brain and kidney of *C. gariepinus*. More so, the level of caspase-9 decreased, while insignificant differences were observed in the levels of creatinine and caspase-3 in the *C. gariepinus*. Histology showed severe distortions in the cyto-architecture of kidneys, gills and brain of fish cultivated in these waters. Waters from Awba dam and Ewekoro area rivers have high metallic contents resulting in bioaccumulation of metals in kidneys, gills and brain of *C. gariepinus* leading to oxidative stress and inflammation, thereby creating a public health concern for the settlements in these areas.

Keywords: Heavy metals, Toxicity, *Clarias gariepinus*, Polluted waters, Cement factory

Introduction

Environmental pollution due to contaminants and pollutant substances that are deposited at high

quantity in the environment beyond its safety limits is on the increase (Maindi, 2018). Global increase in industrialization and urbanization has

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led to increase in pollutants in the environment and, it's a major challenge to human health (Nour *et al.*, 2019). Water pollution is the leading cause of death and diseases, and that it accounts for 1 in 6 deaths which stemmed from poor air quality, unsafe water and toxic chemical pollution (Fuller *et al.*, 2022). The Lancet Commission on pollution and health reported that pollution was responsible for 9 million premature deaths in 2015, making it the world's largest environmental risk factor for disease and premature death (Fuller *et al.*, 2022). This form of environmental pollution occurs when pollutants are directly or indirectly discharged into water bodies without proper treatment. Unfortunately, rivers are being polluted by indiscriminate disposal of sewage, industrial waste and human activities, which affect their physico-chemical characteristics and microbiological quality (Ankita-Mitra, 2014). Among animal species, fish, birds, mammals including humans are more vulnerable to the detrimental effects of these pollutants (Walker *et al.*, 2012).

The African Catfish (*Clarias gariepinus*) is considered a good test organism for heavy metal contamination because of its feeding behaviour and bottom feeding habit (Ankita-Mitra, 2014). The toxic stress and hematological effects of various heavy metals on *C. gariepinus* have been reported (Kori-Siakpere, 2006). Heavy metals can bind to vital cellular components, such as structural proteins, enzymes, nucleic acids, and interfere with their functionality (Oguzie, 2003). These metals can also interact with nuclear proteins and DNA, and may cause direct damage or conformational changes to these bio-molecules or indirectly may damage them via generation of reactive oxygen and nitrogen species (Beyersman, 2002).

Ewekoro cement production facility of the West African Portland Cement (WAPC) PLC established in 1959 is the oldest known cement factory in Nigeria and, it's surrounded by settlements (Itori to the north, Elebute, Alaguntan to the east) which predate the cement company.

The cement factory catchment area is perennially drained by Itori, Elebute and Alaguntan rivers, which are constantly fed from discharges of quarry lakes formed from broken aquifers. These rivers are major tributary of Ogun River, which empties directly into the Lagos Lagoon. In addition, air pollution of cement dust is characteristic of the factory (Olaifa *et al.*, 2004). The damaging effects of dust particles have been attributed to the cement dust, which is characterized by toxic heavy metals (Adejumo *et al.*, 1994). Most studies conducted around Ewekoro cement facility have centered on the heavy metal composition of the dust particles and its health effects on lungs. However, the environmental and toxicological effects of the dust particles on waters from the rivers and groundwater that received effluents from the cement factory have not been investigated. Bakare *et al.*, (2019) revealed the genotoxic effect of water from Awba Dam on *Allium cepa* and mice. These earlier reports inform the choice of this sampling site in the present study. From the aforementioned, this study investigated the biochemical effects of waters from Awba Dam and rivers (Itori, Alaguntan, Elebute), and groundwater around Ewekoro cement factory, Ogun state, Nigeria on selected tissues of African catfish.

Materials and Methods

Sampling sites

The water samples used for this study were obtained from different rivers based on their upstream and downstream location to the industrial site (Cement factory) and Groundwater (Dug-Well water) in close proximity (Distance of 700-1000 meter from the cement factory and 5 dug-well in the community). Ewekoro (6.2, 7.8°N and 3.0, 5.0°E), is surrounded by farm settlements named after the rivers; Itori river to the north, Alaguntan and Elebute rivers to the east. Water samples collected between 8am and 10am from the rivers and groundwater were stored in a 50 L jerry-can container, while de-chlorinated tap water served as

the control. Awba dam (reservoir) in the University of Ibadan (7° 26' N, 3° 53' E), southwestern Nigeria, is located about 160 km from the Atlantic Ocean coast at an altitude of 185 m above sea level (Akin-Oriola, 2003). The dam is located on Awba River that drains the southern part of the University. Awba Dam (AWD) was selected as the non-industrial area of this study due to the commercial fishing activity that covers the entire length of the dam and the domestic use of the dam's water by the University of Ibadan community.

Animals

The African cat fish juvenile used for this experiment (*Clarias gariepinus*, Burchell, 1822) (Average weight of 5.5 ± 0.2 g and length 8.96 ± 0.6 cm) were purchased from Department of Fisheries, Ministry of Agriculture, Agodi, Oyo State Ibadan, Nigeria. The fishes were cared for according to standard guidelines (Bakare *et al.*, 2021).

Study design

After acclimatization, sixty juvenile African catfish (*C. gariepinus*) were randomly assigned into six groups of 10 fishes each. The first group (control) lived on de-chlorinated tapwater, while the second group lived on water from Awba dam, third, fourth, fifth and sixth groups lived on waters from Itori River, Alaguntan River, Elebute River and Groundwater, respectively. The fishes were given access to dry fish pellets and kept for a period of 12 weeks. At the end of the treatment period, the fishes were sacrificed via cold shock.

Preparation of tissues and serum

Blood was collected at the caudal vein of fishes using sterile needles and syringes into 5mL non-heparinized tubes, allowed to clot, and then centrifuged at 3,000 g for 5mins to obtain the serum for biochemical analysis. The fishes were dissected using stainless steel dissection instruments to remove the gills, kidney and brain. The tissues were rinsed in ice cold 1.15% KCl

solution buffer at pH7.2 and weighed. Sections of the weighed gills, kidney and brain were fixed in Boiun's solution for histological analysis while the remaining tissues were homogenized in six volumes of 50mM phosphate buffer of pH 7.4, and the homogenates were subjected to cold centrifugation (-4°C) at a speed of 10,000 g for 15 mins to obtain supernatants which were used for biochemical analyses.

Physico-Chemical and Heavy Metal Analyses

The physiochemical characteristics (pH, temperature, biological oxygen demands, total dissolved oxygen, electrical conductivity and dissolved oxygen) of the dechlorinated tap water and waters from Alaguntan, Elebute and Itori rivers as well as Awba dam and Groundwater were determined with standard methods (APHA, 1985). The levels of heavy metals (Pb, Zn, Fe, Cr, Co, Ni, and Mn.) in organs of *Clarias gariepinus* and waters from sampling sites were determined using Buck scientific flame atomic absorption spectrophotometer (205, 2010/2011 VGP, USA).

Piscine Micronucleus (MN) assay

Five (5) fish per group were used for the MN assay as previously described by Bakare *et al.* (2009). Briefly, peripheral blood smears were made from the freshly collected blood from the caudal vein of the fish on clean microscope slide. The slide smears were air dried and fixed in absolute methanol, stained with 10% May-Grunwald stain and then counterstained with 5% Giemsa stain. Three slides were scored microscopically out of five slides prepared per fish. Total of 1000 erythrocytes were analysed per slides for presence of MN.

Determination of creatinine, urea and lactate dehydrogenase

Serum creatinine and urea levels were determined according to the method of Bartels *et al.*, (1972) and Fawcett *et al.*, (1960), respectively, while the activity of serum lactate dehydrogenase was assayed by method of Weissnar, (1975).

Protein determination

Levels of serum, brain and kidney protein were estimated by the method described by Lowry *et al.*, (1951) using bovine serum albumin (BSA) as a standard.

Assay of Superoxide Dismutase activity

The activity of brain and kidney superoxide dismutase activities were evaluated following the autoxidation of adrenaline in a method described by McCord and Fridovich (1969). The reaction mixture contained an aliquot of 0.2 mL of diluted sample and 2.5 mL of 0.05M carbonate buffer (pH 10.2). The reaction began by the addition of 0.3 mL of freshly prepared 0.3mM adrenaline to the mixture which was quickly mixed by inversion. Optical density was estimated at 480nm every 30 seconds for 150 seconds.

Assessment of Catalase activity

Brain and kidney catalase activities were estimated according to the method described by Aebi (1974). The reaction contained 20 μ L of phosphate buffer (50 mM and pH 7.0), and 10 μ L of 19mM H₂O₂ with 50 μ L of the sample. The mixture was left to incubate at room temperature for 3 mins. The reaction process was stopped when 20 μ L of dichromate in acetic acid solution was added, after which it was subjected to heating in a boiling water bath for 10 mins. The resulting solution was left to cool and the optical density taken at 570 nm.

Reduced glutathione (GSH) determination

The level of brain and kidney GSH was estimated by the method described by Moron *et al.* (1979). Succinctly, 3 mL of 4% sulphosalicylic acid solution was added to the diluted test sample (1mL) to deproteinize it and then centrifuged for 15 mins at 3000g. Thereafter, 0.5mL of the supernatant was added to 4.5 mL of Ellman's reagent and absorbances of the resulting mixtures were taken within 5 mins at 412nm.

Determination of Lipid Peroxidation

Lipid peroxidation was determined by measuring the thiobarbituric acid reactive substances (TBARS) formed as described by Buege and Aust (1978). Briefly, the reaction mixture contains 0.4 mL of sample, 1.6 mL of Tris-KCl buffer and 0.5 mL of 30% TCA. Thereafter, 0.5 mL of 0.75% TBA was added and placed in a water bath for 45mins at 80°C; cooled in ice and centrifuged at 3000g for 15 mins. The optical density of the resulting clear pink solution was measured at 532nm.

Myeloperoxidase Activity

Myeloperoxidase (MPO) is a marker of polymorphonuclear leukocyte accumulation in a living system. Its activity was evaluated by the modified method described by Trush *et al.*, (1994). Briefly, the reaction mixture contains 200 μ L of *O*-dianisidine, 50 μ L of diluted H₂O₂ and 7 μ L of the sample. The activity of MPO was measured at an optical density of 470nm every 30 seconds for 3 mins.

Estimation of Nitric Oxide Level

The levels of nitrite in the liver were evaluated following Griess's reaction as described by Palmer *et al.*, (1987). Briefly, 0.5mL of the sample was incubated with 0.5mL of Griess's reagent [0.2% Naphthyl-ethylenediamine dihydrochloride and 2% sulfanilamide in 5% phosphoric acid] at room temperature for 20mins. The optical density was measured at 550nm spectrophotometrically.

Determination of Caspases-3 and -9 levels These kits used the Sandwich-ELISA principle techniques. Samples were added to the micro ELISA plate wells and combined with the specific antibodies.

Histopathology

A section of the tissues fixed in Bouin's solution was dehydrated in 95% ethanol and cleared in xylene before embedded in paraffin. Micro sections (3-4 μ m) were prepared and stained with

haematoxylin and eosin (H&E) dye, and were examined under a light microscope (Olympus, USA) by a Histopathologist blinded to the study design.

Statistical analysis

Data were presented as Mean \pm Standard Deviation and analyzed by one-way analysis of variance using SPSS version 23.0 (SPSS Inc., USA) and GraphPad Prism 6.0. The differences were taken to be significant at p -value < 0.05 .

Result

The physico-chemical analysis and heavy metal contents of waters from the sampling sites

The physico-chemical analysis revealed that pH, TDS, EC, DO, temperature and BOD were in the range of permissible limits for drinking water and sustainability of aquatic life according to National agency for food and drug administration and control (NAFDAC) and National Environmental Standard Regulation Enforcement Agency (NESREA) (Table 1). The groundwater was turbid, while the Awba dam and the three rivers around Ewekoro cement factory were slightly turbid against the crystal clear de-chlorinated tap water. The taste and colour of the waters from the three rivers, Awba dam, and groundwater were objectionable against the un-objectionable tap water. Waters from Itori river and Awba dam were higher in hardness according to NAFDAC standard. The water samples from the three rivers around Ewekoro cement factory have high levels of Co, Ni, Pb and Cr, which exceeded the permissible limits of WHO and NESREA (Table 2). Water from Awba dam has high levels of Pb and Cr that exceeded the permissible limits of WHO and NESREA.

Body weight, organs' weight and bioaccumulation of heavy metals in organs of C. gariepinus cultivated in waters from sampling sites

There were significant ($p < 0.05$) decreases in body weight-gain in fishes cultured from waters from Awba dam, groundwater and the three rivers around Ewekoro cement factory relative to control (Table 3). There were significant ($p < 0.05$) decreases in the organo-somatic weight of kidneys, brain and gills of *C. gariepinus* cultivated in waters from the three rivers around Ewekoro cement factory and groundwater relative to control (Table 4). The decreasing orders of heavy metals accumulation are: Gills- Fe $>$ Pb $>$ Mn $>$ Co $>$ Ni $>$ Zn $>$ Cr, Kidney- Fe $>$ Pb $>$ Mn $>$ Co $>$ Zn $>$ Ni $>$ Cr and brain- Fe $>$ Pb $>$ Co $>$ Zn $>$ Mn $>$ Ni $>$ Cr (Tables 5, 6 and 7). These metallic bioaccumulation in the organs of the fishes exceeded the permissible limits of WHO and NESREA.

Changes in biochemical indices in C. gariepinus cultivated in waters from sampling sites

Levels of lipid peroxidation products measured as malondialdehyde and nitric oxide, as well as activities of myeloperoxidase and lactate dehydrogenase increased significantly ($p < 0.05$) in the serum of *C. gariepinus* cultivated in water samples from Awba dam, groundwater and rivers around Ewekoro cement factory relative to tapwater (control) (Figures 1 and 2). Similarly, serum urea and creatinine levels significantly ($p < 0.05$) increased in the serum of *C. gariepinus* cultivated in water samples from groundwater and rivers around Ewekoro cement factory relative to control (Figure 3). In figure 4, level of caspase-9 significantly ($p < 0.05$) decreased while caspase-3 was insignificantly ($p > 0.05$) affected in *C. gariepinus* cultivated in water samples from groundwater and rivers around Ewekoro cement factory. The result indicates a significant ($p < 0.05$) increase in peripheral micronuclei formation in erythrocytes of fishes cultivated in Awba dam, rivers around Ewekoro cement factory and

groundwater when compared to tapwater (Figure 5). In figures 6 and 7, oxidative stress marker in kidney and brain; malondialdehyde significantly ($p < 0.05$) increased while antioxidant indices (catalase, superoxide dismutase and reduced glutathione) significantly ($p < 0.05$) decreased in brain and kidney of *C. gariepinus* grown in waters from Awba dam, rivers and groundwater around Ewekoro cement factory.

Changes in histology of tissues from *C. gariepinus* cultivated in waters from sampling sites

Histology revealed significant disruptions of primary and secondary lamellar with fragmentation of the epithelium stalk and base in gills of *C. gariepinus* cultivated in Awba dam, rivers around Ewekoro cement factory and groundwater relative to tapwater (figure 8). In figure 9, kidneys of *C. gariepinus* cultivated in Awba dam, the three rivers and groundwater had diffused degeneration of glomeruli and renal haemorrhage. In figure 10, spongiosis with infiltration of inflammatory cells were seen in brain of *C. gariepinus* cultivated in groundwater and rivers around Ewekoro cement factory.

Table 1: Elemental Analysis of Heavy Metals in *C. gariepinus* brain raised in the laboratory

Table 1: Physico-Chemical analysis of water samples of study sites

PARAMETERS	TAP WATER	AWBA DAM	ITORI	ALAGUNTAN	ELEBUTE	GROUND WATER	NAFDAC	NESREA
pH	7.87	7.24	7.58	7.02	710	6.87	6.5-8.5	6.5-8
TDS (Mg/ L)	280	107	199	143	1.10	0.46		500
EC(s/cm)	410	132	210	254	362	92	NIL	1000
TEMP (°c)	20.2	23.6	21.6	21.6	20.6	21.6	-	-
HARDNESS (Mg/ L)		105.36	135.38	93.21	62.56	33.15	100mg/LMax	-
DO (Mg/ L)	10.13	5.89	6.42	6.05	6.13	6.21	NIL	-
BOD (Mg/ L)	0.5	182.56	102.65	169.78	113.46	109.37	NIL	250
Colour	Unobjectionable	Objectionable	Objectionable	Objectionable	Objectionable	Objectionable	Unobjectionable	-
Odour	Unobjectionable	Unobjectionable	Unobjectionable	Unobjectionable	Unobjectionable	Unobjectionable	Unobjectionable	-
Taste	Unobjectionable	Objectionable	Objectionable	Objectionable	Objectionable	Objectionable	Unobjectionable	-
Turbidity	Clear	Slightlyt	Slightlyt	Slightlyt	Slightlyt	Turbid	Crystal Clear	-

Note: TDS – total dissolved solid, DO dissolved oxygen, BOD biological oxygen demands, EC-electrical conductivity. NESREA- National environmental standards regulations enforcement agency (2011) fisheries and recreation quality criteria standard. NAFDAG- National agency for food and drug administration and control.

Table 2- Heavy metals level in water samples of study sites

METALS	TAP WATER	AWBADAM	ITORI	ALAGUNTAN	ELEBUTE	GROUND WATER	WHO	NESREA
Co (ng/L)	0.001 ± 0.01	0.00 ± 0.01	0.18 ± 0.06	0.12 ± 0.05	0.06 ± 0.01	0.14 ± 0.03	0.005	-
Mn (ng/L)	0.009 ± 0.08	0.00 ± 0.0	0.13 ± 0.03	0.18 ± 0.04	0.07 ± 0.05	0.16 ± 0.02	0.01	0.2
Ni (ng/L)	0.016 ± 0.04	0.08 ± 0.01	1.06 ± 0.17	0.95 ± 0.10	1.04 ± 0.01	0.98 ± 0.00	0.05	-
Pb (ng/L)	0.00 ± 0.01	0.074 ± 0.0	0.20 ± 0.05	0.227 ± 0.06	0.113 ± 0.08	0.411 ± 0.03	0.05	0.05
Fe (ng/L)	0.00 ± 0.00	0.171 ± 0.01	0.033 ± 0.04	0.065 ± 0.01	0.095 ± 0.04	1.244 ± 0.01	0.3	-
Cr (ng/L)	0.00 ± 0.03	0.539 ± 0.03	0.066 ± 0.04	0.001 ± 0.03	0.140 ± 0.01	0.052 ± 0.03	0.05	0.05
Zn (ng/L)	0.01 ± 0.03	0.01 ± 0.01	0.033 ± 0.02	0.015 ± 0.02	0.055 ± 0.02	0.043 ± 0.00	5.0	-

NOTE: Co- Cobalt, Mn- Manganese, Ni- Nickel, Cr- Chromium, Pb -Lead, Fe- Iron, Zn- Zinc, WHO- World Health Organization limit in drinking water. ND- not detected. NESREA- national environmental standard and regulatory agency. Values are Mean ± SD (n=10)

Table 3- Body Weight and length of *C. gariepinus* raised in the laboratory

GROUPS	BODY WEIGHT		WEIGHT DIFFERENCE (g)	INITIAL LENGTH (cm)	FINAL LENGTH (cm)	LENGTH DIFFERENCE (cm)
	INITIAL (g)	FINAL (g)				
TAP WATER	55 ± 0.2	138.52 ± 50.0	132.9 ± 49.8	8.96 ± 0.6	27.02 ± 1.4	18.06 ± 3.2
AWBADAM	51 ± 0.2	138.18 ± 44.4	129.2 ± 43.9*	8.96 ± 0.6	26.95 ± 0.5	17.99 ± 3.3
ITORI	50 ± 0.1	113.15 ± 35.5	108.1 ± 35.4*	8.89 ± 1.1	26.67 ± 1.1	17.18 ± 2.3
ALAGUNTAN	57 ± 0.1	128.22 ± 45.3	122.5 ± 45.2*	8.91 ± 0.9	26.63 ± 0.4	17.69 ± 3.4
ELEBUTE	59 ± 0.2	136.11 ± 43.3	130.8 ± 43.3*	9.43 ± 0.5	27.16 ± 0.5	17.61 ± 1.9
GROUNDWATER	57 ± 0.2	128.34 ± 49.9	122.4 ± 49.8**	8.87 ± 0.2	25.81 ± 0.5	16.13 ± 3.6

Values are Mean ± SD (n=10). Significance - *p < 0.05 as compared to control (Tap water), **p < 0.05 as compared to positive control Awba Dam

Table 4- Body weight and Organosomatic indices of organs of *C. gariepinus* raised in the laboratory

GROUPS	BODY WEIGHT		DIFFERENCE IN WEIGHT (g)	KIDNEY WEIGHT (g)	BRAIN WEIGHT (g)	GILLS WEIGHT (g)	ORGANO-SOMATIC WEIGHT		
	INITIAL (g)	FINAL (g)					KIDNEY (g)	BRAIN (g)	GILLS (g)
TAP WATER	55 ± 0.2	138.52 ± 50.0	132.9 ± 49.8	0.5 ± 0.4	0.2 ± 0.1	0.301 ± 0.0	0.4 ± 0.2	0.2 ± 0.1	0.4 ± 0.1
AWBADAM	51 ± 0.2	138.18 ± 44.4	129.2 ± 43.9*	0.6 ± 0.4	0.1 ± 0.1	0.302 ± 0.0	0.4 ± 0.3	0.1 ± 0.1*	0.2 ± 0.1*
ITORI	50 ± 0.1	113.15 ± 35.5	108.1 ± 35.4*	0.5 ± 0.4	0.1 ± 0.1*	0.302 ± 0.0	0.4 ± 0.4	0.1 ± 0.1*	0.2 ± 0.1*
ALAGUNTAN	57 ± 0.1	128.22 ± 45.3	122.5 ± 45.2*	0.5 ± 0.3	0.1 ± 0.1*	0.301 ± 0.0	0.5 ± 0.3	0.1 ± 0.1*	0.3 ± 0.01*
ELEBUTE	59 ± 0.2	136.11 ± 43.3	130.8 ± 43.3*	0.4 ± 0.3	0.1 ± 0.1*	0.301 ± 0.0	0.3 ± 0.3	0.1 ± 0.1*	0.3 ± 0.1*
GROUNDWATER	57 ± 0.2	128.34 ± 49.9	122.4 ± 49.8**	0.6 ± 0.3	0.1 ± 0.1*	0.302 ± 0.0	0.4 ± 0.4	0.1 ± 0.1*	0.3 ± 0.1*

Values are expressed as Mean ± SD (n=10). Significance - *p < 0.05 as compared to Negative control Tap Water, **p < 0.05 as compared to positive control Awba Dam. Organosomatic weight as %Body Weight.

Table 5- Elemental Analysis of Heavy Metals in Fish Organ Gills Raised in the laboratory

GROUPS	Zn	Pb	Fe	Co	Cr	Mn	N
TAP WATER	0.4±0.05	0.04±0.1	0.2±0.02	0.02±0.01	0±0.01	0.008±0.01	0.03±0.004
AWBADAM	0.7±0.01	0.06±0.1	1.2±0.1	0.1±0.06*	0±0.01	0.1±0.005	0.17±0.005
ITORI	0.8±0.01	0.3±0.03**	2.7±0.02*	0.2±0.01**	0±0.1	0.2±0.006**	0.13±0.003
ALAGUNTAN	1.1±0.23	0.2±0.20*	2.6±0.1*	0.2±0.03*	0±0.1	0.2±0.008**	0.19±0.003
ELEBUTE	0.8±0.05	0.5±0.2**	2.3±0.02*	0.2±0.09**	0±0.06	0.2±0.01**	0.10±0.003
GROUND WATER	1.0±0.04	0.4±0.01**	2.6±0.1*	0.2±0.06**	0±0.01	0.2±0.01**	0.14±0.004
WHO	5.0	0.05	0.3	0.005	0.05	0.01	0.05
NESREA	-	0.05	-	-	0.05	0.2	-

NOTE: Zn- Zinc, Fe- Iron, Pb- Lead, Cr- Chromium, Mn- Manganese, N- Nickel. (WHO- world health organization, NESREA- national environmental standard and regulatory agency) Values are expressed as Mean ± SD (n=10) p Significance - *p<0.05 as compared to negative control tap water. **p<0.05 as compared to positive control Awba Dam

Table 6- Elemental Analysis of Heavy Metals in Fish Organ Kidney raised in the laboratory

GROUPS	Zn	Pb	Fe	Co	Cr	Mn	N
TAP WATER	0.2±0.04	0.05±0.1	0.1±0.03	0.03±0.008	0±0.4	0.04±0.005	0.03±0.002
AWBADAM	0.3±0.01	0.2±0.3	0.1±0.02	0.2±0.006	0±0.04	0.05±0.02	0.009±0.003
ITORI	0.4±0.01	0.4±0.2*	3.2±0.02**	0.2±0.02*	0±0.09	0.2±0.006*	0.07±0.004
ALAGUNTAN	0.6±0.02	0.5±0.1**	1.9±0.03*	0.2±0.006*	0±0.04	0.2±0.005**	0.08±0.003
ELEBUTE	0.5±0.1	0.7±0.2**	3.0±0.02**	0.2±0.02*	0±0.04	0.2±0.006**	0.09±0.003
GROUND WATER	0.4±0.1	0.3±0.1*	3.1±0.01**	0.2±0.03*	0±0.2	0.2±0.005**	0.06±0.002
WHO	0.5	0.05	0.3	0.005	0.05	0.01	0.05
NESREA	-	0.02	-	-	0.05	0.2	-

NOTE: Zn- Zinc, Fe- Iron, Pb- Lead, Cr- Chromium, Mn- Manganese, N- Nickel. (WHO- world health organization, NESREA- National environmental standard and regulatory agency) Values are expressed as Mean ± SD, p Significance - *p<0.05 as compared to negative control Tap water. **p<0.05 as compared to positive control Awba Dam

Table 7- Elemental Analysis of Heavy Metals in *C. gariepinus* brain raised in the laboratory

GROUPS	Zn	Pb	Fe	Co	Cr	Mn	N
TAP WATER	0.3±0.06	0.04±0.1	0.2±0.05	0.1±0.05	0±0.06	0.02±0.05	0.02±0.003
AWBADAM	0.5±0.1	0.4±0.2	0.9±0.03	0.3±0.03	0±0.06	0.2±0.003	0.01±0.002
ITORI	0.4±0.03	0.3±0.08	1.8±0.01**	1.8±0.03**	0±0.07	0.2±0.02**	0.13±0.005
ALAGUNTAN	0.3±0.07	0.3±0.2	1.5±0.01*	1.5±0.01**	0±0.02	0.2±0.002**	0.07±0.004
ELEBUTE	0.2±0.06	0.3±0.1	1.7±0.03**	1.7±0.03**	0±0.02	0.2±0.002**	0.09±0.002
GROUND WATER	0.5±0.09	0.3±0.07	1.4±0.03*	1.4±0.03**	0±0.09	0.2±0.007**	0.13±0.006
WHO	5	0.05	0.3	0.005	0.05	0.01	0.05
NESREA	-	0.05	-	-	0.05	-	0.2

NOTE: Zn- Zinc, Fe- Iron, Pb- Lead, Cr- Chromium, Mn- Manganese, N- Nickel. (WHO- world health organization, NESREA- national environmental standard regulatory agency) Values are expressed as Mean ± SD, p Significance - *p<0.05 as compared to negative control Tap water. **p<0.05 as compared to positive control Awba Dam

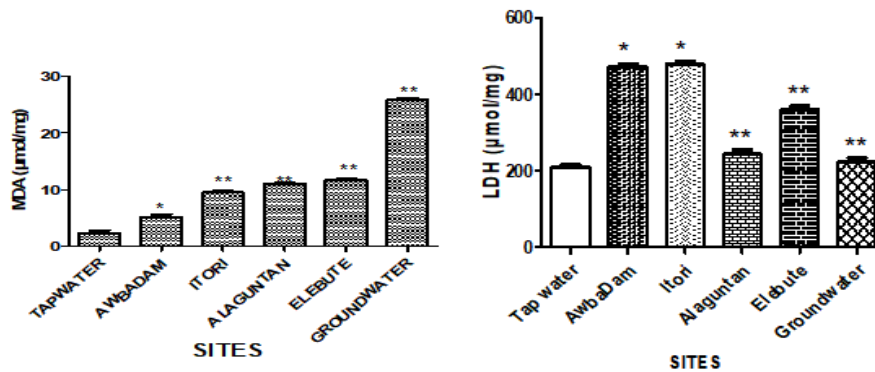


Figure 1- Effect of Heavy Metals on serum Malondialdehyde (MDA) level and Lactate Dehydrogenase (LDH) activity in *clarias gariepinus* exposed to polluted water in the laboratory. Values are expressed as mean \pm SD, N=10. *- significant when compared to control ($p < 0.05$), **- significant when compared to Awba dam ($p < 0.05$)

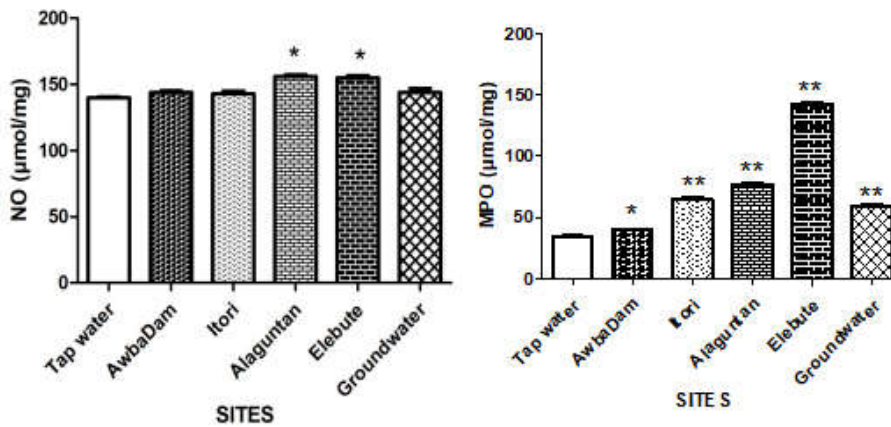


Figure 2- Effect of Heavy Metals on serum Nitric Oxide level and Myeloperoxidase (MPO) activity in *clarias gariepinus* exposed to polluted water in the laboratory. Values are expressed as mean \pm SD, N=10. *- significant when compared to control ($p < 0.05$). **- significant when compared to Awba dam ($p < 0.05$)

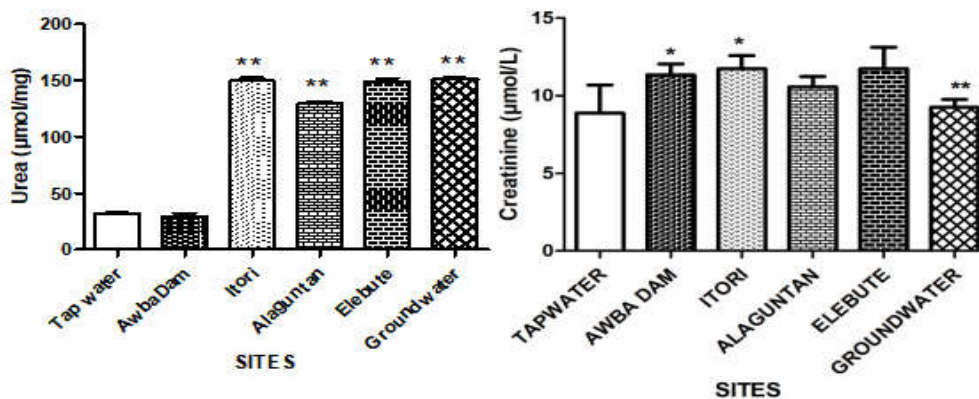


Figure 3- Effect of Heavy Metals on serum Urea and Creatinine levels in *clarias gariepinus* exposed to polluted water in the laboratory. Values are expressed as mean \pm SD, N=10. *- significant when compared to control ($p < 0.05$). **- significant when compared to Awba dam ($p < 0.05$)

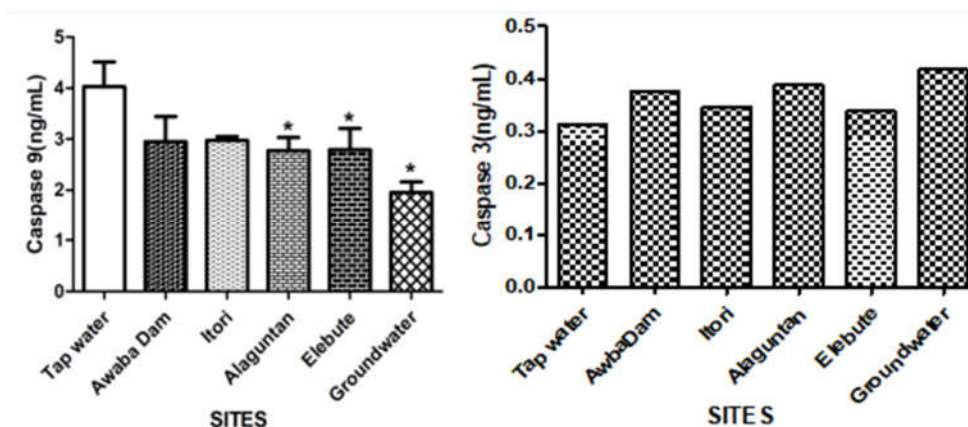


Figure 4- Effect of Heavy Metals on serum Caspase 3 and 9 activities in *clarias gariepinus* exposed to polluted water in the laboratory. Values are expressed as mean \pm SD, N=10. *- significant when compared to control ($p < 0.05$). **- significant when compared to Awba dam ($p < 0.05$)

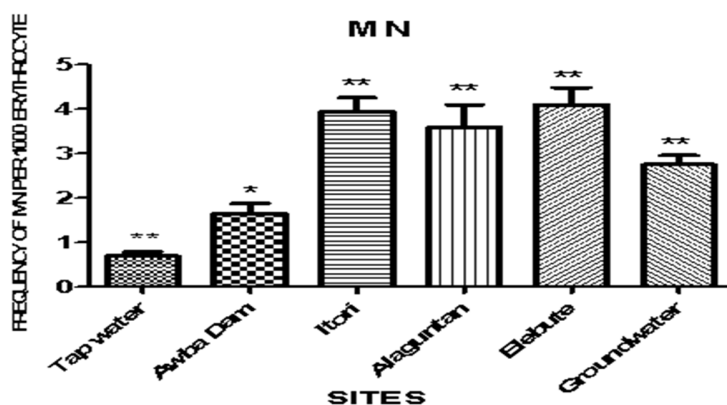


Figure 5- Variation in micronuclei induced in the peripheral erythrocyte observed in *C.gariepinus* exposed to polluted water in the laboratory. Values are expressed as mean \pm SD, N=10. *- Significantly different from the Tap water ($P < 0.05$). **- Significantly different from the Awba Dam ($P < 0.05$)

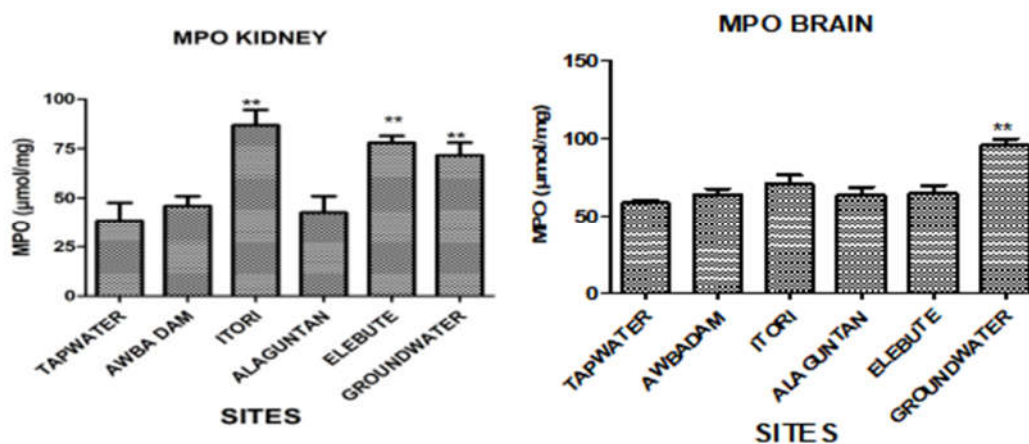


Figure 6- Effect of Heavy Metals on Malondialdehyde (MDA) level in kidney and brain activity in *clarias gariepinus* exposed to polluted water in the laboratory. Values are expressed as mean \pm SD, N=10. *- significant when compared to control ($p < 0.05$). **- significant when compared to Awba dam ($p < 0.05$)

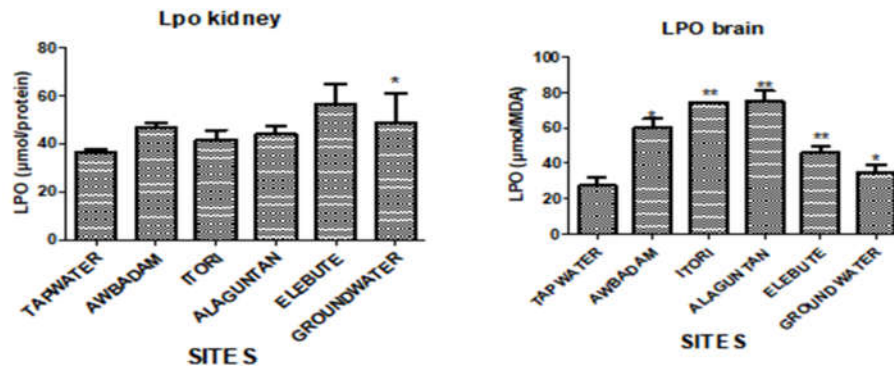


Figure 7- Effect of Heavy Metals on Malondialdehyde (MDA) level in kidney and brain activity in *clarias gariepinus* exposed to polluted water in the laboratory. Values are expressed as mean \pm SD, N=10. *- significant when compared to control ($p < 0.05$). **- significant when compared to Awba dam ($p < 0.05$)

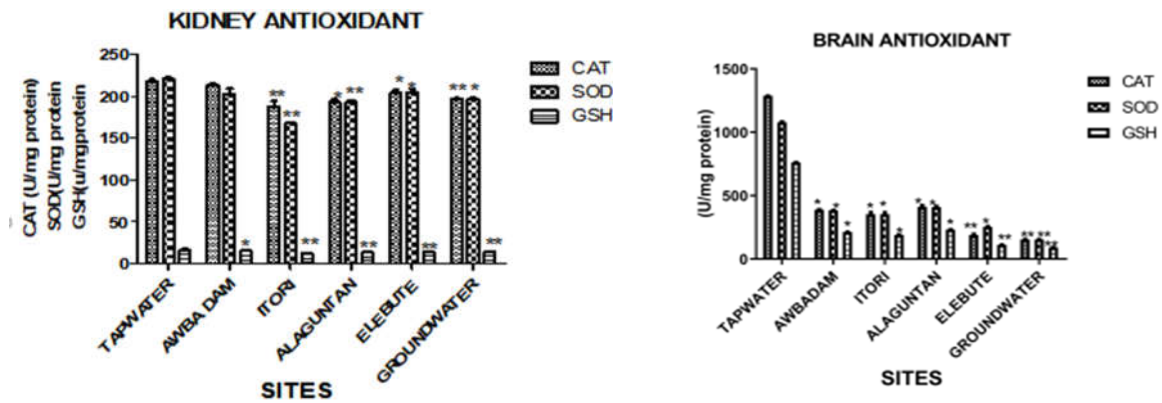


Figure 8- Effect of Heavy Metals on the levels of renal antioxidant indices activity in *clarias gariepinus* exposed to polluted water in the laboratory. Values are expressed as mean \pm SD, N=10. *- significant when compared to control ($p < 0.05$). **- significant when compared to Awba dam ($p < 0.05$)

Figure 9- Effect of Heavy Metals on the levels of brain antioxidant indices activity in *clarias gariepinus* exposed to polluted water in the laboratory. Values are expressed as mean \pm SD, N=10. *- significant when compared to control ($p < 0.05$). **- significant when compared to Awba dam ($p < 0.05$)

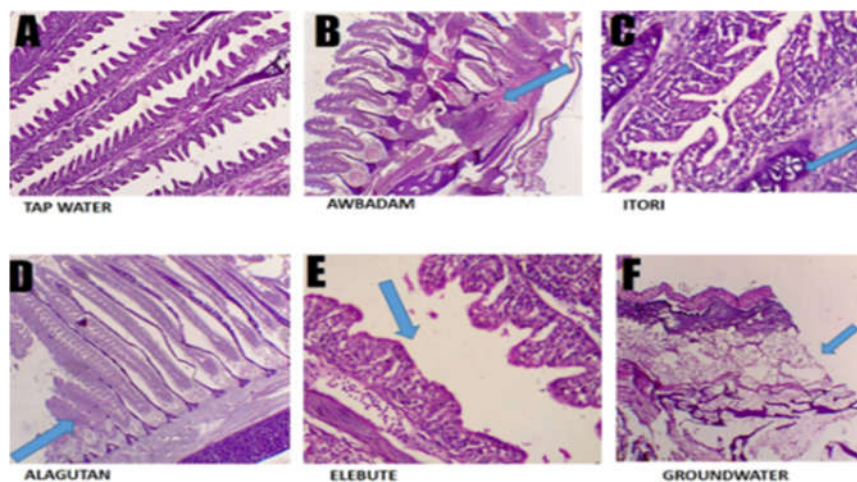


Fig 10: Histological alterations in gills of *C. gariepinus* exposed to polluted water in the laboratory.

(A) Normal primary and secondary lamellar filaments. (B) short and atunted lamellar. (C) congested submucosa. (D) thinning out of the projecting stalk epithelium. (E) fragmentation of the epithelium, stalk and base. (F) severely eroded lamellae

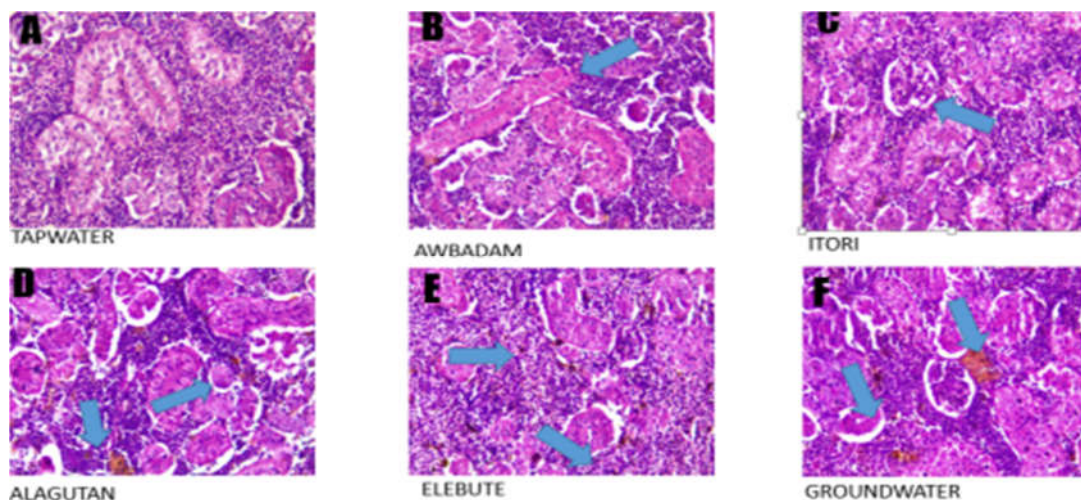


Fig 11: Histological alterations in kidney of *C. grariepinus* exposed to polluted water in the laboratory. A) normal glomeruli and tubular. (B) minimal glomeruli (C) distortion and congestion of vessels. (D) diffuse degeneration of glomeruli. (E) loss of tissue and fragmentation. (f) renal haemorrhage.

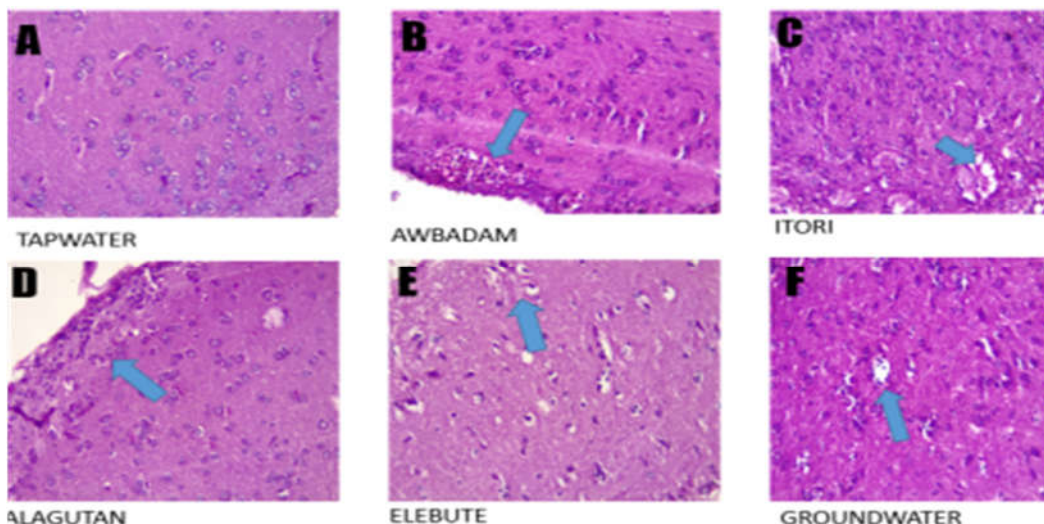


Figure 12: Histological alterations in brain of *C. grariepinus* exposed to polluted water in the laboratory. (A) normal granular and molecular layers. (B) mild spongiosis (C) moderate spongiosis (D) spongiosis. (E) infiltration of inflammatory cells. (F) acute inflammation of neutrophils.

Discussion

Industrial and non-industrial activities initiate undesirable effects and biological dynamics to man and the environment with implication on health and environmental sustainability (Ogunshina *et al.*, 2021). The physio-chemical analysis of waters from Awba dam, the three rivers around Ewekoro cement factory and groundwater showed pH, temperature and electrical conductivity within the permissible range of sustainability of aquatic life according to

NAFDAC and NESREA. This observation is similar to the findings of Adejumo and Adedokun (2012) on Lala, Yobo and Agodo rivers in Ewekoro local government area of Ogun state. The reduced TDS and increased BOD in the waters samples from the sampling sites is an indication of the presence of inorganic pollutants such as heavy metals. The levels of Co, Ni, Pb and Cr in these waters from Awba dam and the rivers exceeded the permissible limits of NESREA and WHO. These high metallic contents may be attributed to cement dust from the factory and also the release of

untreated domestic wastes to the water bodies. This result is in consonance with the findings of Gbadebo and Bankole (2007) who conducted heavy metal analysis of dust particles at different centres during harmattan season. Similar report on the heavy metal analysis of sediments from Awba Dam indicated high levels of As, Cd, Hg and Ni was presented by Adeogun *et al.*, (2016). The significant reduction in body weight-gain was observed in *C. gariepinus* cultured in these waters, and similar to the observation of Bagebal and Tesch (1978) who assumed that fish with heavier weight may be due to better conditions of the surrounding water where it was cultured. The weights of brain and gills of *C. gariepinus* cultured in the water samples significantly low when compared with the control, suggesting physiological damage to these organs might have occurred due to presence of certain pollutants. Thus, the increase in the levels of Pb, Fe, Co and Mn in gills, kidneys and brain of *C. gariepinus* cultured in these water bodies relative to control may be linked to the high metallic contents of the waters from sampling sites as well as the fish has a poor mechanism for digesting and eliminating these heavy metals. This suggests that the *C. gariepinus* cultured in these water bodies may encounter health challenges if the conditions remain. The Pb toxicity observed in the organs of the *C. gariepinus* in this study is very crucial and critical in view of the fact that Pb is one of most lethal metals and toxic to various biological systems including the nervous system, bones, kidney and heart (Flora *et al.*, 2011). These metals are known to induce oxidative damage to organs; heart, kidney, brain (cognitive impairment) and reproductive organs (Sing *et al.*, 2017).

Lipid peroxidation process is initiated in the presence of oxygen and PUFAs during heavy metals reactions in tissues leading to generation of reactive oxygen species (ROS) (Barboza *et al.*, 2018). Our findings confirmed that *C. gariepinus* cultured in these waters had elevated malondialdehyde (MDA), (marker of lipid peroxidation) and lactate dehydrogenase which are indication of heavy metal-induced oxidative toxicity. These results are in consonance with the findings of Farombi *et al.*, (2008) who reported a significant increase in MDA formation in the liver, kidney, gills and hearts of *C. gariepinus* following

exposure to different concentrations of butachlor. Lactate dehydrogenase (LDL) is an enzyme that catalyses the conversion of lactate to pyruvate, it is involved in energy production in heart, liver, muscles, kidney, lungs, and in blood cells (Spengler, 2002). Therefore, significant increase in the serum activity of the enzyme in *C. gariepinus* is indicative of tissues damage occasioned by the presence of heavy metals in these waters. The result is in agreement with the findings of Ajima *et al.*, (2015) who reported a significant increase in alanine and aspartate aminotransferases and LDH in serum and gills of *C. gariepinus* after 42 days of chronic exposure to diclofenac. The myeloperoxidase (MPO) catalyses the hydrogen peroxide mediated oxidation of halide ions, it may also contribute to irritation and pathogenesis of the inflammatory-related diseases (Oday *et al.*, 2014). Nitric oxide (NO) possesses a biphasic action; in low concentration it protects against oxidative damages to cells, while at higher doses it enhances oxidative damages (Joshi *et al.*, 2002). The NO plays important role in many physiological functions including neuro-transmission and vasodilation (Yamamoto, 2015). The activities of MPO and levels of NO in serum of *C. gariepinus* raised in water samples from Itori, Alaguntan and Elebute rivers as well as Awba dam were significantly higher than the control. The levels of urea and creatinine were significantly increased in *C. gariepinus* cultured in water samples from Itori, Alaguntan and Elebute rivers as well as Awba dam. Our observation is corroborated by the findings of Ajeniyi and Solomon, (2014) that urea and creatinine in catfish from 3 different commercial ponds increased with varied length and weight.

Apoptosis is a form of programmed cell death that is regulated by the bcl₂ family and caspase family of proteins (Kelly, 2010). Caspase-9 can remodel mitochondria and increase ROS production by cleaving Bid into tBid (Han, 2006), while caspase-3 is an executioner of apoptosis which regulates inflammation and apoptosis signaling network (Reis *et al.*, 2007). The insignificant differences observed in the levels of caspase-3 and-9 in *C. gariepinus* exposed to waters from rivers around in Ewekoro cement factory and Awba dam confirmed that the process of apoptosis was unaffected in the fish. Genotoxicity is the

alterations in the structure or content of chromosomes or base-pair sequences of DNA (mutagenicity) by exposure to toxic agents such as heavy metals (DeLemos *et al.*, 2007). In order to investigate the genotoxic response to complicated combinations of environmental pollutants in the water samples from the sampling sites, the MN induction assay was conducted. Our results showed a significant increase in the induction of MN in the erythrocytes of *C. gariepinus* grown in these waters. The histological alterations were observed in the gills, brain and kidney of the *C. gariepinus* cultured in these waters relative to the control, which confirms the presence of pollutants in the waters. These observations are similar to the findings of Mansur and Sidky (2002) that linked the physiological disruption of kidney and gills of fish to the presence of heavy metal contaminants in the aquatic environment.

In conclusion, biochemical effects such as induction of oxidative stress, inflammation, formation of erythrocytic micronuclei and histological alterations in gills, kidney and brain of *C. gariepinus* cultured in waters from Awba dam and the three rivers around Ewekoro cement factory were observed in this study, thereby raising a public health concern for residents in Ewekoro area that depend on these water bodies for their domestic purposes.

Conflict of interest

The authors have declared that no conflict of interest exists

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