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ORIGINAL ARTICLE

Biomarkers of oxidative stress in *Clarias gariepinus* for assessing toxicological effects of heavy metal pollution of Abereke river in southwest Nigeria

Oluwatosin Adetola Arojojoye[1] · Ademola Adetokunbo Oyagbemi[2] · Ogundeyi Morufat Gbemisola[1]

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Abstract
Water pollution indices and oxidative stress biomarkers in African sharp-toothed catfish, *Clarias gariepinus* were used to evaluate the health status of Abereke River on the Ilaje coastal zone of Ondo's Niger Delta region. Muscle and liver tissues from fishes harvested from the river and from a clean fish farm were comparatively subjected to heavy metal status and redox stress biomarker analyses. Water samples from the river and fish farm were also subjected to water quality tests. Our findings showed significant elevations in bacterial contaminants, heavy metal pollutants, and particulate matter deposits in Abereke River. In addition, heavy metal deposits were found in tissues of fish harvested from the river correlated with increases in markers of oxidative stress and depletions in antioxidant defense systems. Taken together, water from Abereke River is unsafe for human consumption, and consumption of aquatic organisms from the river may be harmful to health.

Keywords Aquatic pollution · Oxidative stress · Bioaccumulation · Heavy metals · Toxicity

Introduction

Anthropogenic interferences in the balance of local aquatic ecosystems have been reported to lead to high levels of pollution in marine and seawater ecosystems (Machado et al. 2015). The dependence on and growth of the petroleum hydrocarbon industry in Nigeria have been correlated with increased aquatic pollution (Allison et al. 2018). Further, the Nigerian petroleum industry has played a huge role in supplying global energy demand while contributing to local economic development, but this has come at a huge cost (Ite et al. 2018; Mogaji et al. 2018). Moreover, one of the largest oil-producing regions in the world, the Niger Delta, is reported to experience large volumes of oil spill–related pollution with little quantification of the negative impacts on human beings (Obida et al. 2018). Pipeline vandalism, accidental spillages, oil theft, and poor transportation network for crude are major culprits leading to crude and its products being routinely discharged onto farmlands and waterways, with attendant irreversible damage on the eco-system. Over an 8-year period, at least 90 million liters of oil were released into the Niger Delta with approximately 29% of humans living in close proximity to the pipe networks affected (Obida et al. 2018).

These damages from aquatic pollution are cumulative due to several factors including the depletion of dissolved oxygen required by aquatic life for survival (Venturino et al. 2017), the buildup of by-products of pollution, heavy metals, and hydrocarbons in aquatic and plant life (Eroglu et al. 2015), the generation of reactive oxygen and nitrogen species (ROS/RNS) causing oxidative stress-related damage (Abdel-Gawad et al. 2016), the resultant deaths and decomposition of wildlife, and the reduction in general wellness of the water bodies (Jarvela Rosenberger et al. 2017). These all coalesce to make such environments unsuitable for life. For those who depend on coastal bodies for economic and domestic activities, pollution completes a cycle, affecting their health and

✉ Oluwatosin Adetola Arojojoye
tosyne568@yahoo.com

[1] Department of Biochemistry, Faculty of Basic Medical and Applied Sciences, Lead City University, Ibadan, Nigeria

[2] Department of Veterinary Physiology, Biochemistry and Pharmacology, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria

livelihood (Nwankwo and Ifeadi 1988; Ordinioha and Brisibe 2013). Routine biomonitoring of aquatic bodies is crucial in identifying and predicting environmental impacts of anthropogenic and industrial activities (Zhou et al. 2008).

Abereke, one of the sub-towns under the Ilaje local government area of Ondo state is a prime spot for fishmongers and traders due to its location along the coast. It is also known as an oil prospecting community, contributing to Ondo state's status as an oil-producing region in the Niger Delta. We assessed the water quality levels using established markers of aquatic pollution and analyzed fish tissue harvested from the river for health status. We worked with the premise that the Abereke River would contain evidence of chemical and heavy metal leachates from the oil prospection going on inland which would be above standard levels proposed by the World health organization (WHO) as safe for human health. We also hypothesize that aquatic life when evaluated, would also contain such harmful levels of chemicals making them unfit for human consumption.

Materials and methods

Sampling area

The Ilaje River is a delta-like region which borders the Atlantic Ocean, lying on latitude 5° 50′ N–6° 09′ N and on longitude 4° 45′ E–5° 05′ E and is a part of the broader Ondo coastal area. Abereke is one of the areas located along the river which is known for fish farming as a domestic and economic means of livelihood (Fig. 1).

Animals

Ten *Clarias gariepinus*, averaging 220 g, were caught from Abereke area on river Ilaje along the Ilaje coastal community of Ondo State, Nigeria by means of local fishing nets at random points along the river. For reference/control, *Clarias gariepinus*, were similarly harvested from a "clean" fish farm: Ilesanmi fish farm in Ondo State. To the best of our knowledge, this farm was free of industrial effluents or any other pollutants.

Experimental procedure

Fishes were dissected with their target organs (liver and muscle) removed, rinsed in 1.15% KCl and homogenized in 4 volumes of homogenizing buffer (50 mM Tris—HCl mixed with 1.15% KCl and pH adjusted to 7.4). The resulting homogenate was centrifuged at 12,500 g for 10 min to obtain the post-mitochondrial fraction.

Lipid peroxidation was quantified as malondialdehyde (MDA) according to the method described by Farombi et al. (2000). The muscle and hepatic-reduced glutathione (GSH) concentrations were determined according to the method of Jollow et al. (1974) modified by Justarini et al. (2013). The superoxide dismutase (SOD) activity was evaluated by the method of Misra and Fridovich (1972) modified by Oyagbemi et al. (2017). Glutathione-S-transferase (GST) activity was determined according to the method described by Farombi et al. (2008). Catalase activity was estimated according to the method of Sinha (1972) modified by Mahmoud (2016). The pH of the water sample was determined using pH meter Hanna H8921 model. The total suspended solids

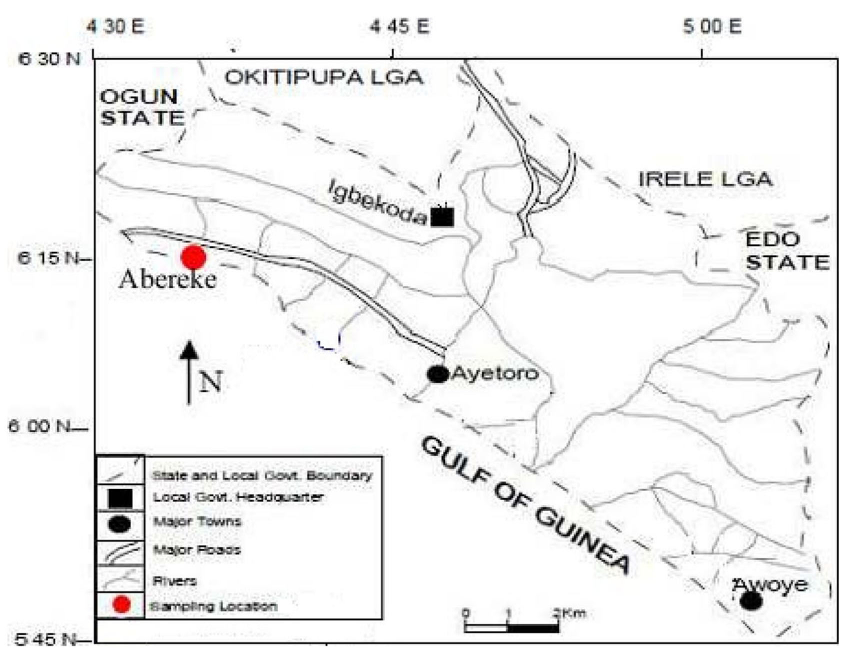

Fig. 1 Map of the Ilaje coastal area showing the study area (Akinnawo et al. 2016)

(TSS) and total dissolved solids (TDS) were determined using the titrimetric method according to the guidelines of A.O.A.C. (2005); conductivity of the water sample was determined using conductivity meter Hanna H8921 model. The heavy metals (lead, cadmium, copper, arsenic, and nickel) contents in the water samples were determined by atomic absorption spectrophotometry using atomic absorption spectrophotometer (AAS) Buck 211 model. The Cl^{-} (mg/L), SO_4^{2-} (mg/L), NO_3- (mg/L), Na + (mg/L), Ca^{2+} (mg/L), and K^{+} (mg/L) in the water sample were determined by flame photometry in accordance with the guidelines of A.O.A.C. (2005). MacConkey agar and salmonella-shigella agar were used for the isolation of coliform and salmonella in the water samples and were counted using colony counter Model 2510 Jenway.

All values for the results are expressed as the mean ± S.D. Parameters obtained from the experimental groups were compared with the control. The data obtained were analyzed using repeated measures one-way analysis of variance (ANOVA) with the Tukey post hoc analysis for the analysis of scientific data using GraphPad Prism® 5.01. Values were considered statistically significant at $p < 0.05$.

Results

From the results in Table 1, the physicochemical parameters and heavy metal concentrations of water sample from Abereke River were significantly higher than that of the control sites and WHO standard.

Table 1 Physico-chemical and heavy metal properties of water samples from Abereke River compared with the control site

Parameters	Control site	Abereke River	WHO standard*
PH	7.2	5.8	6.5–8.5
Total Solids (mg/L)	521.3	1817.6	500
TSS (mg/L)	325.6	1095.8	30
TDS (mg/L)	196.0	721.8	500
Total Hardness	9.8	156.7	100
Pb (mg/L) US/CM	0.082	17.8	0.05
Cd (mg/L)	0.009	6.2	0.05
Cu (mg/L)	0.28	4.6	1.0
As (mg/L)	0.17	72.8	0.01
Cl^{-} (mg/L)	56.5	79.5	250
SO_4^{2-} (mg/L)	3.4	63.7	400
NO_3^{-} (mg/L)	1.8	42.6	10
Na^{+} (mg/L)	11.7	31.3	200
Ca^{2+} (mg/L)	26.5	53.6	50
K^{+} (mg/L)	63.4	84.7	12

*WHO World Health Organization standard for drinking water (WHO 2004)

Table 2 Bacterial load comparison between the control site and Abereke River

	Control site	Abereke River
Total viable count	3.2×10^3	7.2×10^5
Coliform count	NIL	1.4×10^3
Salmonella count	NIL	2.3×10^2

From the results in Table 2, total viable count was higher in water sample from Abereke River compared with control, while coliform and salmonella were found in Abereke water sample but absent in water sample from the control site.

From the results in Table 3, the concentrations of lead, cadmium, arsenite, copper, and nickel in the liver and muscle of *Clarias gariepinus* from Abereke River were significantly higher than that of the control.

From the results in Fig. 2, malondialdehyde and glutathione concentration were significantly higher in the liver and muscle of *Clarias gariepinus* from Abereke River compared with control.

From the results in Fig. 3, the activity of the antioxidant enzyme superoxide dismutase (SOD) was significantly lower in the liver and muscle of *Clarias gariepinus* from Abereke River compared with control. The activity of GST was also significantly lower in the liver of *Clarias gariepinus* from Abereke River compared with control, but GST activity was significantly higher in the muscle. Catalase activity was significantly higher in both the liver and muscle.

Discussion

Anthropogenic influences are responsible for dwindling health and life forms in water bodies (Machado et al. 2015).

Table 3 Heavy metal concentration in hepatic and muscle tissue of *Clarias gariepinus* harvested from Abereke River when compared with the control site

	Fish organ	Control site	Abereke River
Pb (mg/L)	Liver	0.02 ± 0.004	0.84 ± 0.06**
	Muscle	0.006 ± 0.001	0.56 ± 0.08**
Cd (mg/L)	Liver	0.003 ± 0.003	0.06 ± 0.007**
	Muscle	0.0004 ± 0.0002	0.09 ± 0.02**
As (mg/L)	Liver	0.05 ± 0.006	0.86 ± 0.06**
	Muscle	0.023 ± 0.005	0.61 ± 0.17**
Cu (mg/L)	Liver	2.7 ± 0.5	5.0 ± 0.6**
	Muscle	0.6 ± 0.3	3.3 ± 1.2**
Ni (mg/L)	Liver	0.75 ± 0.06	8.7 ± 0.6**
	Muscle	0.34 ± 0.09	7.69 ± 0.68**

Results are expressed as mean ± S.D

*indicate significant difference from control at $p < 0.05$

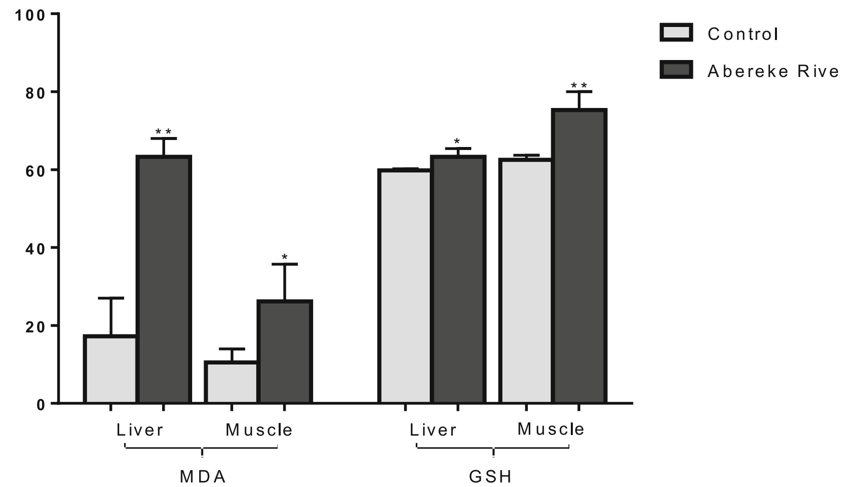

Fig. 2 Malondialdehyde and glutathione concentration in the liver and muscle of *Clarias gariepinus* from Abereke River compared with control. MDA (malondialdehyde, µmole of MDA formed/mg protein), GSH (glutathione, µmol/mg protein). Asterisks indicate significant difference from control at $p < 0.05$

In the Niger Delta region of Nigeria, this manifests primarily as oil production in local communities (Obida et al. 2018). An unfortunate consequence of this is the contamination of aquatic bodies with polycyclic aromatic hydrocarbons, heavy metals, and radioactive isotopes which have deleterious effects on human health (Okogbue et al. 2017; Ite et al. 2018). The concentration of heavy metals in Abereke River water samples was significantly higher than the acceptable limits for use as drinking water (Table 1). The concentrations of lead, cadmium, copper, and arsenite were significantly higher by 217, 689, 16, and 428-folds respectively in water samples from Abereke River when compared with control (Table 1). The trend of heavy metal concentration was found as follows: Cd > As > Pb > Cu. Cadmium and arsenic are of no importance to the human body and are actually considered extremely harmful at very small concentrations (Chang et al. 1996). The heavy metal lead has been demonstrated to cause irreversible damage to the liver, kidney, and erythrocytes at low doses (Omobowale et al. 2014; Oyagbemi et al. 2015). Copper as a metal is an important co-factor in redox processes involving catalase and superoxide dismutase as well as being required in several other physiological processes (Stern 2010). Repeated exposure to these heavy metals has been reported to activate ROS production and initiate oxidative stress (Jomova et al. 2011). This can progress to DNA damage, cell cycle disruption, cancer, and cell death (Stevens et al. 2010).

Other physicochemical parameters evaluated from Abereke water sample were well above the acceptable limits for potable water (WHO 1984). High concentrations of chloride, sulphate, nitrate, sodium, calcium, and potassium ions were recorded in water samples when compared with control. When consumed frequently, these ions could precipitate electrolyte imbalances in the body with attendant metabolic risks. Furthermore, there were increases in particulate matter in Abereke River sample when compared with the control. There were about 349% increase in total solids and 337% and 368% increases in total suspended solids (TSS) and total dissolved solids (TDS), respectively, in water samples from Abereke River when compared with the control sample (Table 2).

Bacterial analyses revealed active bacteria to be higher in Abereke water sample when compared with those found in the control water sample. Coliforms and salmonella visible in Abereke water sample were not detected in the control water sample (Table 2). This further confirms that water from Abereke River should not be used for domestic purposes, it

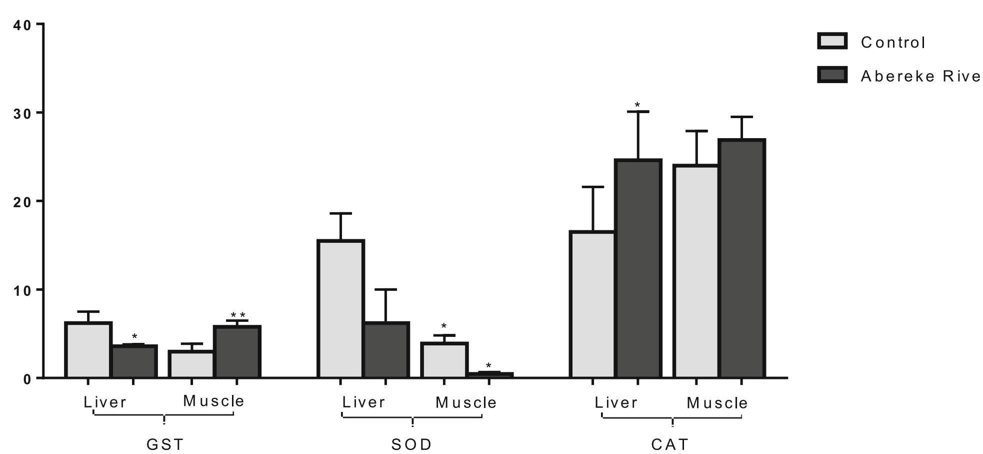

Fig. 3 Antioxidant profile in the liver and muscle of *Clarias gariepinus* from Abereke River compared with control. SOD (superoxide dismutase, units/mg protein); GST (glutathione-S-transferase, mmole1-chloro-2, 4-dinitrobenzene-GSH complex formed/min/mg protein); CAT (µmole H_2O_2/min/mg protein). Asterisks indicate significant difference from control at $p < 0.05$

could pose additional health hazard of gastrointestinal discomforts and diseases such as typhoid and diarrhea if consumed.

Just like the heavy metal pollution seen in the Abereke water sample, a similar trend was observed in tissues of *Clarias gariepinus* harvested from the Abereke River. The principle of biomonitoring presumes that the presence of aquatic organisms in a polluted environment can lead to bioaccumulation of pollutants such as heavy metals, bacteria, etc. from the surrounding water, making them bio-indicators of environmental pollution (Zhou et al. 2008; Okay et al. 2016). Our results seem to confirm this finding. Hepatic and muscle tissues of *C. gariepinus* harvested from Abereke River showed significant increases ($p < 0.001$) in the concentration of heavy metals in both the liver and muscle samples when compared with the control (Table 3). In both tissues, however, the liver had higher levels of heavy metal accumulation. This probably reflects the functions of the liver as a detoxification organ with storage and metabolism properties (El-Moselhy et al. 2014). Metallothionein is a natural binding protein found in the liver which acts by binding to copper and retaining it within the liver to serve as an enzymatic co-factor for physiological processes (Gorur et al. 2012). However, cadmium displaces metallothionein bound metals making it more commonly found in hepatic tissue (Capaldo et al. 2016).

Bioaccumulation of heavy metals led to increased malondialdehyde deposits, products of lipid peroxidation in both hepatic and muscular tissues (Fig. 2). Alongside, increases were observed in reduced glutathione, a non-enzymic antioxidant. Elevated malondialdehyde contents agree with previous works which showed such as a response to oxidative stress from heavy metals (Farombi et al. 2007; Sanchez et al. 2007). Heavy metals induce the generation of ROS/RNS within tissues which then destroy nucleic acids, proteins, and lipid membranes (Tchounwou et al. 2012).

In this study, the activity of the antioxidant enzyme superoxide dismutase (SOD) was significantly lower in the liver and muscle of *Clarias gariepinus* from Abereke River compared with control.

The activity of GST was also significantly lower in the liver of *Clarias gariepinus* from Abereke River compared with control, but this was not so in the muscle as the activity of GST was significantly higher in the muscle, this might be in response to redox stressors. A similar trend was observed in catalase (CAT) activity for both hepatic and muscle tissues (Fig. 3).

Antioxidant enzymes are bio-indicators of oxidative stress in aquatic organisms, and they could be induced in response to pollutants (Borković et al. 2005). The initial depletion in GST and SOD of hepatic and muscular tissues could be due to heavy bioaccumulation of heavy metals within these tissues. This leads to increased production of superoxide anions to convert the harmful radicals to the less harmful form: hydrogen peroxide (H_2O_2). The enzymes are then mopped up in the antioxidant response, leading to the observed decrease (Fig. 2). Conversely, the rise in CAT activity is probably an adaptive response against ROS-mediated oxidative stress. Oxidative stress has been known to mediate an upregulation in antioxidant system response through the Nrf2-Keap 1 pathway. This increases antioxidant mobilization and facilitates an increased response to clear out the ROS stressors (Baird and Dinkova-Kostova 2011),

Conclusively, there were increases in markers of oxidative stress coupled with alterations in antioxidant status in fish samples from Abereke River compared with control. There were also high concentrations of heavy metals in both water samples and tissues of *C. gariepinus* from Abereke River, confirming existing models of fishes as biomarkers of aquatic pollution and raising valid health concern issues for inhabitants of these communities who rely on the river for livelihood and consume aquatic products from the river.

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