

Oxidative Stress Biomarkers of Two Fish Species from Different Sites of Ikpoba River

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Abstract

The study assessed metals at three (3) different sites of Ikpoba River and oxidative stress biomarkers in gills, brain and kidney of two fish species, African catfish and Tilapia. The metals in the sediments were analysed using flame atomic absorption spectrophotometer (AAS). A total of four hundred and sixty eight (468) fish per species was collected from the three different sites every week for fifty-two (52) weeks and known weight of the brain, gills and kidney were recovered for enzyme assays. The highest level of lead, zinc, copper, iron, nickel and manganese was recorded in the Abattoir site followed by Capitol and the Guinness site. The study recorded decreased antioxidant enzymes, aldehyde oxidase and lactate dehydrogenase activities in the tissues/organs of fishes in different site-exposed fish species when compared with control. Malondialdehyde and uric acid (UA) levels were significantly ($p < 0.05$) increased in the tissues/organs of fishes relative to control except for kidney of Tilapia which recorded decreased UA. Fishes from Abattoir site contained the highest level of stress enzymes when compared with Guinness site. The study shows an increased deterioration of Ikpoba River and suggests that high metal content caused oxidative stress in fishes which manifested as alteration of antioxidant enzyme activities and other oxidative biomarkers. In conclusion, the results showed a high but tolerable concentration of metals in Ikpoba River. Alteration in antioxidant enzyme activities and other biomarkers of oxidative stress observed in the two fish species, indicates increasing treatment by pollutants. We recommend public awareness on dangers of toxic metals and promulgation of laws for effluents discharge into Ikpoba River to protect the populations [aquatic organisms (fish) and final consumers (humans)] from the deleterious effects and further deterioration.

Keywords

Biomarkers, Fish, Ikpoba River, Metals, Oxidative Stress

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1. Introduction

An intact environment is appropriate for all living organisms. However, industrial effluents and other anthropogenic activities are major sources of contaminants which are discharged into water bodies. These contaminants may induce oxidative stress [1, 2] and pose threat to aquatic organisms such as fishes. Fishes are major sources of protein

and they contain essential minerals, vitamins, unsaturated fatty acids and Omega-3 which help in reducing blood cholesterol and heart malfunction (arteriosclerosis) [3, 4].

The presence of toxic heavy metals in fishes can counteract their beneficial effects if they adversely affect the health of organisms [5, 6, 7]. At elevated concentrations, heavy metals have been shown to induce oxidative stress [1, 2] and accumulate in fishes causing reduction in growth rate and

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fecundity [8], cumulative behaviour [9] and can result in health hazards when consumed by humans [4].

Ikpoba River is one of the major rivers in southern Nigeria and supplies water to most places in Edo State and environs which is home to over 2 million people. Edo State lies roughly between longitude 06° 04'E and 06° 43'E and latitude 05° 44'N and 07° 34'N. High concentration of heavy metals have been reported in different water bodies in Nigeria [10] and the world at large [11]. This is also true for Ikpoba River as investigations have revealed contamination with heavy metals [12].

Increasing anthropogenic and industrial stress on the river is likely to increase contamination load. It is therefore necessary to establish continuous monitoring of the river in order to assess the quality of fish therein meant for human consumption, on the one hand and the health of the aquatic ecosystem, on the other; since it is reported that most degenerative diseases are due to prolonged exposure to environmental pollution [13].

In the last few decades, most studies on aquatic pollution investigated distribution of metals in different tissues of fish [10] and their potential risks for human consumption [3, 14]. Much attention, in the opinion of these researchers, has not been given to the effects of accumulated pollution load on fish health and biomarkers. Since assessment of contaminants in aquatic environment is not enough indication of its state of pollution, it is, therefore, important to consider levels of contamination of Ikpoba River in biological systems. Two fish species [African catfish (*Clarias gariepinus*) and Tilapia (*Oreochromis niloticus*)] were used for this study. This choice is predicated on the fact that Tilapia and African Catfish are major sources of protein in the local diets of Edo State indigenes in Nigeria. The aim of the study is to investigate the current metal levels of Ikpoba River and the level of stress biomarkers (antioxidant enzymes, aldehyde oxidase, lactate dehydrogenase, uric acid and malondialdehyde levels) of selected organs/tissues in African catfish and Tilapia.

2. Materials and Methods

2.1. Sample Collection and Pre-treatment

The samples (sediment, water, fishes) were collected at three (3) different sites located at different distances and directions from the University of Benin. Three (3) samples each of water, fish, and sediments were collected from three different sites every week for fifty-two (52) weeks. The sites were: Site B: Abattoir at the side of Ikpoba Slope Bridge, Benin City. Site C: Guinness (by the side of Guinness Nigeria Plc), Oregbeni Quarters, Benin City. Site D: Capitol (behind

Dentistry Quarters, University of Benin, Benin City), Iguana Village. Site A: Control (Fish farm of Department of Fisheries, Faculty of Agricultural Science, University of Benin, Benin City, Edo State, Nigeria).

Water was collected in a pre-cleaned and acidified glass bottles. On spot fixation of water was done to measure dissolved oxygen (D.O). Total solids (T.S), total dissolved solids (T.D.S), and total suspended solids (T.S.S) were determined using standard techniques [15]. The pH was recorded at the sites using pH strips. Sediment samples were collected at two (2) different depths between (0 – 15) cm and (15 – 30) cm in 1 L polythene containers with screw caps and taken to Edo State Environmental Laboratory, Sapele Road, Benin City, Edo State for analysis.

Three (3) samples each of two (2) different species of fish; African catfish and Tilapia were collected every week for 52 weeks per site (total of 468 fish per species from the 3 sites) and used for the experiment. The fish species were collected with the help of local fishermen around the sites who used fish nets and hooks. They were conveyed in a 100-litre bowl to the laboratory; and one each was taken to the Department of Fisheries, Faculty of Agricultural Science, University of Benin for identification. Fish weight and length were recorded. Known weight of the brain, gills, and kidney were homogenized, centrifuged and supernatants used for enzyme assays. Metal contents were analyzed using sediment samples from the three sections with a Varian spectre AA10 Atomic absorption spectrophotometer with an air/acetylene flame equipped with an auto print out system and metal hollow cathode lamps. Wavelengths were varied for different metals. Standard solutions for the metals ($1000\mu\text{g L}^{-1}$) were prepared from analytical grade for the different metal compounds manufactured by May & Baker Limited, Dagenham, England. Procedural blanks were analysed in the same way as the samples and concentrations were determined using standard solutions. The working standards were prepared by serial dilution of standard stock solutions and were used for calibration of the instrument. Accuracy of the method used was assessed by the analysis of twenty six replicate samples. Percent recovery experiments on metal samples spiked with authentic releasing agents were carried out as part of the analytical data quality assurance. Values obtained were in excess of 92%. The limits of detection were as follows: Pb = 0.08ppm; Ni = 0.05ppm; Zn = 0.005ppm; Mn = 0.03ppm; Fe = 0.05ppm; Cu = 0.005ppm; and Cd = 0.01ppm. The data generated by laboratory analysis of the sediments were summarized by simple descriptive statistics. The level of concentration of each metal in each sampling point were summed up and the mean was taken to be the representative value in that site. To examine the safety levels of the heavy metals in the sediments of the Ikpoba River, the mean value

of the metals found in the sites were compared with WHO standard [16],

2.2. Biochemical Analysis

Thiobarbituric acid reactive substance (TBARS) assay was carried out to determine malondialdehyde levels (lipid peroxidation) according to the method of Hunter *et al.* [17] as modified by Guttridge and Wilkins [18]. The levels of MDA were quantitated using a molar extinction coefficient of 1.56×10^5 M/cm and expressed as $\mu\text{mol MDA g}^{-1}$. Uric acid levels were assayed by colorimetric method described by Fossati *et al.* [19]. Superoxide dismutase (SOD) activity assay was carried out according to the method of Misra and Fridovich [20] based on the ability of the enzyme to inhibit oxidation of epinephrine by superoxide anion. Enzyme activity was expressed as units/mg tissue weight. Catalase activity was assayed by the method of Cohen *et al.* [21]. Aldehyde oxidase enzyme activity was assayed according to the method of Johnson *et al.* [22]. Accuracy and reliability was maintained by using analytical grade (BDH, May & Baker, or Sigma) reagents for preparation of standard solutions and analyses. All glasses and plastics were acid-washed. Buck scientific standard solutions were used to calibrate equipment. Procedural blank samples were subjected to similar extraction method using the same amounts of reagents. All analyses represent mean \pm standard error of mean (SEM) of determinations from total samples.

2.3. Data Analysis

Statistical evaluations of all data were done using one-way analysis of variance (ANOVA) to test for differences in groups, while Duncan's multiple comparisons test was used

to determine significant differences between means at $p < 0.05$. Instat-Graphpad software, San Diego, California, USA, was used for this analysis.

3. Results and Discussion

Physico-chemical properties of the Ikpoba River water, Benin City is shown in Table 1. The physicochemical properties of the Abattoir and Guinness section of the Ikpoba River water were published in previous study [23]. The River water pH ranged between 6.21–6.55, which shows slight acidity. Acidic pH has been reported in different rivers in Nigeria and the world [24]. Several researches have indicated that water acidity (lower pH) directly affects metal accumulation rates by fishes. Water acidity can directly affect bioaccumulation of metals in fishes, by damaging gill epithelia which may become more permeable to metals or indirectly by changing solubility of metal compounds [4]. Dissolved oxygen concentration (DOC) ranged between 0.67-3.04 mg L⁻¹. The levels of total suspended solute (TSS) ranged between 100-420 mg/l. The dissolved salt content in Ikpoba River was significantly greater ($p < 0.05$) than that of distilled water. Biological oxygen demand was below normal for fish health. The level of hardness fell below acceptable limits adopted in Nigeria. Raja *et al.* [25] reported that water hardness (mainly due to calcium carbonates) affects metal transport across gill epithelium and reduced copper accumulation in gills of fish [4]. The elevated level of dissolved salt content in Ikpoba River may be due to high rate of contamination of the river water. Other parameters occurred within acceptable limits for river water adopted in Nigeria [24].

Table 1. Physico-chemical properties of Water analysis from different sites of Ikpoba River.

	pH	Turbidity (NTU)	TSS (mg/l)	Cl (mg/l)	NO ₃ (mg/l)	Acidity (mg/l)	Alkalinity (mg/l)	Hardness (mg/l)	BOD (mg/l)
B	6.21	39	190	35.5	0.00	110.00	30.00	2.13	2.02
C	6.37	16	100	35.5	0.00	125.00	35.00	2.50	0.67
D	6.55	8	420	24.84	0.00	90.00	55.00	2.04	3.04

Source: [23]

Values are means of determinations (n = 52), BOD = Biological Oxygen Demand, NTU = Neihelometric Turbidity Unit, TSS = Total Suspended Solute

Table 2 shows the metal analysis on sediment samples collected from the different sites of Ikpoba River. The metal analysis on sediment of the Abattoir and Guinness section of the Ikpoba River were published in previous study [23]. The levels of Lead (Pb) (35.8 mg/kg) and Zinc (Zn) (120mg/kg) were beyond recommended values set by WHO [26]. Previous studies reported high metal contamination in Ikpoba River [27, 28] and increase concentration of Fe, Cu, Mn, Zn, Ni, Cd, chromium (Cr) and lead (Pb) in two fish species (*Mormyrops deliciosus* and *Mormyrus macrophthalmus*) netted from two locations on the Ikpoba River [28]. The

present study reports even higher levels of some of the metals (Pb, Ni, Cu, Zn, Fe, and Mn) metals in Ikpoba River. This report therefore suggest substantial increase in the levels of metal contamination of the river over time. This reported levels of the metals may not be unconnected to the increasing human and industrial activities in the sites the metals were measured. It may be conceivable that the increase in the levels of metals may have reduced fertility, mobility and increased mortality [29].

Heavy metals toxicity induces oxidative stress in fish by generating reactive oxygen species (ROS) through Fenton

chemistry and by donating electrons to oxygen. ROS in form of free radicals or toxic metabolites are major entities that encourage tissue and organ damage. Free radicals are generated by aerobic metabolism as well as when there is stress. Heavy metals are reported to stimulate release of free radicals which alter cellular characteristics like membrane

lipids. Since lipids are important constituent of organs/tissues, peroxidative damage to cellular membrane lipids and fatty acids may lead to membrane fragility and permeability, a likely consequence of oxidative stress [1, 2, 30].

Table 2. Metal analysis on sediment samples collected from different sites of Ikpoba River.

	Pb (ppm)	Ni (ppm)	Zn (ppm)	Mn (ppm)	Fe (ppm)	Cu (ppm)	Cd (ppm)
B(0–15) cm	10.00	14.00	231.00	72.00	27.59	0.04	<
(15–30) cm	60.00	27.00	211.00	70.00	27.82	0.02	<
C(0–15) cm	<	<	59.00	4.00	7.60	<	<
(15–30) cm	<	<	14.00	6.00	3.60	<	<
D(0–15) cm	<	<	119.00	28.00	21.62	5.24	<
(15–30) cm	<	<	42.00	18.00	20.00	2.00	<

Source: [23]

Values are means of determinations (n = 26), B = Abattoir; C = Guinness; D = Capitol, < = less than detection limit; DT = Detection Limit, DT for Pb = 0.08ppm; Ni = 0.05ppm; Zn = 0.005ppm; Mn = 0.03ppm; Fe = 0.05ppm; Cu = 0.005ppm; Cd = 0.01ppm.

Uric acid levels were significantly increased in all sites when compared with control (Tables 3 and 4) in all organs/tissues with fishes from capitol revealing marked increase in these activities. Activities of uric acid levels were not significantly (p>0.05) increased in African catfish (Table 3) from the sites when compared with Tilapia from same sites. Increase in uric acid in fishes from different sites of Ikpoba River may be attributed to the action of accumulated chemical

contaminants (i.e. heavy metals) on renal tubules that may cause disturbances and pathological changes in kidney functions [31]. It may also be due to its extracellular antioxidants properties which enable uric acid scavenge oxygen radicals once produced and forms stable complex with iron ions as a preventive antioxidant [30]. The observed increase may also be due to increased defense against possible contamination of Ikpoba River.

Table 3. Lactate dehydrogenase, oxidative stress enzymes, malondialdehyde and uric acid levels in organs/tissues of African catfish from three different sites of Ikpoba River and control.

Tissue/Organ	Group/Assay	A Control	B Abattoir	C Guinness	D Capitol
Brain	Catalase	2.72±0.15 ^a	7.64±0.80 ^b	11.09±0.20 ^c	15.75±0.42 ^d
	SOD	4.61±1.08 ^a	3.72±0.46 ^a	3.72±0.46 ^a	4.24±0.23 ^a
	Aldehyde Oxidase	0.31±0.08 ^a	0.47±0.08 ^a	0.46±0.35 ^a	1.12±0.20 ^b
	LDH	19.59±0.21 ^a	26.12±0.86 ^b	32.87±1.34 ^c	38.81±1.43 ^d
	MDA	3.05±0.20 ^a	4.97±0.25 ^b	17.26±0.33 ^c	19.67±0.36 ^d
	Uric Acid	7.73±0.28 ^a	14.89±1.19 ^b	15.63±0.30 ^{bc}	20.42±1.02 ^d
Gills	Catalase	3.58±1.73 ^a	0.03±0.01 ^b	3.00±0.92 ^{bc}	1.23±0.54 ^d
	SOD	4.58±0.59 ^a	1.33±0.54 ^b	4.43±0.46 ^{bc}	5.42±1.15 ^{ad}
	Aldehyde Oxidase	2.36±0.97 ^a	8.17±1.56 ^b	7.63±0.04 ^{bc}	9.82±1.98 ^{bd}
	LDH	27.54±0.79 ^a	28.93±4.09 ^{ab}	38.04±2.34 ^c	22.35±0.55 ^d
	MDA	11.49±1.47 ^a	18.73±3.76 ^b	16.40±5.46 ^c	27.52±1.48 ^d
	Uric Acid	1.09±0.02 ^a	24.61±9.69 ^b	32.73±6.31 ^c	21.67±0.36 ^d
Kidney	Catalase	8.45±0.12 ^a	5.65±2.34 ^b	3.08±0.73 ^c	5.04±1.26 ^{bd}
	SOD	4.25±0.17 ^a	14.15±8.09 ^b	4.40±0.50 ^a	3.74 ±1.02 ^a
	Aldehyde Oxidase	3.45±0.23 ^a	2.34±1.03 ^a	2.01±0.12 ^a	2.12 ±1.02 ^{ab}
	LDH	32.12±5.59 ^a	45.23±2.56 ^b	52.56±3.21 ^c	59.60±10.4 ^d
	MDA	10.83±0.64 ^a	3.22±0.33 ^b	9.03±1.30 ^c	8.00±2.33 ^d
	Uric Acid	1.46±0.53 ^a	7.33±4.34 ^b	12.49±3.26 ^c	9.56±1.72 ^d

Results are expressed as means ± SEM of determinations (n = 52). Means carrying different notations are statistically different at p < 0.05.

Table 4. Lactate dehydrogenase, oxidative stress enzymes, malondialdehyde and uric acid levels in organs/tissues of Tilapia from three different sites of Ikpoba River and control.

Tissue/Organ	Group/Assay	A Control	B Abattoir	C Guinness	D Capitol
Brain	Catalase	8.66±2.04 ^a	7.64±0.76 ^a	3.62±1.42 ^b	16.31±2.01 ^c
	SOD	3.93±1.16 ^a	1.69±0.33 ^b	2.93±1.67 ^a	3.82±1.44 ^a
	Aldehyde Oxidase	0.28±0.05 ^a	0.67±0.09 ^a	0.20±0.06 ^a	1.30±0.08 ^a
	LDH	57.01±2.10 ^a	78.70±2.68 ^b	75.90±2.73 ^b	81.92±4.73 ^c
	MDA	1.28±0.05 ^a	6.67±0.08 ^b	7.61±1.97 ^b	9.78±0.16 ^c

Tissue/Organ	Group/Assay	A Control	B Abattoir	C Guinness	D Capitol
Gills	Uric Acid	0.73±0.28 ^a	12.49±2.19 ^b	12.63±3.00 ^b	17.18±9.55 ^c
	Catalase	3.83±1.62 ^a	2.59±1.10 ^a	57.70±22.37 ^b	28.40±46.80 ^c
	SOD	3.72±1.11 ^a	1.27±0.30 ^b	1.94±0.16 ^b	5.12±1.02 ^c
	Aldehyde Oxidase	2.93±0.75 ^a	2.93±1.15 ^a	1.56±0.17 ^a	4.18±1.34 ^b
	LDH	70.38±2.58 ^a	108.70±2.68 ^b	110.90±9.73 ^b	99.92±0.73 ^c
	MDA	8.01±2.18 ^a	4.96±0.85 ^b	7.40±2.27 ^a	17.77±1.67 ^c
	Uric Acid	2.30±0.52 ^a	13.88±4.99 ^b	21.99±1.86 ^c	12.09±0.47 ^d
	Catalase	4.73±0.58 ^a	8.07±0.28 ^b	62.80±8.50 ^c	42.30±1.76 ^d
Kidney	SOD	3.13±0.36 ^a	23.55±11.78 ^b	5.56±0.23 ^c	3.89±0.11 ^a
	Aldehyde Oxidase	4.68±0.34 ^a	1.34±0.24 ^b	15.44±2.24 ^c	10.52±1.37 ^d
	LDH	67.72±2.59 ^a	89.70±2.68 ^b	92.90±9.73 ^c	121.92±9.73 ^d
	MDA	7.67±1.18 ^a	8.11±2.26 ^a	7.36±0.37 ^a	1.89±0.17 ^b
	Uric Acid	1.03±0.53 ^a	3.09±0.46 ^b	16.46±2.74 ^c	12.09±0.47 ^d

Results are expressed as means ± SEM of determinations (n = 52). Means carrying different notations are statistically different at $p < 0.05$.

Antioxidant enzymes activities were significantly altered in all sites when compared with control (Tables 3 and 4) in all organs/tissues with fishes from capitol revealing marked increase. CAT activity of kidney and gills were also markedly increased both in Guinness and Capitol sites respectively. Only brain SOD activity showed a decrease in all the sites when compared with control values. While gills SOD activity showed a decrease in abattoir and Guinness sites, kidney SOD revealed an increase. Activities of these enzymes were not as increased in African catfish (Table 3) from the sites when compared with Tilapia from the same site.

The most intracellular sensitive enzymatic index in cell stress brought about by ROS and oxidative stress is SOD and CAT activities [30]. The decrease observed in brain and gills SOD activities of both fish species may indicate toxic effects of ROS. Inhibition of some antioxidant enzymes in gill and brain of catfish may interfere with their biotransformation function and ultimately compromise the survival of the fish. The results show that contaminated river water exerted its toxic effects by promoting oxidative stress in the tissues/organs. This observation is in agreement with studies carried out by Adjene *et al.* [32] for efavirenz treated rats and the report of Abdelazim *et al.* [33] in the muscles of the fish Nile Tilapia treated with zinc oxide nanoparticles. Activities of antioxidant enzymes are significantly modified in organs/tissues during intoxication, where a decrease in activity may indicate oxidative modification of enzymatic proteins and/or decrease in synthesis rate. This study however, showed inconsistent alteration in activities of antioxidant enzymes and levels of other biomarkers in tissues studied (especially brains and gills). The data obtained from assay of SOD and CAT activities in brain and gills of African catfish and Tilapia from abattoir and Guinness site is in agreement with the report of Wilhelm *et al.* [34]. The apparent increase in activity of this enzyme could be a result of response to oxidative stress in order to mop up ROS induced by heavy metals detected in the sites [35, 36]. Antioxidant enzymes activities which showed marked

increase may be as a result of increased defense against possible contamination of Ikpoba river water. The increase may also have arisen as a result of the adaptive response to excess free radicals. The decrease observed may be due to enhancement of protein synthesis as a confounding factor, where it may be capable to inactivate enzymes [30, 36]. These confounding factors may explain the inconsistency in relative level of enzymes (both increase and decrease) that has been observed in this study.

Lactate dehydrogenase (LDH) activities were significantly ($p < 0.05$) increased in all sites when compared with control (Tables 3 and 4) in all organs/tissues with fishes from capitol revealing marked increase in these activities. Gills and kidney showed the most marked increase in LDH activity in fishes from the three sites. The high LDH activity may indicate elevated energy demand to overcome stress for growth and metabolic processes in the fishes as a result of contamination stress. The limitation or absence of oxygen as indicated in reduced biological oxygen demand, may have induced conversion of aerobic respiration to anaerobic respiration (fermentation) hence increase in the enzyme LDH [37].

Aldehyde oxidase (AO) activities were significantly increased in all sites when compared with control (Tables 3 and 4). However, kidney AO activity decreased in African catfish in all sites and Tilapia from Abattoir site when compared with other organs/tissues both in the Guinness and Capitol sites. Activities of enzymes were not as increased as in African catfish (Table 3) from the sites when compared with Tilapia (Table 4) from same sites. Kidney AO activity in Tilapia increased significantly when compared with other organs in Guinness and Capitol sites. Aldehyde oxidase possesses wide substrate specificity and plays important role in metabolism of environmental contaminants [38]. AO has been implicated in pathophysiology of alcohol liver injury, reperfusion tissue injury and synthesis of retinoic acid. Alteration of retinoic acid synthesis has been implicated in the etiology of degenerative diseases like Parkinson's disease

and schizophrenia. Since AO is a source of oxygen radicals, its increase in this study may contribute to increased production of ROS in the fishes of Ikpoba River. The increase in AO activity may be considered a more specific biomarker in monitoring environmental stress due to contamination because oxidative stress occurs when antioxidant enzymes defence is overwhelmed by increased production of ROS leading to peroxidation of lipids, proteins and DNA [13].

MDA levels were significantly increased in some sites when compared with control (Tables 3 and 4). This observation is in agreement with the report of Abdelazim *et al.* [33]. However, kidney of Tilapia in abattoir site and kidney of African catfish in all three sites observed significant decrease. Lipid peroxidation has been postulated to be the destructive process in cell stress due to H₂O₂ administration and failure of antioxidant defense mechanisms to prevent formation of excessive free radicals [30]. The increase in MDA levels in gills and brain of fishes from the different sites of Ikpoba River compared with fishes from control site may indicate lipid peroxidation. The decrease in MDA level observed in kidneys of both fish species may be the compensatory effects of antioxidant enzymes and uric acid defence. The alteration of significant and non-significant increase and decrease in MDA levels in the fish species from the different locations of this study on Ikpoba River may be indicative of deterioration of the river.

4. Conclusion

The results showed that a high but tolerable concentration of Pb, Zn, Cu, Mn, Fe and Ni may have exerted a little toxic effect on the fishes. However, the alteration in antioxidant enzyme activities and other biomarkers of oxidative stress, as markers of pollution, in the African catfish and Tilapia may indicate that the river is increasingly treated by pollutants and this may endanger the populations that depend on it. We recommend that effluents should be treated before they are discharged into Ikpoba River to protect the populations [aquatic organisms (fish) and final consumers of fish (humans)] that depend on it from the deleterious effects of further deterioration.

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Conflict of Interest

The authors declare that they have no conflict of interest.

Ethical Approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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