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Toxic metals (Cd and Pb) induced dysfunctioning of antioxidant system in marine fish *Sphyraena barracuda* (Edwards, 1771) collected from Kpeme, South of Togo

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Pollution of aquatic systems has become a recurring problem nowadays. The main goal of this study is to assess the impact of Cd and Pb on the antioxidant system of *Sphyraena barracuda* collected at Kpeme of South Togo. Two enzymatic biomarkers (catalase and glutathione-S-transferase) and two non-enzymatic biomarkers (malondialdehyde and glutathione) of oxidative stress were measured in various organs like liver, heart, gills and kidney of *Sphyraena barracuda*. The results indicated that stress was induced by Cd and Pb in these organs through lipid peroxidation and glutathione production. However, there was an alteration of the antioxidant system by low glutathione-S-transferase and catalase activities in the gills. Whereas, in other organs like heart, liver, and kidney, higher activity of glutathione-S-transferase and lesser activity of catalase was observed. From the results, it is very clear that Cd and Pb altered the antioxidant system of fish in comparison to the control samples.

[**Keywords**: Antioxidant systems, Cd and Pb, *Sphyraena barracuda*, Togolese phosphate]

Introduction

Anthropogenic environmental pollution represents a major problem in sustainable development. One of the major sources of pollution is industrial activity. The impacts of industries on the environment are diversified and include air pollution, due to dust or gaseous emissions, and soil and aquatic ecosystem pollution, caused by the release of effluents and solid waste[1]. In Togo, industrial effluents and solid waste are dumped into the sea at the lake region of Kepme by the SNPT (New Phosphate Treatment Society); these wastes contain trace elements such as Cd, Pb[2-4], and fluoride (Fig. 1). Bioaccumulation of xenobiotics in marine floral and faunal species is observed when these pollutants end up in the marine environment[5]. Cd and Pb contents observed in the soil, marine sediments, phosphate ores, seawater, and seafood from our study area are summarized in Table 1[(refs. 3,5-7)]. According to the previous studies, sludge weighing about 5100 tons was directly released into the marine environment, which contains 28 ppm and 2.3 ppm of Cd in dry form and liquid sludge, respectively[7]. The data thus express the degree of exposure of aquatic species to the pollutants in this area.

The marine waters of the Togolese coast are therefore subjected to intense pollution at Kpeme because of the presence of the toxic metals (Cd and Pb)[2-7]. It is clear that toxic metals are the most problematic of all contaminants because of their ubiquitous character in the biosphere, their high persistence, and high toxicity[8,9]. Being non-biodegradable, toxic metals are harmful to the environment and have the capacity to accumulate in living organisms[10]. Beyond the health risk on humans through the food chain, the alteration of aquatic organisms deserves special attention not only to protect species but also to contribute to food security. The SNPT phosphate waste disposal model therefore represents a danger through pollution of the marine ecosystem and subsequent harmful effects on marine organisms[2-5]. Toxic metals promote cellular oxidative stress in organisms by decreasing anti-radical protection and activating free radical production pathways[8,10]. Indeed, when the body functions well, there is an equilibrium between the mechanism of reactive oxygen species production (pro-oxidant system) and their elimination[8,10]. This equilibrium following exposure to environmental pollution can be broken in favor of the pro-oxidant system thus establishing an oxidative stress[10].

The numerous adverse effects of metals have led many researchers in both developed and developing

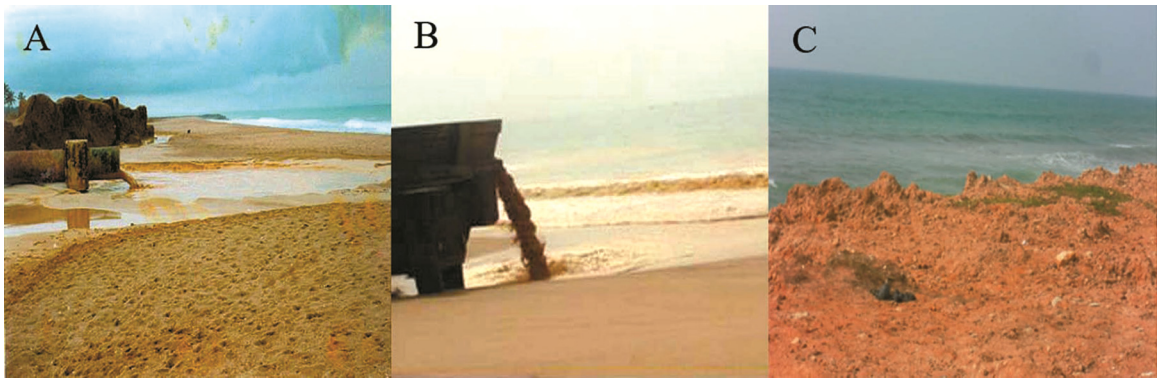

Fig. 1 — Reject of phosphate sludge (A and B) and wastes (C) into the sea at Kpeme

Table 1 — Cd and Pb content range in soil, phosphate ores, marine sediments, seawater, and seafood in the study area (ppm)

	Environmental Samples				
Pollutants	Soil	Phosphate sediments	Marine sediments	Seawater	Seafood
Cd content Range (ppm)	1.27-42.53[6]	2.00-09.00[3]	2.00-44.00[3,7]	3.50-12.00[7]	0.10-1.68[7]
Pb content Range (ppm)	2.58-16.05[6]	-	22.0-176.0[3,7]	0.33-6.97[5]	5.99-8.49[7]

countries to investigate the aquatic ecosystem contamination by toxic metal pollutants[11,12]. The adverse effects in the Togolese coastal ecosystem due to the bioaccumulation of toxic metals are not yet studied. However, in case of pollution, the effects observed at the level of the populations are manifested for a long duration of exposure and for a high concentration of the pollutants. Thus, the effect becomes identifiable at the human level in the impacted zones when the intoxication exceeds the remediation limits. In addition, the gradual decline in the quantities of fishery products raises concerns about the threat of marine fauna in this area in Togo.

The present work fits into this context and aims to evaluate the impact of the presence of toxic metals (Cd and Pb) on the antioxidant system of marine fish *Sphyraena barracuda* at Kpeme, where sludge, effluents, and solid waste are released into the sea after phosphate ore treatment. Fishes have been used for assessing the aquatic ecosystem quality, and thus can be used as bioindicators of aquatic ecosystem monitoring[13]. At the Togolese coast, the marine fish *S. barracuda* is very popular among consumers because of its good taste and less bone content with more flesh. Thus, the cadmium and lead contents in the organs such as heart, liver, gills, and kidney of *S. barracuda* were determined at Kpeme and Gbodjome. The biochemical impact of these metals was evaluated through enzymatic (glutathione-S-transferase and catalase) and non-enzymatic (glutathione and malondialdehyde) bioindicators of the aquatic environmental stress that are biological markers of aquatic pollution[14-16]. This evaluation assessed the integrity of the antioxidant system of *S. barracuda* affected by Cd and Pb.

Materials and Methods

Our area of study is situated to the south of Lome–Aneho national road, from the village of Gbodjome (west) to Kpeme at an approximate distance of 11 km. By its geographical location, this complex is part of the prefecture of the lakes, located at 66 m altitude and between 06°22'00" N and 01°40'00" E in the Togo maritime region. The geographical description of the two villages in this study is as follows: Kpeme (06°2'45.9" N and 01°30'16.1" E) is the site of the phosphate treatment plant. After this treatment, sludge and solid wastes are released into the sea and also on the land (Fig. 1); Gbodjome (06°11'39.6" N and 01°25'03.8" E) is a village located to the west at 10 – 11 km from Kpeme and it is not directly affected by the release of waste from the plant (Fig. 2). This locality is considered as control. The essential characteristic of this geographical space is that it houses the phosphate ore processing plant whose dust is discharged into the atmosphere, as well as waste and sludge are released into the sea and on soils/land which contain toxic metals (Cd and Pb) and fluoride (Fig. 1).

S. barracuda samples were collected from the sea at Kpeme (South of Togo) and were moved to the laboratory on the same day using ice-cold containers (0 – 4 °C). In order to minimize stress, the fish samples were kept alive for at least 24 hours. Fishes used as control samples were collected from

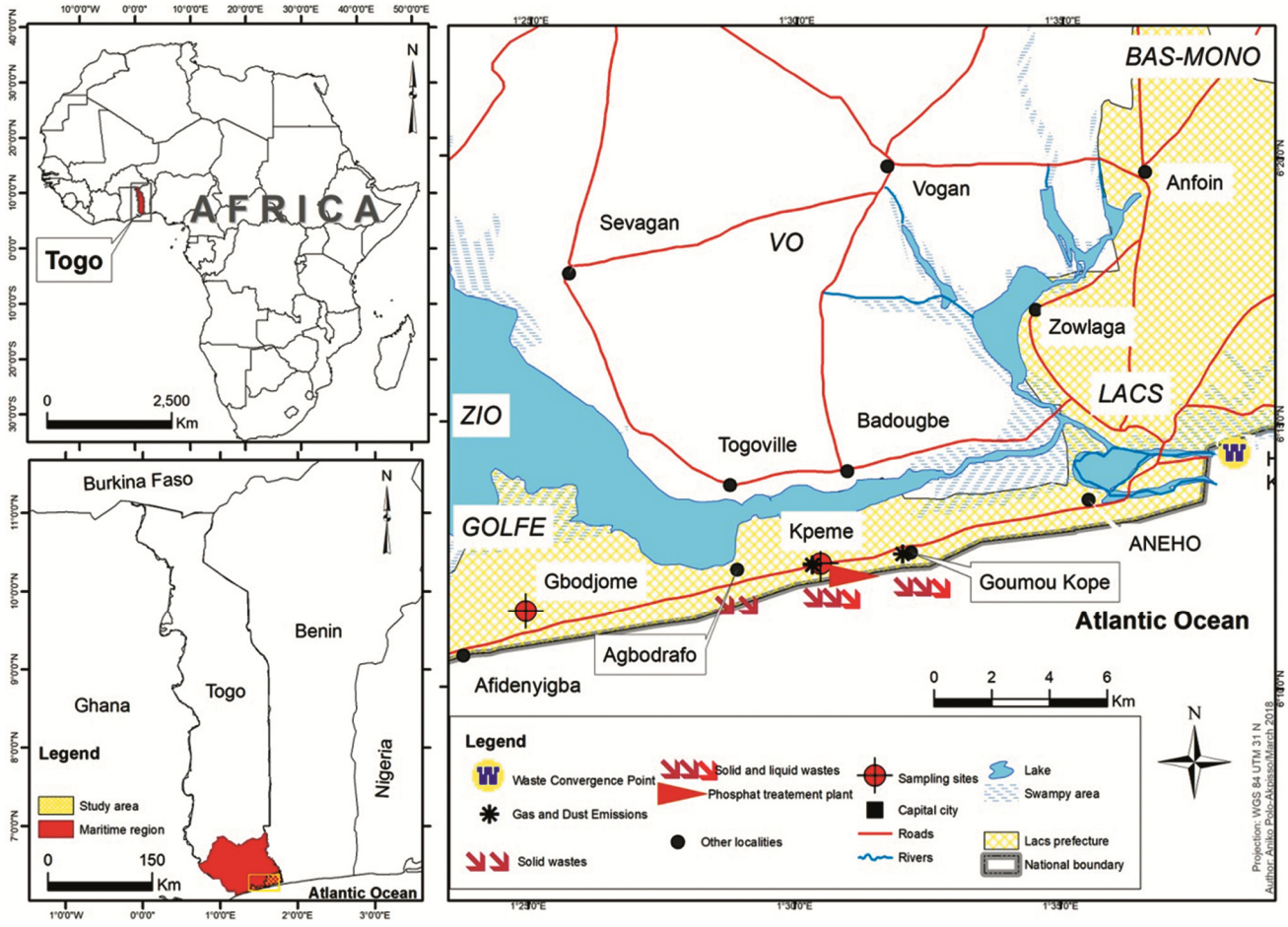

Fig. 2 — Map showing the study area with the prospected localities

Goumoukope locality which is not directly affected by the release of waste from the plant. The fish samples (20) weighed in the range of 457 – 529 g (Fig. 3). Samples of seawater were also collected at these two stations at the same time. Dissection of the fishes was performed to quickly remove the fish organs such as heart, liver, kidney, and gills, and the post-mitochondrial fraction was processed as follows: the organs were washed in ice-cold 1.15 % KCl solution, blotted, and then weighed. Then the samples were homogenized by four volumes of homogenizing buffer (50 mM Tris-HCl mixed with 1.15 % KCl and pH adjusted to 7.4), using Teflon Homogenizer. Centrifugation of the homogenate was perfomed at 10,000 g at a temperature of 0 – 4 °C for 20 min in a Beckman L5-50B centrifuge. The supernatant was decanted and stored at -20 °C for further analysis.

The Cd and Pb concentrations in the blood were determined by using a Bulk Scientific Atomic Absorption Spectrophotometer, AES, 2000 series. A mixture of air and acetylene was used as the flame. For quality control and quality assurance, standards for each of the metals were aspired into the flame in the order of 0.0 ppm, 0.8 ppm, and 1.6 ppm. A standard curve was plotted with the resultant values. Then, the tissue samples were aspirated into the flame, and the values were obtained by extrapolation from the standard curve[8].

The content of total protein in the samples was estimated using the method of Lowry *et al.*[17] by using a standard protein, here bovine serum albumin (BSA)[17]. The estimation of lipid peroxidation was made by measuring the thiobarbituric acid reacting substances (TBARS) as previously described[14]. Malondialdehyde (MDA) was quantified by using Σ = 1.56 x 105 M-1/cm[18].

Glutathione (GSH) was measured in the 10,000-g supernatant fraction of the heart, liver, kidney, and gills homogenates of *S. barracuda* as suggested by Jollow *et al.*[19] at 412 nm using 5,5'-dithio-bis-(2-nitrobenzoic acid) (DTNB)[19]. The activity of

glutathione-*S*-transferase (GST) was determined by adopting the method of Habig *et al.*[20] using 1-chloro-2,4-dinitrobenzene as a substrate[20]. The reaction mixture comprised of 1.7 ml of 100-mM phosphate buffer (pH 6.5) and 0.1 ml of 30-mM CDNB. A 5-min pre-incubation of the reaction mixture was performed at 37 °C; then the reaction was started by the addition of 0.1 ml diluted sample, and the absorbance was measured for 5 min at 340 nm. An enzyme-less reaction mixture was used as blank. The specific activity of glutathione-S-transferase is expressed as nmoles of GSH–CDNB conjugate formed/min/mg protein using an extinction coefficient of 9.6 mM^{-1} cm^{-1}. Activity of catalase (CAT) was analyzed according to the standard procedure (Greenwald[21]) by following the absorbance of hydrogen peroxide at 240 nm, with a pH 7.0, and temperature 25 °C[21].

All results were expressed as mean ± ESM and analyzed using the GraphPad Prism software version 7.0. The experimental samples were compared using the one-way ANOVA. When significant differences were observed ($P < 0.05$), the polluted areas were compared with the control using the Student's *t*-test. SAS (The SAS System for windows, v8; SAS Institute Inc., Cary, NC) was used to perform all the statistical analyses.

Fig. 3 — *Sphyraena barracuda* samples

Results

The concentrations of metals in the heart, liver, kidney, and gills of *S. barracuda* from the control station (Gbodjome) and Kpeme are tabulated in Table 2[(refs. 22-25)]. Varying levels of heavy metal accumulations in the organs of the fish were observed. Higher level of metal accumulation was observed in Pb followed by Cd in all the organs. The concentration trend of the metals in the organs is as follows: Pb) Liver > Kidney > Gills > Heart; and Cd) Liver > Gills > Kidney > Heart. The Cd and Pb concentrations in all the organs of fishes collected at Kpeme (Table 2)[22-25] were non-compliant with the acceptable limit for consumption by international standards (FAO, UNEP, and WHO)[22-25]. In addition, while comparing the fish from Kpeme with the control samples, the level of metals in the Kpeme fish organs were higher than the control samples (Gbodjome) (Table 2)[22-25]. Bioaccumulation factor also varied as follows: Pb) Liver > Kidney > Gills > Heart; and Cd) Liver > Gills > Kidney > Heart (Table 3).

The MDA formation levels in *S. barracuda* are illustrated in Figure 4. In all the organs of fishes, MDA levels were observed to be significantly higher in the Kpeme fishes compared to Gbodjome fishes.

Table 3 — Cd and Pb contents in seawater and bioaccumulation factor (BAF) of *Sphyraena barracuda* organs (ppm)

		Various Organs			
Metals	Content in seawater (ppm)	Liver	Kidney	Heart	Gills
Pb	Control : 0.33±0.02	09.09	-	-	03.03
	Test : 6.97±0.930***	108.17	90.10	53.08	80.34
Cd	Control : ND	-	-	-	-
	Test : 1.09±1.030	108.26	94.49	10.09	103.67

Contents are expressed as mean of 10 fishes ± SD; ***Significantly different from control, $P < 0.001$; ND = Not detected; and BAF determined through the ratio of the content in the organ to that of the seawater.

Table 2 — Cd and Pb contents in *Sphyraena barracuda* organs (ppm)

		Various Organs			
Metals	Stations	Liver	Kidney	Heart	Gills
Pb Acceptable limit : 0.29[22-25]	Control	0.03±0.008	ND	ND	0.01 ± 0.05
	Test	7.54±0.012***	6.28 ± 0.004	3.70 ± 0.001	5.60±0.004***
	% Change	25033	-	-	55900
Cd Acceptable limit : 0.05[22-25]	Control	0.01 ± 0.0002	ND	ND	0.01 ± 0.001
	Test	1.18±0.003***	1.03 ± 0.080	0.11±0.005	1.13±0.010***
	% Change	11700	-	-	11200

The results are expressed as mean of 10 fishes ± SD; ***Significantly different from control, $P < 0.001$; and ND = Not detected.

The concentration of MDA was considerably higher in the gills. The increase in lipid peroxidation percentage compared with control for liver, kidney, heart, and gills was 168 %, 93 %, 53 %, and 148 %, respectively. The concentrations of GSH in the various organs of *S. barracuda* are shown in Figure 5. A significant increase in the levels of GSH was observed in the liver, kidney, and heart, except the gills of fish from Kpeme compared to that of control fishes from Gbodjome. Increased percentages in GSH levels were observed in liver (71 %), kidney (73 %), and heart (43 %). A 39 % decrease in the GSH value was observed in the gills compared to control (Fig. 5).

The GST activity levels in the liver, kidney, and heart of *S. barracuda* from Kpeme were higher compared to control ($P < 0.001$), whereas there was a significant decrease in the GST activity level in the gills ($P < 0.001$). In the liver, kidney, and heart there was an increase in the level by 53 %, 55 %, and 39 %, respectively. A significant decrease (38 %) was seen in the gills ($P < 0.001$) of fish from Kpeme compared to that of control fishes from Gbodjome (Fig. 6). CAT activities were ($P < 0.001$) reduced significantly by 26 %, 21 %, and 37 % in the liver, kidney, and gills, respectively; and less significant ($P < 0.05$) reduction (09 %) was observed in the heart of *S. barracuda* from Kpeme in comparison to control fishes from Gbodjome (Fig. 7).

Discussion

Due to extended anthropogenic activities, the industrial effluents are considered as one of the prime sources of elevated levels of toxic metals including Pb and Cd in certain aquatic bodies[1,26]. Marine water is known to be polluted at Kpeme (South of Togo) due

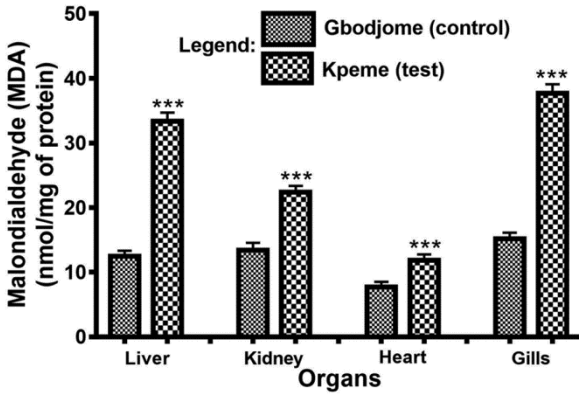

Fig. 4 — Levels of malondialdehyde (MDA) in the liver, kidney, heart, and gills of *Sphyraena barracuda* from the control station (Gbodjome) and Kpeme. Values are mean ± SD of 10 fishes. Significantly different from control ***$P < 0.001$

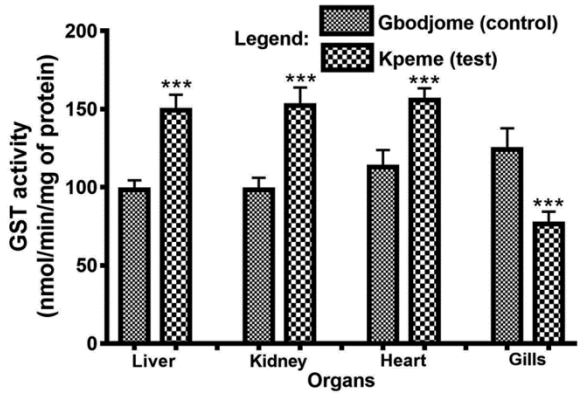

Fig. 6 — Activities of glutathione-S-transferase (GST) in the livers, kidney, heart, and gills of *Sphyraena barracuda* from the control station (Gbodjome) and Kpeme. Values are mean ± SD of 10 fishes. Significantly different from control ***$P < 0.001$

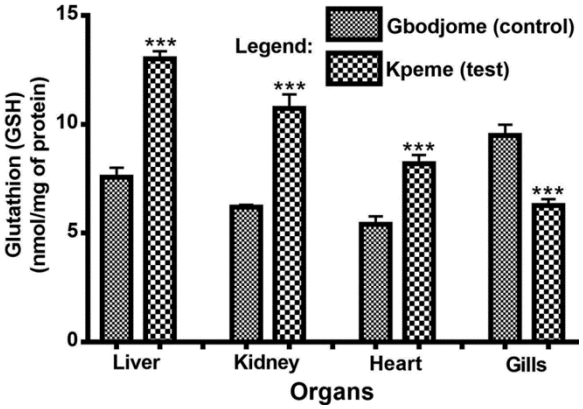

Fig. 5 — Levels of glutathione (GSH) in the livers, kidney, heart, and gills of *Sphyraena barracuda* from the control station (Gbodjome) and Kpeme. Values are mean ± SD of 10 fishes. Significantly different from control ***$P < 0.001$

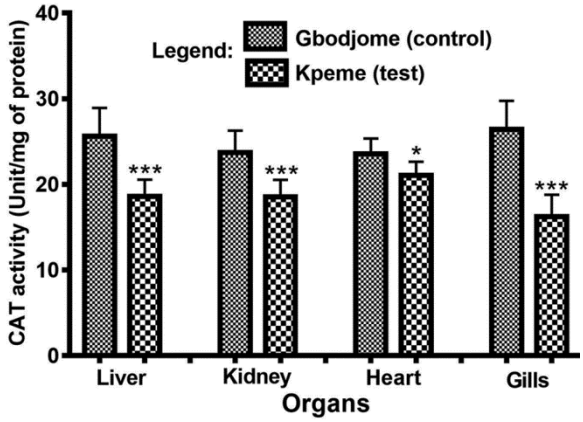

Fig. 7 — Activities of catalase (CAT) in the livers, kidney, heart, and gills of *Sphyraena barracuda* from the control station (Gbodjome) and Kpeme. Values are mean ± SD of 10 fishes. Significantly different from control ***$P < 0.001$; *$P < 0.05$

to increasing industrial activities of SNPT plant. Studies indicated the presence of toxic metals in Togolese phosphates[2-7]. It has also been shown that solid wastes and effluents discharged into the sea at Kpeme after phosphate treatment by the plant contain these trace metal elements. Subsequently, many authors reported the contamination of marine waters, sediments, and marine products with toxic metals to show the human health risk[5]. The impact of toxic metals on marine fauna in Kpeme has been neglected, and no research activity has focused on this impact. However, it is noted from year to year a drop in catch of fishery products in this zone and certain species become rarer[7]. Data are therefore lacking in relation to the biochemical impact of toxic metals pollution on marine fauna in this area. These data are important not only for the purpose of contributing to the preservation of species, but also to sustainable human development. The present study assessed the concentration of heavy metals in *S. barracuda* which is a commercially important fish in Togo and is preferred widely for consumption. Further, certain biomarkers of oxidative stress have been explored which can be used as surrogate biomarkers of aquatic pollution. The results are indicative of accumulation of high concentrations of Cd and Pb in the liver, kidney, heart, and gills of *S. barracuda* from Kpeme area which influences the lipid peroxidation induction and the antioxidant enzymes alteration in the organs of fish.

The level of Pb in the liver and kidney samples of fish were in the range from 6.28 ppm to 7.59 ppm. The liver and kidney contained the highest concentrations of these metals. Presumably, due to the presence of metal-binding proteins (metalothioneins and apoliprotein A-I), the liver and the kidneys possess the ability to accumulate heavy metals[27]. Similar concentrations of these metals in the liver and kidney of fish samples have been reported elsewhere[8]. In this study, the reported cadmium concentration is consistent and within the range reported in other studies which have evaluated metal contamination in fishes. For example, Asharaf[12] reported a 0.41-ppm concentration of Pb in the kidney of *Epinephelus microdon* fish from Arabian Gulf. In many previous studies, lower concentrations of Cd in kidney have been reported[8-9]; and this could be due to the decreased tendency of Cd species towards the available active sites (N- and/or O-donor atoms) in the kidney tissues to form tetrahedral or square planar Cd (II) complex species. The metal concentrations in all the organs of the fish were found to be higher than the permissible levels set by international standards for consumption[22-26]. The concentrations of Pb and Cd in the gills are explained by the physiological role of this organ in fish. Indeed, the gills come in first contact with the polluted water because of the branchial breathing. This result agrees well with *Clarias gariepinus* from Ogun river, polluted by industrial activities[8].

Our findings prove a significant elevation of lipid peroxidation in all the organs. Lipid peroxidation plays an integral role in the numerous pathologies in which oxidative stress is involved, occurring through a chain reaction that contributes to membrane damage in cells. In the present study, increased concentration of heavy metals observed in the various organs could be the reason for the increase in lipid peroxidation. It is well documented that metals catalyze the formation of reactive oxygen species (ROS) which are capable of damaging biological molecules such as DNA, proteins, and lipids[28]. Lipid peroxidation was greater in the gills and liver followed by kidney and was lesser in the heart. Peroxidation indicates the stress induced by pollutants in the organs. If this stress is significantly more in the gills, it is because they are in permanent contact with the polluted water. The importance of stress follows that of bioaccumulation of Cd and Pb. Further, in all the organs except the gills, the higher activity of GST and the redox-sensitive thiol compound GSH was noted.

The increase in GSH levels with concomitant elevation in the activity of GST in the organs is an indicative of adaptive and protective role of this biomolecule against oxidative heavy-metal-induced stress. The result of this study agrees well with the study from Panipat river in India where *Wallgo attu* fish was studied[28]. This variation of GSH and GST activity confirms that of MDA. The decrease in the levels of antioxidant enzymes, GSH, and GST in the gills could be reason for the good lipid peroxidation activity. Metal accumulation is higher in the gills, as they are the first line of contact with the contaminated water and have thin epithelial cells that can be easily penetrated[29]. Under acute oxidative stress, the toxic effects of the pollutants may easily overcome the antioxidant defenses[8].

The CAT system being one of the first line of defenses against oxidative stress, faces an increased activity when it is confronted by the environmental pollutants[8,11]. However, the activity of CAT in all the

organs was found to be decreased in this study. The flux of superoxide radicals, with their CAT activity inhibition properties, could be attributed to the decreased CAT activity. A similar CAT activity decrease has been reported earlier in *Cyprinidae* fish from Seyhan dam lake of Turkey[29], *Acipenser ruthenus* from the Danube river of Serbia, and in *Clarias gariepinus* from Ogun river in Nigeria[8].

Thus, lipid peroxidation allowed us to show that the presence of Cd and Pb in organs induces oxidative stress whose intensity is proportional to bioaccumulation. The GSH detoxification system shows that the gills are more overwhelmed by pollutants because of their role in branchial breathing. The activity of catalase, less expressed in this study, shows an inhibition of the antioxidant system due to the intensity of the bioaccumulation of toxic metals.

Conclusion

In summary, this study reveals higher accumulation of Cd and Pb in the liver, kidney, heart, and gills of *S. barracuda* from Kpeme, possibly due to the increased level of industrial pollution. This study also reports the biochemical dysfunction of *S. barracuda* due to the alterations in antioxidant enzyme activities and other biomarkers of oxidative stress. In addition, the results provide evidence for the use of enzymatic and non-enzymatic biomarkers of oxidative stress as sensitive indicators of aquatic pollution.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

MM & RR conceived and designed the experiments; and MM & AG conducted the experiment and collected the data. MM, RR & AG: Data analysis and wrote the paper; and MM & RR involved in data analysis and language editing.

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