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Effects of Simulated-Leachate on the Redox Status of Cellular System of *Clarias gariepinus*

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Abstract
Different pollutants, including leachates have been reported to affect physiological and morphological properties. In the present study, leachate was simulated from municipal open dumps and was used to contaminated water at various concentration (0, 5, 10, 15, 20) %v/v. Clarias gariepinus were exposed to the various concentrations of simulated-leachate and were designated groups A, B, C, D and E respectively. Fish in group A is control as they were not exposed to simulated-leachate. Reduced glutathione (GSH) malondialdehyde (MDA), catalase (CAT) and superoxide dismutase (SOD) were assayed in selected tissues. The concentration of GSH was significantly lower ($p<0.05$) in the liver, kidney and gills of fish exposed to varying concentrations of simulated-leachate (5, 10, 15, 20) %v/v) compared to the control. MDA concentration of tissues of fish exposed to simulated-leachate was significantly higher ($p<0.05$) compared to the control fish. Activities of CAT and SOD of tissues of fish exposed to simulated-leachate increased as the exposure concentration increased. These results show that simulated-leachate exposure resulted in oxidative damage in fish and suggest the role of free radicals in its mechanism of toxicity. The results also revealed that the gill is a redox-sensitive tissue like the kidney and liver.
Keywords: Simulated-leachate, redox, cellular system, *Clarias gariepinus*

Introduction
Waste is defined as any matter, whether solid, liquid, gaseous or radioactive, which is discharge, emitted or deposited in the environment in such volume, constituency or manner as to cause an alteration of the environment (1). Waste management in Nigeria is still at its infancy various governments have over the years set up environmental sanitation authorities to tackle waste problems but the problems seems not to have been touched at all (2). Therefore, the country is fast becoming a dump site for locally generated refuse and highly toxic imported wastes. Wastes is dumped everywhere in most of the cities in Nigeria-Ibadan, Lagos, P-Harcourt, Warri, Benin etc. waste disposal is the final placement where wastes are being dumped or processed which may be chemical deposit, toxic, radioactive household waste, plastic been it and approved place or method (3). The various ways of dealing with waste problems in Nigeria include open dumps, sanitary land-fill, incineration and the use of facility of processing waste into manure. Disposing of waste in open dumps is the most commonly practice in Nigeria. It is uncovered, unsightly and open to scavengers (4). Open dumps entails the disposal of wastes along corners in market fronts, in burrowed pits or in drainage system.

During the cause of managing waste through open dumps, it is deduced that leachate is being generated (5). It occurs as a result of heavy precipitation which percolates through the wastes that has been gathered in a dump site dissolving soluble waste constituents both toxic and non-toxic along its path. The leachate which collects undergoes aerobic and/or anaerobic degradation (6). Even in the process of managing waste through a facility to generate manure it is still detected that leachate is being generated after micro-organisms have acted on the wastes. Leachate quality varies throughout the operational life of the open dumps and long after its closure (7). During the early stages of waste degradation and leachate generation the composition is acidic and high in volatile fatty acids (8). The acid leachate may dissolve other components of the wastes, such as heavy metals. The leachate also contains high concentrations of ammoniacal nitrogen and has both a high organic carbon concentration and a biochemical oxygen demand (BOD) (2,9).

The contamination of aquatic ecosystem by leachate streams and runoffs from open dumps has gained increasing attention in recent decades. The acute and chronic exposure and accumulation of these pollutants can result in tissue burdens that produce adverse effect not only in the exposed organisms, but also consumer organisms including human beings; therefore, it seems essential to study detrimental effects of such hazardous pollutants so as to formulate the strategies for safe guarding aquatic organisms. In assessing the toxic effects of pollutants in aquatic

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organisms the use of redox parameters, such as enzymic and non-enzymic antioxidants, have become more useful in recent times, as a result of the intimate relationship between fish and its aqueous environment (10-15).
Clarias gariepinus are tolerant to a wide range of water and laboratory conditions and has detritivorous behavior. This means that the fish can be in contact with xenobiotics from different ways of interacting with algae from stone or sediment. These characteristics make this particular specie an interesting model for ecotoxicological and biochemical studies. Moreover, catfish are valuable bio-indicators of contamination because of their large distribution, being open swimmers, capacity to react against ecological pollution and food source for human. The present study, therefore, is to investigate the effect of leachate simulated from municipal open dumps on the redox status of cellular system of *C. gariepinus*.

Materials and Methods

Chemicals and solvents are of analytical grade and are products of Sigma-Aldrich Inc, St. Louis, USA.
Solid wastes were collected from the official dump site along Delta Steel Company (DSC) Expressway, Udu, Delta State, Nigeria. Leachate simulation was carried out following the ASTM method (2). The physicochemical properties of simulated-leachate were carried out following standard method (16) and Atomic Absorption Spectrophotometer was used for the determination of heavy metals. Microbial analysis involving isolation and identification of bacteria in the simulated-leachate was done using the procedure described by Olutola et al (17).
One hundred and fifty species of *C. gariepinus* with the mean weight of 67.9±5.8 g and standard length mean length of 21.4±3.9cm were used for the experiment. They were purchased from a reputable fish farm in Delta State, Nigeria. The fish were kept in transparent plastic tanks filled with dechlorinated tap water and made to acclimatize in laboratory conditions for two weeks. The experimental fish were managed in accordance with the guidelines for handling experimental animals. They were fed (3% w/w) with commercial feeds. Water quality was measured according to the method of APHA/AWWA/WEF (16). The temperature of the experimental water was 25.9±0.8°C, pH was 7.2±0.4 dissolved oxygen was 7.1±0.2 mg/L, free carbon dioxide was 5.7±0.3 mg/L and alkalinity was 106.4 mg/L. Water was changed every day.
Five plastic aquaria (56 x 28 x 28cm) with 30 L of dechlorinated water were contaminated with varying concentration of simulated-leachate, and designated as follows:
A: dechlorinated tap water free of simulated-leachate
B: water contaminated with 5% v/v simulated-leachate
C: water contaminated with 10% v/v simulated-leachate
D: water contaminated with 15% v/v simulated-leachate
E: water contaminated with 20% v/v simulated-leachate
After the period of acclimation, the fish were randomly distributed into the five plastic aquaria (A – E) ten fish per aquarium. Each of these treatments had three replicates. The control group of fish were kept in aquarium A while aquaria B-E contained the test group of fish reared in water contaminated with varying volumes of simulated-leachate. The experiment lasted for fifty-six days.
Afterwards, the fish were sacrificed by medullar transection (18); blood was obtained by severing the caudal peduncle and dissected within 3 min on ice. The blood was collected in non-heparinised bottles and centrifuged at 3,500 rpm for about 15 min using refrigerated centrifuge RC650s and the serum samples obtained were preserved at -8^{o}C until required for analyses. The liver, kidney and gills were quickly removed and the post-mitochondria fraction was prepared as follows: The kidney, liver and gills were washed in ice-cold 1.15% KCl solution, blotted and weighed. They were then homogenized in 4 volumes of homogenizing buffer (50 nM Tris – HCl mixed with 1.15% KCl and the pH adjusted to 7.4), using Teflon homogenizer. The resulting homogenate was centrifuged at 10,000 g for 20 min in a Beckman L5-50B centrifuge at 0-4°C. The resulting supernatant was decanted and stored -20^{o}C until further analysis.

Total Protein Estimation

The total protein content of the various fractions was estimated by the method of Lowry et al. (19), using bovine serum albumin as standard.

Biochemical Assays

MDA determination was based on method described by Bird et al. (20). MDA reacts with thiobarbituric acid to give a red complex which is measured spectrophotometrically at 535nm. The method described by Jollow *et al* (21) was used to determine reduced glutathione (GSH) concentration. The absorbance was read at 412nm. Catalase activity was determined according to the method of Sinha (22). The method is based on the fact that dichromate in acetic acid is reduced to chromic acetate when heated in the presence of H_2O_2 with the formation of perchloric acid as an unstable intermediate. The chromic acetate was then measured spectrophotometrically at 570nm. The activity of

superoxide dismutase (SOD) was determined by the method of Misra and Fridovich (23). This method is based on the ability of SOD to inhibit the autoxidation of epinephrine at pH 10.2. The absorbance was measured at 480nm.

Statistical Analyses

All numerical results were obtained from the five (5) groups (control and treated). Data obtained were presented as mean±SEM and subjected to statistical analysis using a one way analysis of variance (ANOVA) by employing the method of Steel and Torrie (24). Significant difference between the treatment means was determined at 95% confidence level using Duncan's Multiple Range Test (25).

Results

The concentration of GSH was significantly lower ($p<0.05$) in the liver, kidney and gills of fish exposed to varying concentrations of simulated-leachate (5, 10, 15, 20) %v/v) compared to the control (Group A) (Figure 1). It was also found that GSH concentrations in the tissues of fish exposed to 5%, 10% and 15% simulated-leachate representing groups B, C and D were not significantly different ($p>0.05$) from one another. However, at 20% simulated-leachate concentration, GSH concentrations of tissues of fish (Group E) were found to be significantly higher ($p<0.05$) than those of the fish exposed to lower concentrations of simulated-leachate.

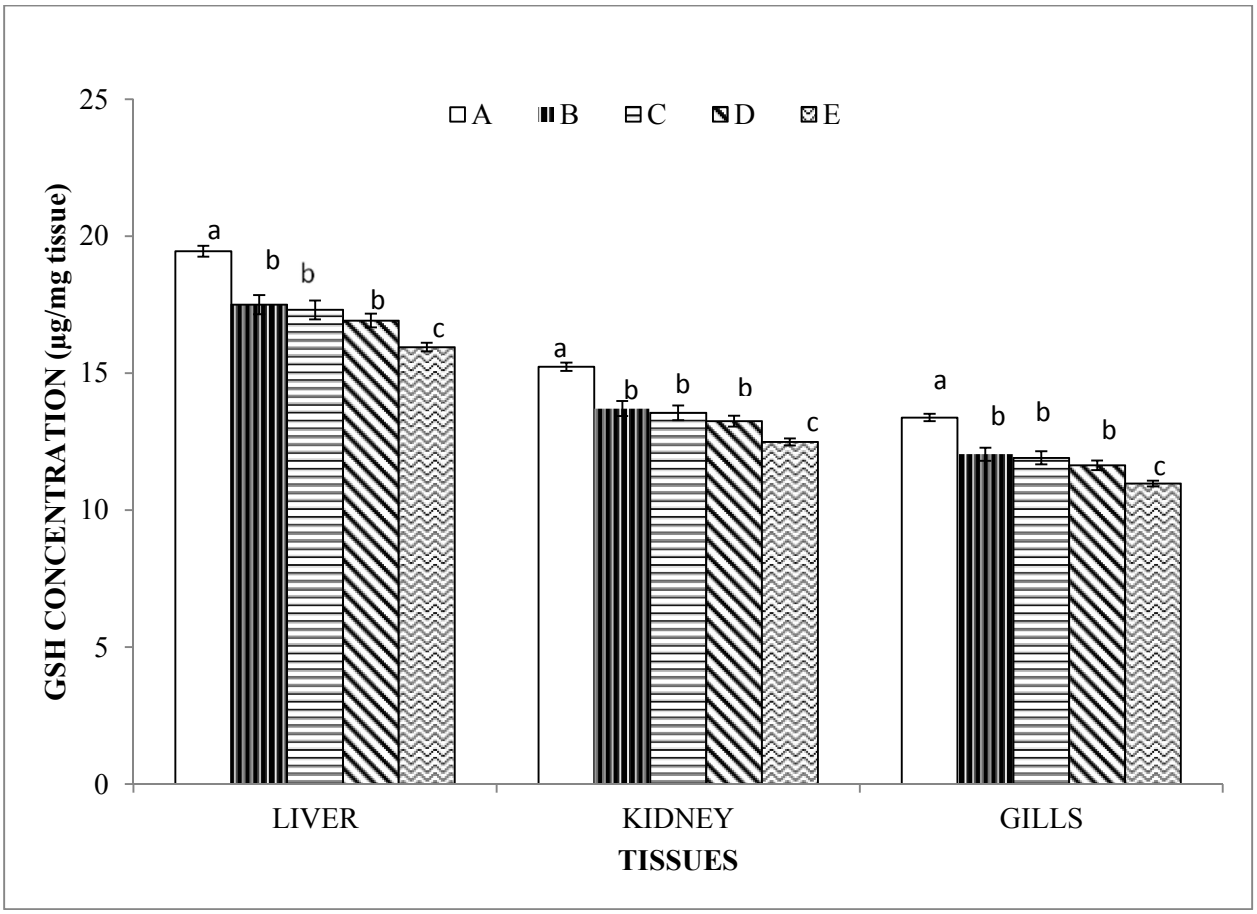

Figure 1: Concentration of reduced glutathione (GSH) (µg/mg tissue) of selected tissues of Clarias gariepinus cultivated in water contaminated with simulated-leachate. Bars bearing different superscripts are significantly different (P<0.05). Plotted data are means of three (3) determinations ± SEM.

Figure 2 presents concentration of MDA in the tissues of fish exposed to simulated-leachate. MDA concentration of tissues of fish exposed to simulated-leachate was significantly higher ($p<0.05$) compared to the fish in group A. The concentration of MDA of fish in group B (5%v/v) was not significantly different ($p>0.05$) compared to the fish in group A (Control). Conversely, MDA concentrations in tissues of fish exposed to higher concentration of simulated-

leachate (10%, 15% and 20%) were found to increase significantly ($P<0.05$) as the concentration of simulated-leachate increased.

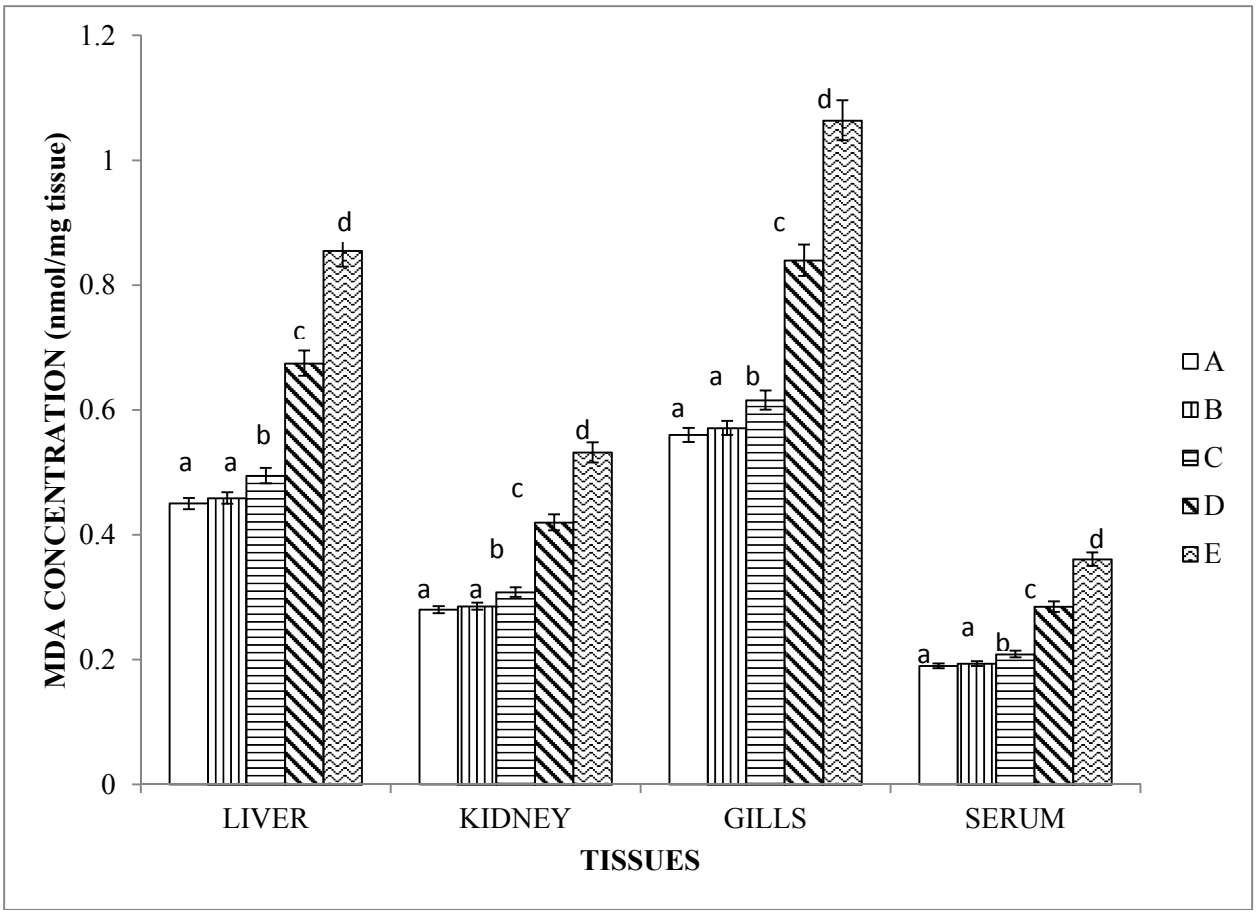

Figure 2: Concentration of malondialdehyde (MDA) (nmol/mg tissue) of selected tissues of Clarias gariepinus cultivated in water contaminated with simulated-leachate. Bars bearing different superscripts are significantly different (P<0.05). Plotted data are means of three (3) determinations ± SEM.

There was a significant increase ($p<0.05$) in CAT activity in the tissues of fish exposed to simulated-leachate at concentrations above 5% compared to fish in group A (Control) (Figure 3). Activity of CAT of tissues of fish exposed to simulated-leachate increased as the exposure concentration increased. The increase in activity of CAT of tissues of fish in group E, in particular, is about 34% relative to the control.

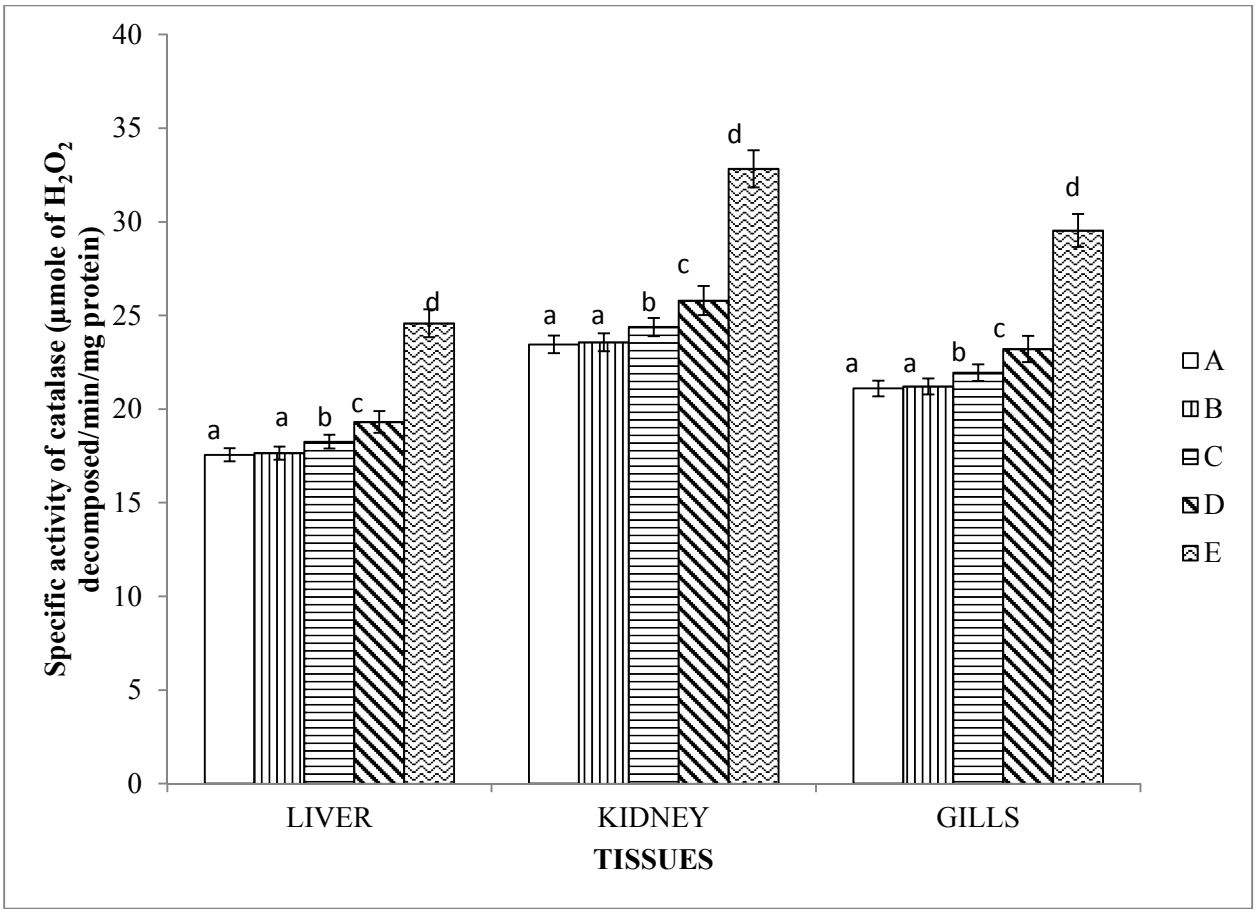

Figure 3: Specific activity of catalase (µmole of H_2O_2 decomposed/min/mg protein) of selected tissues of Clarias gariepinus cultivated in water contaminated with simulated-leachate. Bars bearing different superscripts are significantly different (P<0.05). Plotted data are means of three (3) determinations ± SEM.

Table 1 shows the activity of SOD of tissues of fish exposed to simulated-leachate. There was a significant increase ($p<0.05$) in the activity of SOD of liver and gills of fish exposed to simulated-leachate at concentration above 10%. However, SOD activity of kidney of fish exposed to 10%v/v simulated-leachate (Group B) was found to be significantly higher ($p<0.05$) compared to the control.

Table 1: Specific activity of superoxide dismutase (SOD) (Unit/mg protein) of selected tissues of *Clarias gariepinus* cultivated in water contaminated with simulated-leachate

Group	Liver	Kidney	Gill
A	12.34 ± 0.50^{a}	0.87 ± 0.02^{a}	9.38 ± 0.54^{a}
B	12.59 ± 0.48^{a}	0.89 ± 0.03^{a}	9.57 ± 0.62^{a}
C	13.57 ± 0.52^{a}	0.96 ± 0.02^{b}	10.32 ± 0.77^{a}
D	18.51 ± 0.67^{b}	1.31 ± 0.04^{c}	14.07 ± 0.58^{b}
E	22.21 ± 0.66^{c}	1.57 ± 0.03^{d}	16.88 ± 0.92^{c}

Values in the same column bearing different superscripts are significantly different ($P<0.05$). Tabulated data are means of three (3) determinations ± SEM

Discussion

Earlier documented report on the effect of simulated-leachate on rat cellular system revealed that it could lead to oxidative damage (26), ill health particularly anaemia (27), damage kidney cells and impair renal function (28). The first documented report on the effects of pollutants contaminated *Clarias gariepinus* on growth and haematological properties of rats revealed evidence of tissue damage and increased energy demand as the main mechanism of stress

induced by contaminated water (29). The present study, however, attempts to elucidate effects of simulated-leachate on the redox status of *Clarias gariepinus*.

The usefulness of glutathione in fish for evaluating the detoxification of xenobiotics has been reported by Adeyemi (30). In vitro examinations proved that the free thiol group of glutathione reacts with xenobiotics to form conjugates. The conjugates reveal toxic properties. Glutathione (GSH) is a metabolizing phase II enzyme involved in the biotransformation of xenobiotics (31) and as a redox sensitive thiol compound, GSH has a protective role against noxious chemicals and is known to be a substrate for the activity of GST (32). Glutathione is responsible for the regulation of intracellular levels of lipid peroxidation and also act as a reactant in conjugation with electrophilic substances, therefore a change in GSH level may be a very important indicator of the detoxification ability of an organism (33). In addition to being a necessary cofactor for GST activity, GSH is an effective protectant capable of detoxifying oxyradicals (34). Evidence from various pathological and toxicological conditions such as chemical induced oxidative injury, aging and degenerative disease indicate that GSH is a primary component of the protection system of cells against oxidative and free radical damage (32). The apparent significant decrease ($p<0.05$) in GSH levels of tissues of fish exposed to simulated-leachate in this study (Figure 1) suggests a condition of oxidative stress induced by the simulated-leachate or its metabolites.

Lipid peroxidation is one of the major mechanisms involved in oxidative cell injury and an increase in Malondialdehyde (MDA) level is frequently observed during oxidative stress and has generally been used as a marker of oxidative damage (35). It is also a major oxidation product of peroxidized polyunsaturated fatty acids and increased MDA content can be related to degradation of an environment due to poor water quality (36). The significant increase in lipid oxidation (MDA) may indicate the susceptibility of lipid molecules to reactive oxygen species and the extent of oxidative damage imposed on these molecules. The increased MDA levels of tissues of fish found in this study (Figure 2) lend credence to the data on GSH which portends that antioxidants defenses are impaired or overcome. Oxidative stress may exert effects on biomolecules like proteins, lipids and DNA. This may apparently explain the significant increase in the MDA activity recorded in this study.

The significant increase ($p<0.05$) in the activities of catalase (CAT) (Figure 3) and superoxide dismutase (SOD) (Table 1) found in this study laid to rest any doubt that may arise from the earlier submission that simulated-leachate affects the redox status of *C. gariepinus* by inducing oxidative stress. SOD is a group of metalloenzymes that play crucial antioxidant role and constitute a defense system against natural and chemical pollutants by catalyzing the dismutation of the highly reactive superoxide anion radical (O^{2-}) which is an important agent of oxygen toxicity to the less reactive species H_2O_2 (37). CAT is a peroxisomal haemoprotein that catalyzes the removal of H_20_2 formed during the reaction catalyzed by SOD. Earlier studies established that antioxidants enzymes are induced during conditions of oxidative stress as a defense mechanism (11,38). Elevated levels of antioxidant defense enzyme systems (SOD and CAT) observed in this study may be due to the fact that under oxidative stress, the toxic effects of pollutants may trigger the production of antioxidant defenses to overcome stressful conditions generated by such pollutants.

Conclusion

The results of this study clearly show a relationship of simulated-leachate exposure and induction of antioxidant enzymes (CAT, SOD), elevation of levels of lipid peroxidation product (MDA) as well as depletion of reduced glutathione (GSH). It further indicates that exposure of *C. gariepinus* to simulated-leachate induced oxidative stress which overwhelmed the antioxidant defense of the fish. The results also revealed that the gill, which is the first point of contact with environmental pollutants, is a redox-sensitive tissue like kidney and liver.

Conflict of interest

The author declares that there are no conflicts of interest.

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