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Olusosun Landfill Leachate Induced Oxidative Stress in African Cat Fish, *Clarias gariepinus*.

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Abstract

An understanding of the toxicity of landfill leachate is crucial to know their potential effects upon human health and the ecosystems. In this study the effects of Olusosun landfill leachate (OLL) from Ojota in Lagos State, Nigeria on oxidative stress indices in African cat fish, *Clarias gariepinus* was investigated. Thirty *Clarias gariepinus* were equally divided into five groups. Control groups were exposed to fresh water and fish in the other groups were exposed to 10%, 20%, 40% and 60% OLL respectively for a period of seven days. Levels of malondialdehyde (index of lipid peroxidation), reduced glutathione, hydrogen peroxide generation and activities of Glutathione-S-transferase (GST), Glutathione peroxidase (GPX), Catalase and Superoxide dismutase (SOD) were determined in the Post Mitochondrial Fractions (PMFs) of the liver, kidney and gills of the fish. From the results, significant increase in lipid peroxidation, hydrogen peroxide generation, Catalase and GST activities were observed in all the organs of the fish exposed to OLL, however a decrease in reduced glutathione concentration was observed in these organs. An increase in SOD activity was observed in the liver and kidney of the fish but was depleted in the gills. In conclusion it may be inferred from this study that the significant induction of lipid peroxidation, hydrogen peroxide generation and alterations in antioxidant status in the organs of *Clarias gariepinus* in response to Olusosun landfill leachate exposure indicate that the leachate induced oxidative stress in the fish. This toxic effect may be passed on to man through the food chain

Keywords: Leachate, Oxidative Stress, Toxicity, *Clarias gariepinus*

Introduction

Wastes are usually disposed in landfills and open dumps in Nigeria. Most of the landfills in Nigeria are located in residential areas without regard to safety and health hazards. Infiltration and percolation of rainfall, groundwater, run off or flood water into and through the waste layers of an existing or abandoned landfill site generate leachate (Kjeldsen *et al.*, 2002). Leachate generated from landfills could cause environmental pollution, developmental birth anomalies and pollution of surface and groundwater sources (Dolk *et al.*, 1998; Elliot *et al.*, 2001; Longe and Balogun, 2010). Leachate contain a range of chemical compounds, which may leach to the groundwater and pose serious risks to ecosystems and human health if the chemicals migrate to surface waters or to drinking water sources (Oshode *et al.*, 2008). The contamination of fresh water systems with a wide range of pollutants including leachates has become a matter of concern over the last few decades (Canli *et al*., 1998). Movement of landfill leachate into ground and surface waters is of primary concern for aquatic ecosystems. Aquatic organisms may become naturally exposed to leachate as a result of surface leachate break-outs or through contamination of groundwater in connection with the water bodies in which they live (Dewhurst *et al.*, 2003; Slack *et al.*, 2007). Chemical pollutants contained in the leachate could be assimilated by any aquatic species, and may pass through the food chain and bioaccumulate over long-term exposure (Sanchez-Chardi and Nadal, 2007; Sanchez-Chardi *et al.*, 2007 and Long *et al.*, 2010). Fish and sea foods are a major source of human exposure to contaminants from

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runoffs and leachate (USEPA, 2002). Fish should be a major test organism in ecotoxicological studies because of their link to man in the food chain. Of all aquatic species, fish are particularly sensitive to waterborne contamination and are recognized as bioindicators for water quality monitoring.

Leachates have been shown to be toxic to aquatic organisms including fishes. Leachate samples from six municipal solid waste landfills in the United States were shown to be toxic to *Ceriodaphnia dubia* (Ward *et al.,* 2002). Landfill leachate collected from a closed industrial waste site in the United Kingdom was also shown to be acutely toxic to freshwater crustaceans, amphipod *Grammarus pulex* and isopod *Asellus aquaticus* (Bloor *et al.,* 2005). Olivero-Verbel *et al.* (2008) reported the acute toxicity of leachates collected from a municipal solid waste landfill in Colombia to Brine Shrimp *Artemia franciscana*. Osaki *et al.*, 2006 determined the toxic potency of landfill leachates to the larvae and adult of Japanese Medaka (*Oryzias latipes*) and Sisinno *et al.*, 2000 also evaluated toxicity of municipal dump leachate using zebrafish (*Brachydanio rerio*).

Unfortunately published work on toxicity of landfill leachates using fish in Nigeria is scanty. Little or no attention is given to the issue of contaminated fishes, despite the fact that most of the ponds where fishes are cultivated are contaminated by landfill leachate (Adeyemi *et al.*, 2007a). People, oblivious of this risk, consume fish ad libitum regardless of the source of cultivation. Therefore, all manners of clinical manifestations (such as kidney failure, increased incidences of cardiovascular diseases), which were not rampant before are now common clinical cases. *Clarias gariepinus* which is a source of food to Nigerians is cultivated by many for commercial and subsistence purposes. Most water sources where *Clarias gariepinus* is cultivated are contaminated by landfill leachate and also serve as recipients to runoffs from open dumps where domestic and industrial wastes are deposited (Alegbeleye *et al.*, 1991).

To the best of our knowledge, there is no report so far on the effect of Olusosun landfill leachate on markers of oxidative stress in *Clarias gariepinus.* This study reports for the first time the effect of Olusosun landfill leachate on oxidative stress indices in the organs of African Catfish, *Clarias gariepinus*.

Materials and Methods Chemicals

Adrenaline, 1-chloro-2, 4-dinitrobenzene (CDNB), 5', 5'-dithiobis-2-nitrobenzoic acid (DTNB), reduced glutathione (GSH), hydrogen peroxide (H_2O_2), Thiobarbituric acid (TBA), were obtained from Sigma Chemical Company, (USA). All other chemicals were of analytical grade.

Study Area and Leachate Sampling

The sampling site/study area is Olusosun landfill site situated in Ojota Area, Northern part of Lagos State, Nigeria. Its geographical coordinates are 6° 35' 16" North, 3° 22' 56" East. Olusosun landfill is the biggest among the three landfills in Lagos State and started operation in the year 1992. Olusosun landfill site is the deposit of household and industrial wastes generated in Lagos State. It is about 42 hectares in size with a life span of 35 years and receives an average of 1.2 million tons of waste annually (Lasisi, 2011). Raw leachate was collected from leachate wells and thoroughly mixed. The leachate sample was then filtered to remove debris and stored until use. The sample was designated Olusosun Landfill Leachate (OLL).

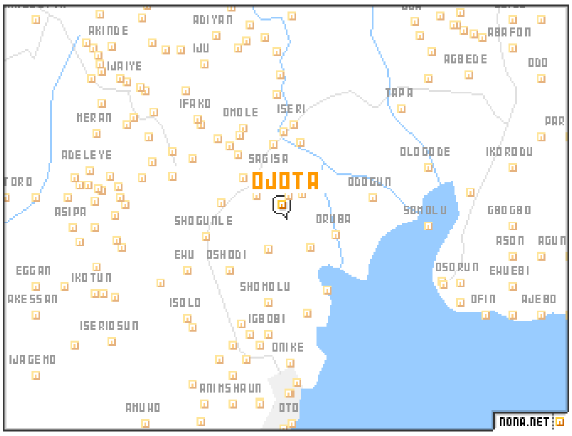

Figure 1: Map of the study area

Assessment of the physicochemical parameters and heavy metals of Olusosun Landfill Leachate

The leachate sample was analyzed for standard physico-chemical properties, including pH, total alkalinity, total acidity, total hardness, biological oxygen demand (BOD), chemical oxygen demand (COD), dissolved oxygen (DO), and phosphates according to American Public Health Association Standard methods for the examination of water and wastewater (APHA, 1998). The levels of metals such as copper, lead, iron, cadmium, chromium, nickel, manganese, mercury, arsenite and cobalt were also analyzed according to the American Public Health Association Standard methods for the examination of water and wastewater (APHA, 1998) and United

States Environmental Protection Agency Method (USEPA, 1996)

Animal Exposure and Experimental Design

Thirty *Clarias gariepinus* (450g-500g) obtained from a standard fish farm, Durantee fisheries in Ibadan, Oyo State, Nigeria were divided into five groups with six fish in each group. Group 1 (control) animals were exposed to fresh water for seven days. Groups II-V animals were exposed to 10%, 20%, 40% and 60% OLL respectively for seven days. The fish were sacrificed on the 8th day and the liver, gills and kidney were removed. The organs were washed in ice cold 1.15% KCl solution, blotted and weighed. They were then homogenized in 4 volumes of homogenizing buffer (50mM Tris-HCl mixed with 1.15% KCl and pH adjusted to 7.4), using a Teflon homogenizer. The resulting homogenate was centrifuged at 12,500g for 10 minutes in a Beckman L5-50B centrifuge at 4°C to obtain the post mitochondrial supernatant fraction that was used for biochemical assays.

Biochemical Assays

Glutathione (GSH) level was determined in the post mitochondrial fraction of the liver, gills and kidney of the fish according to Jollow *et al*. (1974) at 412 nm using 5, 5-dithio-bis-2-nitrobenzoic acid (DTNB), Glutathione peroxidase (GPx) activity was determined according to the method of Rotruck *et al*., 1973, Glutathione *S*-transferase (GST) activity was determined by the method of Habig *et al*. (1974) using 1 chloro 2, 4 dinitrobenzene as substrate. The specific activity of glutathione S-transferase is expressed as nanomoles of GSH-CDNB conjugate formed/min/mg protein using an extinction coefficient of 9.6mM-1cm-1. Superoxide dismutase (SOD) activity was determined by the method described by Misra and Fridovich (1972). Activity of catalase (CAT) was determined according to the method of Sinha (1972). Lipid peroxidation, measured as malondialdehyde (MDA) was determined according to the method described by Farombi *et al*. (2000).

Statistical Analysis

Results were expressed as Mean ± Standard Deviation. Student's t test was used to determine differences between groups. Levels of statistical significance were determined by analysis of variance (ANOVA), using microcal origin 6.0 software and p-values < 0.05 were considered significant.

Results

Physicochemical Characteristics and Concentration of Heavy Metals in Olusosun Landfill Leachate

The physicochemical characteristics and concentration of heavy metals detected in OLL are shown in tables 1 and 2. The results shows that certain sample-constituents and heavy metals were present in the leachate at concentrations beyond the permitted limits set by international regulatory authorities and the physicochemical parameters of the leachate sample were not in compliance with regulatory standards (FEPA, 2001 and USEPA, 1996).

TABLE 1: PHYSICOCHEMICAL PARAMETERS OF OLL

PARAMETERS	LEACHATE SAMPLE	FEPA	USEPA
PH	4.6	6-9	6.5-8.5
Total Acidity	476	200	-
Total Alkalinity	628	250	20
Total Hardness	121.4	-	0.75
BOD	205.6	50	-
COD	425.2	-	410
Dissolved Oxygen	64.8	2000	500
Colour	Dark brown	-	-

*All values are in mg/l except pH
*FEPA- Federal Environmental Protection Agency permissible limits for drinking water
*USEPA-United States Environmental Protection Agency permissible limits for drinking water
*BOD: Biochemical oxygen demand, COD: Chemical oxygen demand.

TABLE 2: Concentration of Heavy Metals detected in OLL

PARAMETERS	LEACHATE SAMPLE	FEPA	USEPA
Copper	6.2	0.3	1.0
Lead	13.2	0.01	0.015
Cadmium	0.36	0.05	0.05
Arsenite	0.12	-	-
Cobalt	0.44	-	-
Chromium	0.26	0.05	0.1
Mercury	0.41	0.1	-
Nickel	0.003	<0.1	-
Iron	0.07	0.05	0.3
Zinc	0.21	<0.1	-

*FEPA- Federal Environmental Protection Agency permissible limits for drinking water

*USEPA-United States Environmental Protection Agency permissible limits for drinking water

Lipid Peroxidation

Figure 2 shows the levels of malondialdehyde (MDA) in the organs of *Clarias gariepinus* exposed to Olusosun landfill leachate (OLL). Malondialdehyde level increased in a concentration dependent manner in the liver, kidney and gills of the fish following exposure to OLL showing that lipid peroxidation occurred in these organs. There were 11.1%, 13.1%, 43.6% and 53% increases in MDA in the liver; 8.0%, 37.6%, 40.0% and 55.2% increases in the kidney and 19.5%, 24.8%, 54.8% and 74.9% increases in the gills following exposure to 10%, 20%, 40% and 60% OLL respectively when compared with control.

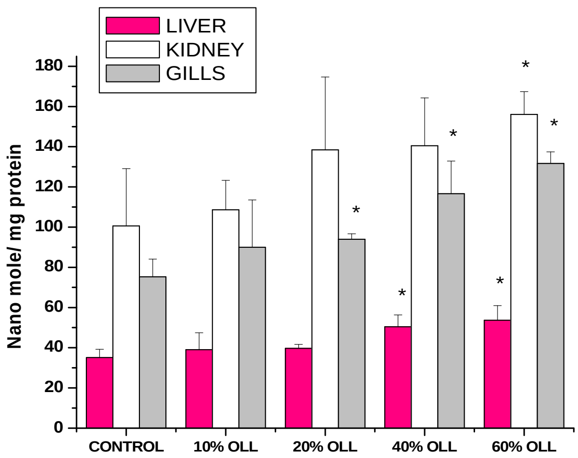

Figure 2: Effect of Olusosun landfill leachate on MDA level in the organs of *Clarias gariepinus*. Significantly different from control,* p<0.05.

Hydrogen Peroxide Generation

Concentration of hydrogen peroxide generated in the organs of *Clarias gariepinus* after exposure to OLL is shown in figure 3. Exposure to OLL caused an increase in hydrogen peroxide generation in the organs of *Clarias gariepinus* when compared with control. There were statistically significant (p<0.05, p<0.001) increases in hydrogen peroxide generation in the kidney and gills of the fishes following exposure to 10%, 20%, 40% and 60% OLL but the increase in hydrogen peroxide generation in the liver was not statistically significant (p>0.05) except at 60% leachate concentration.

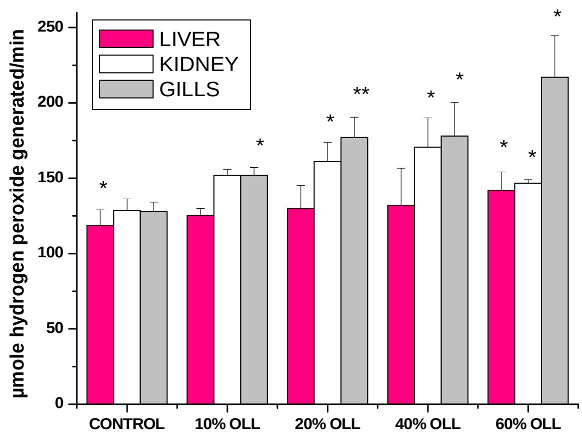

Figure 3: Effect of Olusosun landfill leachate on H_2O_2 generation in the organs of *Clarias gariepinus*. Significantly different from control,* p<0.05, **p<0.001.

Glutathione Level

Figure 4 shows the levels of Glutathione (GSH) in the organs of *Clarias gariepinus* after exposure to OLL. Exposure to OLL significantly (p< 0.05) depleted GSH in the liver, kidney and gills of the fish when compared with control. Glutathione level decreased by 8.8%, 33.9%, 34.2% and 41.7% in the liver; 9.9%, 28.5%, 30.5% and 37.1% in the kidney; 9.5%, 45.7%, 50.2% and 53.2% in the gills after exposure to 10%, 20%, 40% and 60% OLL respectively.

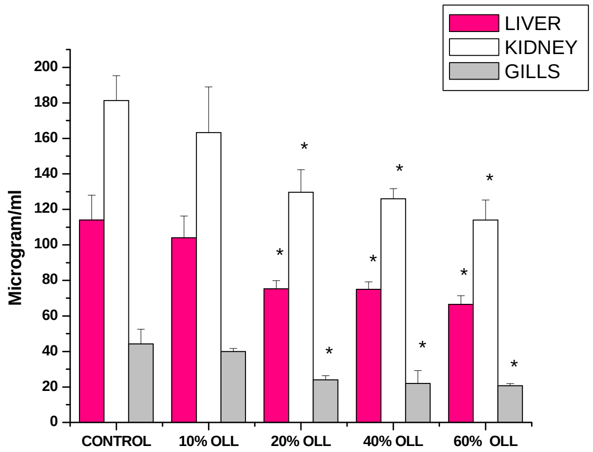

Figure 4: Effect of Olushosun landfill leachate on glutathione concentration in the organs of *Clarias gariepinus.* Significantly different from control,* p<0.05;** p<0.001.

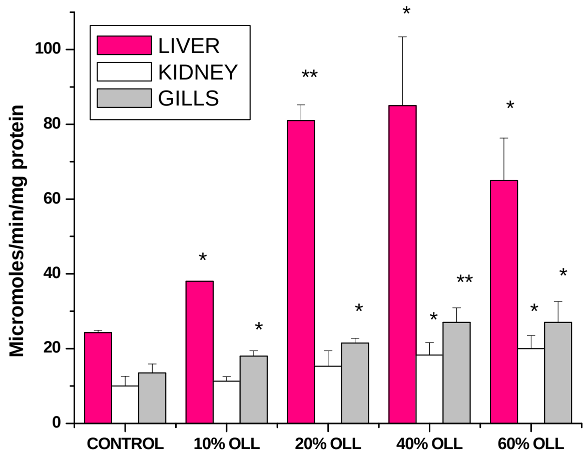

Figure 5: Effect of Olusosun landfill leachate on glutathione-S-transferase (GST) activity in the organs of *Clarias gariepinus.* Significantly different from control,* p<0.05;** p<0.001.

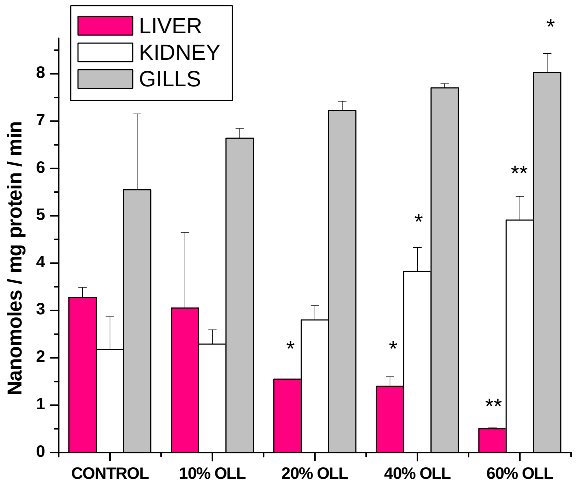

Figure 6: Effect of Olusosun landfill leachate on glutathione peroxidase activity in the organs of *Clarias gariepinus*. Significantly different from control,* p<0.05, ** p<0.001.

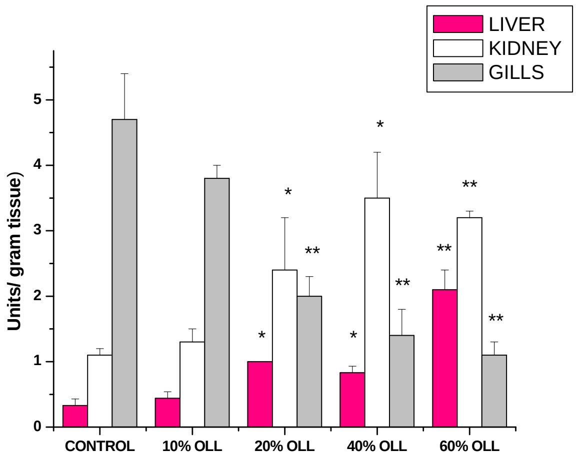

Figure 7: Effect of Olusosun landfill leachate on superoxide dismutase (SOD) activity in the organs of *Clarias gariepinus*. Significantly different from control,* p<0.05;** p<0.001.

Antioxidant Enzymes

There was induction of Glutathione-S-Transferase activity (figure 5) in the liver, kidney and gills of the fish following exposure to OLL. The enzyme's activity increased significantly (p<0.05, p<0.001) in the liver by 56.4%, 233.3%, 249.8% and 167.5%; in the gills by 33.3%, 59.3%, 100% and 100% and in the kidney by 13.0%, 53.0%, 83.0% and 100% after exposure to 10%, 20%, 40% and 60% OLL respectively. Glutathione Peroxidase activity was depleted in the liver and kidney of the fish but was induced in the gills ((figure 6). There were statistically significant increases (p<0.05, p<0.001) in Superoxide Dismutase activity in the liver and kidney and a statistically significant (p<0.001) decrease in the gills of the fish after exposure to OLL when compared with control (figure 7). There was a significant (p<0.05) and a concentration dependent induction of catalase activity in the liver, kidney and gills of the fish after exposure to OLL (figure 8). There were 2.6%, 26.3%, 32.1% and 44.9% increases in catalase activity in the liver; 8.3%, 27.3%, 35.6% and 46.2% increases in the kidney and 18.5%, 26.1%, 33.2% and 50.2% increases in the gills after exposure to 10%, 20%, 40% and 60% OLL respectively when compared with control.

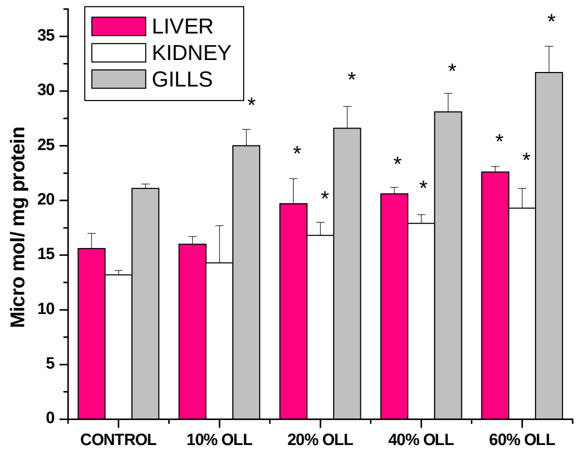

Figure 8: Effect of Olusosun landfill leachate on catalase activity in the organs of *Clarias gariepinus.* Significantly different from control,* p<0.05;** p<0.001**.**

DISCUSSION

The results of this study showed that the physicochemical parameters and heavy metals of OLL were higher than permissible limits set by regulatory authorities (FEPA, 1991; USEPA, 1996). A relatively low pH of 4.6 recorded for the leachate sample showed that the leachate was acidic and indicated presence of metals in the samples particularly toxic metals. As acidity increa ses, most metals become more water soluble and more toxic, this may increase the concentration of soluble and bioavailable free metal ions, which are more capable of causing internal toxic effects (Ostman *et al.,* 2008; Walker *et al.*, 2006; Reithmuller *et al*., 2001). The high Biological oxygen demand (BOD) and Chemical oxygen demand (COD) values observed in the leachate sample show that it is toxic and indicative of the presence of high organic matter in the leachate. Samples with such high BOD values have been demonstrated to have corresponding high mutagenic activities (Omura *et al*., 1992). Biological oxygen demand is useful in evaluating the pollution strength of water and so gives measure of the amount of oxygen required by micro- organisms to decompose organic matter in samples under specific set of conditions (Akinwunmi, 2000). The high value for total hardness of OLL may be due to leaching of Ca and Mg ions into the leachate. High concentrations of heavy metals such as Cu, Cr, Cd, Hg, Co, Pb, As, and Zn in Olusosun landfill leachate confirm that toxic wastes are dumped in Olusosun landfill , the metals perhaps might be from disposed off of battery cells, electronic wastes, used aerosol cans and other materials with certain degree of toxicity. This may be hazardous to the ecosystem and public health since metals are cumulative toxicants that pose danger to organisms and bioaccumulate in the food chain. Other studies have reported similar concentrations of these metals in landfill leachates (Dave and Nilsson, 2005; Osaki *et al.,* 2006; Oygard *et al.,* 2007; Oman and Junestedt, 2008; Maja *et al*., 2011; Daniel *et al*., 2011; Olanrewaju *et al*., 2012).

Also from the results of this study, a significant(p<0.05) and dose dependent increase in lipid peroxidation as MDA formation was observed in the liver, kidney and gills of the fish following exposure to OLL. This suggests that the fish were under oxidative stress after exposure to various concentrations of OLL. Oxidative stress biomarkers are important tools in ecotoxicology and are useful in environmental monitoring systems (Pandey *et al*., 2003). Environmental stressors may induce oxidative stress, leading to generation of free radicals and alteration in antioxidants, the scavenging enzyme system, and lipid peroxidation (Akhgari *et al*., 2002). This result is in consonance with previous studies that also reported increase in lipid peroxidation and oxidative stress in tissues of different species of fresh water fishes, after being exposed to environmental stress (Adeogun *et al*., 2012; Rajamanickam and Muthuswamy , 2009; Farombi *et al*., 2007; 2008; Jos *et al*., 2005; Achuba and Osakwe, 2003). Besides acting as a mediator in oxidative stress, higher levels of lipid peroxidation affect cellular functions; culminating in disease processes (Munkinttrick *et al*., 1998; 2000; Bailey *et al*., 1992; Baile *et al*., 1996). The levels of hydrogen peroxide generation, a marker of increased reactive oxygen species (ROS), were also found to be elevated in all the organs of the fish which is also an indication of oxidative stress.

To attenuate the negative effects of ROS, fish possess an antioxidant defense system like other vertebrates that utilizes enzymatic and non-enzymatic mechanisms (Rajamanickam and Muthuswamy, 2009). Exposure to OLL led to a significant (p<0.05) and dose dependent decrease in glutathione concentration in all the organs of the fish; this suggests feedback inhibition or oxidative inactivation of the protein due to excess ROS generation. The depletion of GSH level in all the organs of the fish would probably be to enhance the risk of oxidative stress in the organs, and induce membrane damage as evidenced by high accumulation of MDA in all the organs. The depletion of GSH may be attributed to the heavy metals in the leachate. Heavy metals and microorganisms release free radicals that are able to abstract electrons from oxidizable substrates (such as GSH) thereby exposing the tissue to oxidative damage (Kidd, 1991). The penetration of OLL into the gills, liver and kidney of the fish depleted GSH through the generation of free radicals which led to the induction of lipid peroxidation in these organs. Farombi *et al*., 2008 also observed decrease in GSH level in the kidney and gills of *Clarias gariepinus* exposed to Butachlor which is in consonance with the present observation.

The enzyme GST was found to increase significantly ($p<0.05$; 0.001) in the liver, kidney and gills of *Clarias gariepinus* after exposue to OLL indicating activation of detoxification mechanism. Induction of GST activity is stimulated by large variety of pollutants including metals. Elevation in the activity of GST in the organs of the fish suggests an adaptive and protective role of this biomolecule against oxidative stress induced by OLL. This result is similar to the findings of Rajamanickam and Muthuswamy, 2009 and Pandey *et al*., 2003 who also reported increase in GST activity in the organs of fresh water fishes after exposure to pollutants.

Glutathione peroxidase activity was induced in the kidney and gills of *Clarias gariepinus* exposed to OLL but the activity of this enzyme was depleted in the liver of the fish. The increase in GPX activity in the kidney and gills indicates the protective role of the enzyme against lipid peroxidation in these organs and this probably reflects an adaptation to the oxidative conditions to which the fish have been exposed and the decrease in the liver may be an indication of enzyme inhibition or oxidative inactivation of the enzyme in this organ. Glutathione peroxidase terminates the chain reaction of lipid peroxidation by removing lipid hydroperoxides and H_2O_2 from the cell membrane (Roberta and Timothy, 1995). Increase in GPX activity recorded in the kidney and gills of *Clarias gariepinus* exposed to OLL may be due to excessive production of H_2O_2 in these organs as a result of OLL induced toxicity. Effective induction of these enzymes in cells and tissues would help to clear the peroxides accumulated after exposure to OLL. An increased oxidative stress has been suggested to enhance the antioxidant enzyme activity in animals, as a protective action towards the oxidative stress (Halliwell and Gutteridge, 2001).

The defensive free radical scavenger, superoxide dismutase (SOD), triggered a significant ($p<0.05$; 0.001) induction response in the liver and kidney of the exposed fish but was inhibited significantly ($p<0.001$) in the gills of the fish. The increased superoxide dismutase activity in the liver and kidney of the fish may be explained as a compensation mechanism against OLL intoxication, to convert the superoxide radical generated by the leachate to H_2O_2, thereby reducing the oxidative damage to cells in the liver and kidney. This result is similar to the observation of Dimitrova *et al*., 1994 in fishes after exposure to lead and Zinc. Specific tissues and organs can display different antioxidant defense status (Wihelm *et al*., 1993) depending on the organism and the circumstances involved (habit, habitat, age, thermoregulatory capacity, metabolic rate and nutritional status, among others). This may explain the decrease in SOD activity observed in the gills. The decrease may also be due to the fact that the gill being the first point of contact with environmental xenobiotics are more exposed to contaminated water and as such metals and other toxicants in leachate can penetrate through their thin epithelial cells to produce reactive species, thereby inactivating the antioxidant defenses. Fernandes and Mazon, (2003) observed that fish gills are the prime target organ of all pollutants due to their extensive surface in contact with the external medium and the reduced distance between the external and internal medium. The decrease in SOD activity in the gill was concentration dependent which means as the concentration of OLL increased, the antioxidant capacity of the animal may have been overwhelmed and hence the observed decrease in enzyme activity.

There was significant ($p<0.05$) increase in catalase activity in all the organs of the fish. This result is consistent with the results of Adeogun *et al.*, 2012; Cheung *et al*., 2004; Li *et al*., 2003 and Cossu *et al*., 2000. The induction of catalase activity in the organs of the fish may be explained as a compensation mechanism against leachate intoxication. The increase in catalase activity in these organs may be a response to the hydrogen peroxide produced by SOD activity since catalase is responsible for the detoxification of hydrogen peroxide to water. Increase in the activity of catalase and SOD is usually observed in the face of environmental pollutants since SOD-CAT system represents the first line of defense against oxidative stress (Dautremepuits *et al*., 2004; Pandey *et al*., 2003).

In conclusion, it may be inferred from this study that the significant induction of lipid peroxidation, hydrogen peroxide generation and alterations in antioxidant systems in the organs of *Clarias gariepinus* after OLL exposure indicate that OLL induced oxidative stress in the organs of the fish. This implies that animals and human beings could also be subjected to the same toxic effect if they feed on leachate contaminated fishes. This is of great concern since *Clarias gariepinus* is the most widely consumed fish in Nigeria. The organ specific responses to antioxidants indicate interdependency of these antioxidants to scavenge the reactive oxygen radicals. Also, these results show that the liver, kidney and gills of *Clarias gariepinus* are active in generation of oxidative stress and antioxidant responses after exposure to OLL, which can be used as biomarkers of aquatic pollution.

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