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# Municipal landfill leachate induces hepatotoxicity and oxidative stress in rats

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**Abstract**

Human beings are more often exposed to complex mixtures of hazardous chemicals than single toxicant. The present study investigated the effects of Olushosun municipal landfill leachate (OMLL) from Ojota in Lagos State of Nigeria on hepatic function and some biomarkers of oxidative stress in adult rats. Physicochemical characteristic analysis of OMLL showed that while total alkalinity, total acidity, total hardness, biochemical oxygen demand and chemical oxygen demand were 3-fold, 2-fold, 4-fold and 1-fold, respectively, concentrations of heavy metals analysis showed that copper, lead, cadmium, arsenic, cobalt, chromium and mercury were 9-fold, 4-fold, 21-fold, 1320-fold, 7-fold, 5-fold and 4-fold, respectively, higher than acceptable limits by regulatory authorities. The OMLL was administered at 0, 10, 20, 30 and 40% concentrations to adult male rats for 14 days. Following exposure, serum was collected for serum biochemistry assays and liver was collected to determine the antioxidant status. Exposure of animals to 10, 20, 30 and 40% OMLL resulted in 3%, 31%, 52% and 83% increase in aspartate aminotransferase activity, whereas it elevated alanine aminotransferase activity by 10%, 25%, 30% and 49%, respectively, when compared with the control. While OMLL administration significantly increased catalase activity, a sequential decrease in reduced glutathione level and in superoxide dismutase and glutathione-*S*-transferase activities with concomitant increase in malondialdehyde level were observed, when compared with the control. Collectively, the hepatotoxicity of OMLL could be due to the induction of oxidative stress and may suggest possible health hazards in subjects with occupational or environmental exposure.

**Keywords**

Olushosun leachate, heavy metals, oxidative stress, hepatotoxicity, rats

## Introduction

Hazardous wastes are those that hold a substantial potential risk to human health or environment when improperly treated, stored, transported, disposed off or otherwise managed. Lagos State is an African megacity located in southwestern Nigeria on the west coast of Africa, within latitudes 6°23′N and 6°41′N and longitudes 2°42′E and 3°42′E. Lagos State accounts for more than 70% of the nation's industrial and commercial establishments. Physically it is the smallest but most highly populated state in the country, with an estimated population of over 10 million inhabitants, which is more than 10% of the total population in Nigeria. Water is the most significant topographical feature in this state; water and wetlands cover over 40% of the total area within the state and additional 12% is subject to seasonal flooding (Iwugo et al., 2003). In Nigeria, the propensity of residents to generate waste seems to have heightened in recent times as a result of accelerated industrialization, urbanization and population growth. Landfilling and open

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dumping of wastes are very common methods of managing these wastes (Alimba et al., 2006).

One of the major problems arising from landfill/ waste dump sites is the generation of leachates. The generation of leachate is caused principally by precipitation percolating through waste deposited in a landfill. The precipitation can be from rain, melted snow or the waste itself. Once in contact with decomposing solid waste, the percolating water becomes contaminated and if it then flows out of the waste material it is termed leachate (Monroe, 2001). Small amounts of leachates can pollute large volume of ground and surface waters, rendering them unsuitable for domestic use and other purposes (Lee and Jones-Lee, 1996). Additional leachate volume is produced during the decomposition of carbonaceous materials including methane, carbon dioxide and a complex mixture of organic acids, aldehydes, alcohols and simple sugars. The amount of leachate produced is directly linked to the amount of precipitation around the landfill (Lee and Jones-Lee, 1994). Public health concerns occur often among communities located near municipal solid waste (MSW) landfills. Due to high population density in the city, the rate of solid waste generation is very high and the facilities required for proper disposal are not adequate. In some areas where landfill sites are designated for the disposal of the waste, the leachates resulting from the decomposition of the biodegradable matters in the wastes find their way into the ground/surface water, because there are no means of collecting the leachates. Hence, heavy contamination of the water resources results (Iwugo et al., 2003).

Heavy metals and organic compounds that are present in leachates are assimilated by aquatic species, pass through food chain and bioaccumulate upon long-term exposure (Sang and Li, 2004). Much of the damage produced by toxic metals stems from the proliferation of oxidative free radicals they generate (Pouls, 1999). Li et al. (2006) reported the oxidative damage induced in hearts, kidneys and spleens of mice by landfill leachates. A leachate prepared from municipal sludge dose-dependently inhibited the mitotic index (MI) and increased the frequency of chromosome aberrations in the bone marrow of mice (Tewari et al., 2005); in addition, increased frequencies of micronucleated polychromatic erythrocytes and DNA damage were observed in mouse bone marrow cells (Li et al., 2004; Tewari et al., 2005). Genotoxicity of leachates from the sediment of river Matanza-Riachuelo, one of the most polluted rivers of Latin America, has been reported (Magdaleno et al., 2008).

In Nigeria, previous studies have implicated land-based urban and industrial waste sources in the anthropogenic heavy metal enrichment of the Lagos lagoon and groundwater with cadmium (Cd), cobalt (Co), copper (Cu), iron (Fe), manganese (Mn), nickel (Ni), lead (Pb) and zinc (Zn) (Ajao and Fagade, 1990; Longe and Enekwechi, 2007; Okoye et al., 1991), and these metals have also been reported to exceed the maximum permissible concentration for drinking water (Abolude et al., 2009). Moreover, high levels of Cd, chromium (Cr), Fe, Zn and Cu have been reported in paint flakes and soil (Nduka et al., 2006, 2007, 2008; Orisakwe et al., 2004). The possible long-term deleterious effects of these heavy metals among occupationally exposed painters in Nigeria have also been reported (Nduka et al., 2007; Orisakwe et al., 2007). Widespread contamination of the environment with lead (Pb) is consistent with the results of epidemiological studies, which have found elevated blood Pb levels in a large proportion of Nigerian children (Nduka and Orisakwe, 2010).

The recycling rate for rechargeable batteries containing Co, Ni, Cr and Cd in Nigeria is zero as there is no form of material recovery currently taking place. Recent study on the waste portable rechargeable batteries used in mobile phones revealed that the Co and Ni concentrations of the batteries are so high and far exceeded the toxicity threshold limit concentrations used in the toxicity characterization of solid waste by about 40 folds the limit concentrations of each of the metals (Nnorom and Osibanjo, 2009). Mercury (Hg) has no known function in human biochemistry or physiology and does not occur naturally in living organisms. Hence, intake of Hg from contaminated food and water even at very low concentrations can be very harmful because of its bioaccumulation. Exposure to arsenicals, which is used as herbicides, fungicides and rodenticides may cause soil, air and water pollution. Previous study from our laboratory showed that high concentration of Cd, Zn, Pb, Cu and arsenic (As) accumulated in the liver, kidney, heart and gills of fish from Ogun River located between two heavily industrialized cities, Lagos and Sango-Ota, in Nigeria (Farombi et al., 2007).

Exposure to leachate has been shown to increase lipid peroxidation and activities of antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase and catalase (CAT) in heart, kidney and spleens in mice (Guangke et al., 2005; Li et al., 2006). Tire leachates have been shown to have profound effects both on cytochrome P450 A1 content and on ethoxyl-resorufin-o-deethylase (EROD) activity, as well as upregulation of antioxidant defences in mice (Stephensen et al., 2003). Most landfill sites receive a mixture of municipal commercial and mixed industrial wastes. Several xenobiotics enter the body through gastrointestinal tract and after absorption are transported by the hepatic portal vein to the liver; thus the liver is the first organ perfused by chemicals that are absorbed in the gut (Adedara et al., 2011). To date, research has largely concentrated on hepatotoxicity, since the liver plays a major role in the metabolism of xenobiotics and consequently the primary target of most toxic responses. The present study investigated, for the first time, the effects of municipal landfill leachate from Olushosun landfill Ojota in Lagos State of Nigeria, on hepatic function and some biomarkers of oxidative stress in male albino rats of the Wistar strain.

## Materials and methods

### Sampling site and leachate sampling

The sampling site, Olushosun landfill Ojota is located on the Northeastern part of Lagos State of Nigeria (latitude 6°34′N and longitude 3°24′E). The site was swampy prior to the landfilling operations, which commenced in 1977 (LAWMA, 1998). It covers about 42 hectares of land, with an excavation of about 18 m deep into the landfill area. Raw leachate was collected from leachate wells (holes in the ground) and was thoroughly mixed. Thereafter, the leachate sample was filtered to remove debris, the pH was measured and the sample stored at 4°C until use. It was designated Olushosun municipal landfill leachate (OMLL).

### Physicochemical parameters and heavy metal analysis

The physical and chemical properties of the OMLL were determined in accordance with the standard methods (Federal Environmental Protection Agency (FEPA), 2001; United States Environmental Protection Agency (USEPA), 1996). Standard physical and chemical properties including chemical oxygen demand (COD), biochemical oxygen demand (BOD), total alkalinity, total acidity and total hardness were analyzed. The concentrations of seven heavy metals namely Cu, Pb, Cd, As, Co, Cr and Hg were estimated in the leachate sampling using atomic absorption spectrophotometer. The levels of these metals were assessed because of their reported occurrences in both solid and liquid wastes in Nigeria (Farombi et al., 2007; Longe and Balogun, 2010; Longe and Enekwechi, 2007; Nduka et al., 2007).

### Chemicals

Epinephrine, glutathione, 5,5′-dithio-bis-2-nitrobenzoic acid, hydrogen peroxide, thiobarbituric acid and 1-chloro-2,4-dinitrobenzene (CDNB) were purchased from Sigma Chemical Co (St. Louis, MO, USA). All other reagents were of analytical grade and were obtained from the British Drug Houses (Poole, Dorset, UK). Kits for serum aminotransferase activities were purchased from Random Laboratory Limited, United Kingdom.

### Animal protocol

A total of 25 healthy adult male Wistar rats weighing approximately 160–200 g obtained from the Department of Biochemistry, University of Ibadan, Ibadan, Nigeria, were randomly assigned to 5 groups of 5 animals per group. They were housed in plastic suspended cages placed in a well-ventilated rat house, provided rat pellets and water *ad libitum* and subjected to natural photoperiod of 12-h light:dark cycle. All the animals received humane care according to the criteria outlined in the ‘Guide for the Care and Use of Laboratory Animals’ prepared by the National Academy of Science and published by the National Institute of Health. Ethic regulations have been followed in accordance with National and institutional guidelines for the protection of animal welfare during experiments (PHS 1996).

Four different concentrations (10, 20, 30 and 40%) of OMLL were prepared according to the groups, and the rats in each group were administered 1 ml of OMLL via intaperitoneal injection for 14 consecutive days (Brusick, 1980). Corresponding group of animals were administered with the same volume of distilled water via the same route and served as control. Rats were killed by cervical dislocation 24 h after the final treatment and blood was collected by cardiac puncture. The livers were quickly removed, weighed and placed on ice bath. The blood was allowed to clot and centrifuged at low speed (3000*g*), at room temperature for 15 min. The body weights of rats were taken before exposure to various treatments and prior to killing.

### Biochemical assay

Liver samples were homogenized in 50 mM Tris-HCl buffer (pH 7.4) containing 1.15% potassium chloride, and the homogenate was centrifuged at 10,000*g* for 15 min at 4°C. The supernatant was collected for the estimation of CAT activity using hydrogen peroxide as substrate, according to the method of Clairborne (1995). SOD was assayed by the method described by Misra and Fridovich (1972). Protein concentration was determined by the method of Lowry et al. (1951). Reduced glutathione (GSH) was determined at 412 nm using the method described by Jollow et al. (1974). Lipid peroxidation was quantified as malondialdehyde (MDA) according to the method described by Farombi et al. (2000) and expressed as micromoles of MDA/g tissue. Serum activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined by the method of Reitmann and Frankel (1957).

### Statistical analysis

All values are expressed as the mean ± standard deviation. Levels of statistical significance were analysed by analysis of variance (ANOVA), followed by Student's $t$ test to compare the means between different treatment groups. Difference was considered significant for $p < 0.05$.

## Results

### Physicochemical characteristics and heavy metals concentration in OMLL

With exception of pH value, the physicochemical parameters such as total alkalinity, total acidity, total hardness, BOD and COD as well as concentrations of heavy metals in OMLL were significantly ($p < 0.05$) higher than the acceptable limits by regulatory authorities—USEPA and FEPA (Tables 1 and 2).

**Table 1.** Physicochemical characteristics of Olushosun municipal landfill leachate (OMLL)[a]

| Parameters | OMLL | FEPA | USEPA |
|---|---|---|---|
| pH | 7.24 | 6–9 | 6.5–8.5 |
| Total alkalinity | 628.00 | 250 | 20 |
| Total acidity | 476.00 | 200 | — |
| Total hardness | 121.40 | — | 0–75 |
| BOD | 205.60 | 50 | — |
| COD | 425.20 | — | 410 |

FEPA: Federal Environmental Protection Agency (1991), permissible limits for drinking water, USEPA: US Environmental Protection Agency, BOD: biochemical oxygen demand, COD: chemical oxygen demand.
[a]All values are in mg/l except pH.

**Table 2.** Concentration of heavy metals detected in OMLL

| Parameters | OMLL | FEPA | USEPA |
|---|---|---|---|
| Copper | 6.20 | 0.30 | 1.0 |
| Lead | 13.20 | 0.01 | 0.015 |
| Cadmium | 0.36 | 0.05 | — |
| Arsenate | 0.12 | — | — |
| Cobalt | 0.44 | — | — |
| Chromium | 0.26 | 0.05 | 0.10 |
| Mercury | 0.41 | 0.1 | — |

OMLL: Olushosun municipal landfill leachate, FEPA: Federal Environmental Protection Agency, USEPA: U.S. Environmental Protection Agency.

### Body weight and serum biochemical indices

The results on the body weight and serum biochemical indices are presented in Figure 1. During the exposure period, the percentage gain in body weight of controls and 10% OMLL-treated animals were 1% and 6%, respectively, whereas the percentage loss in body weight in 20, 30 and 40% OMLL-treated animals were 1%, 3% and 8%, respectively. Serum activities of AST and ALT were increased in a dose dependent manner following acute exposure of rats to OMLL. Exposure of animals to 10, 20, 30 and 40% OMLL resulted in significant increase in AST activity by 3%, 31%, 52% and 83% as well as ALT activity by 10%, 25%, 30% and 49%, respectively, when compared with the controls.

### Antioxidant status in the liver

Effects of OMLL on hepatic antioxidant status and lipid peroxidation are shown in Figures 2-4. Following exposure to OMLL, a significant ($p < 0.05$) decrease in hepatic glutathione (GSH) level and in activities of SOD and glutathione-*S*-transferase (GST) were observed in all treated groups. While 10, 20, 30 and 40% OMLL-treatment resulted in decreased GSH level by 25, 57, 71 and 76%; SOD activity decreased by 41, 61, 63 and 67%; GST activity decreased by 21, 63, 71 and 75%, respectively, when compared with the control animals. However, CAT activity and level of lipid peroxidation were markedly elevated in a dose-dependent manner following OMLL administration.

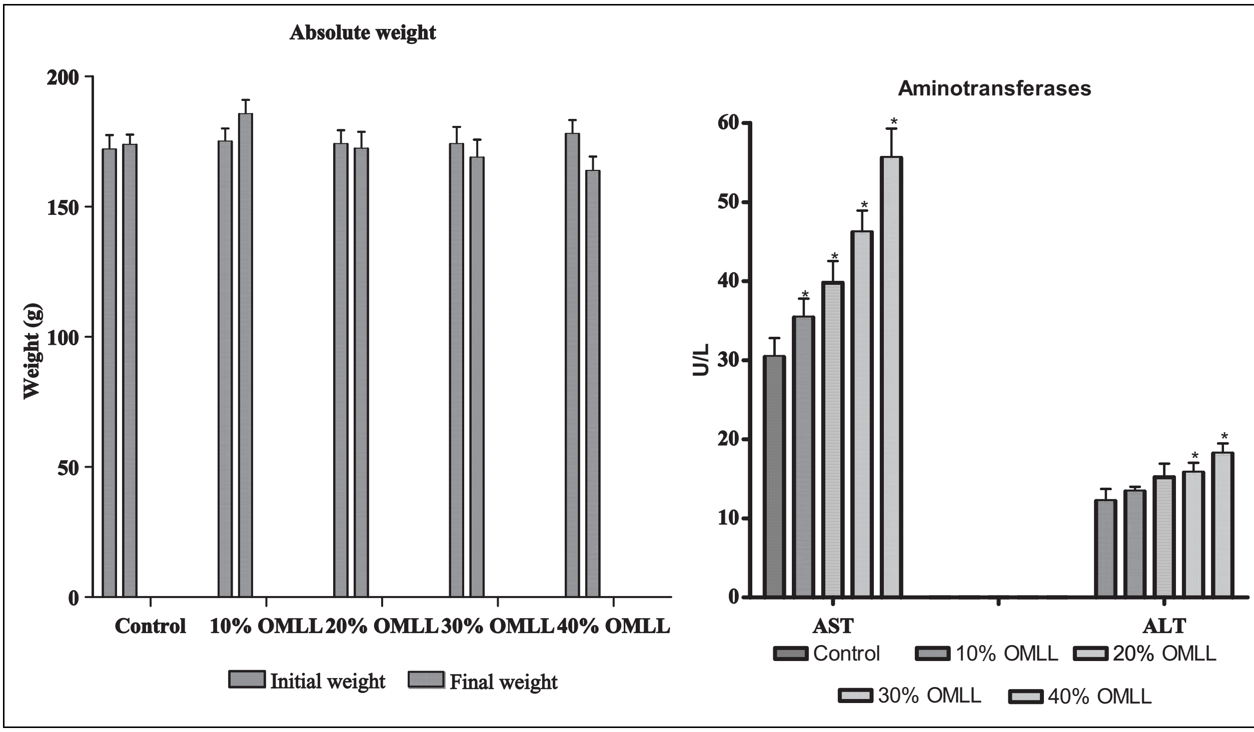


**Figure 1.** (A) Effect of Olushosun municipal landfill leachate (OMLL) on the body weight of rat after 14 days. (B) Effect of OMLL on serum activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in rats after 14 days.

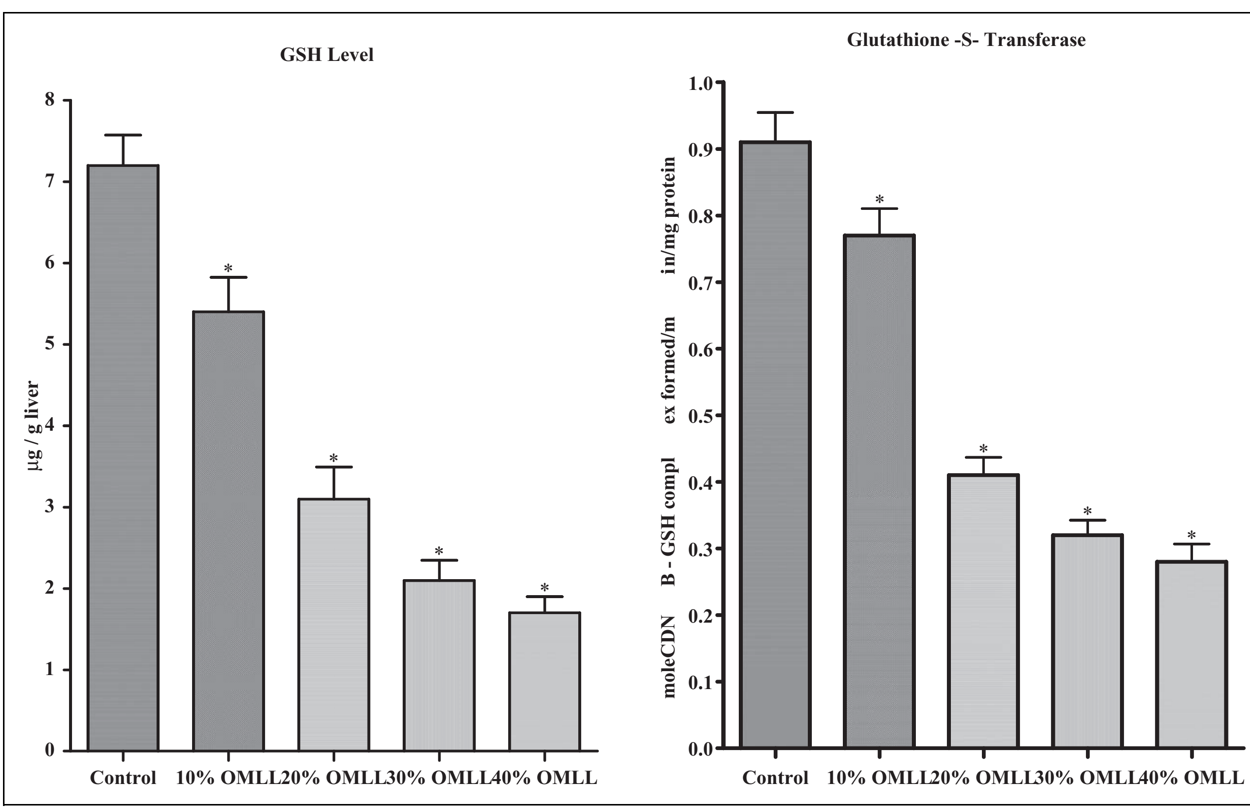


**Figure 2.** Effect of Olushosun municipal landfill leachate (OMLL) on reduced glutathione (GSH) level and glutathione-S-transferase activity in the livers of rats after 14 days.

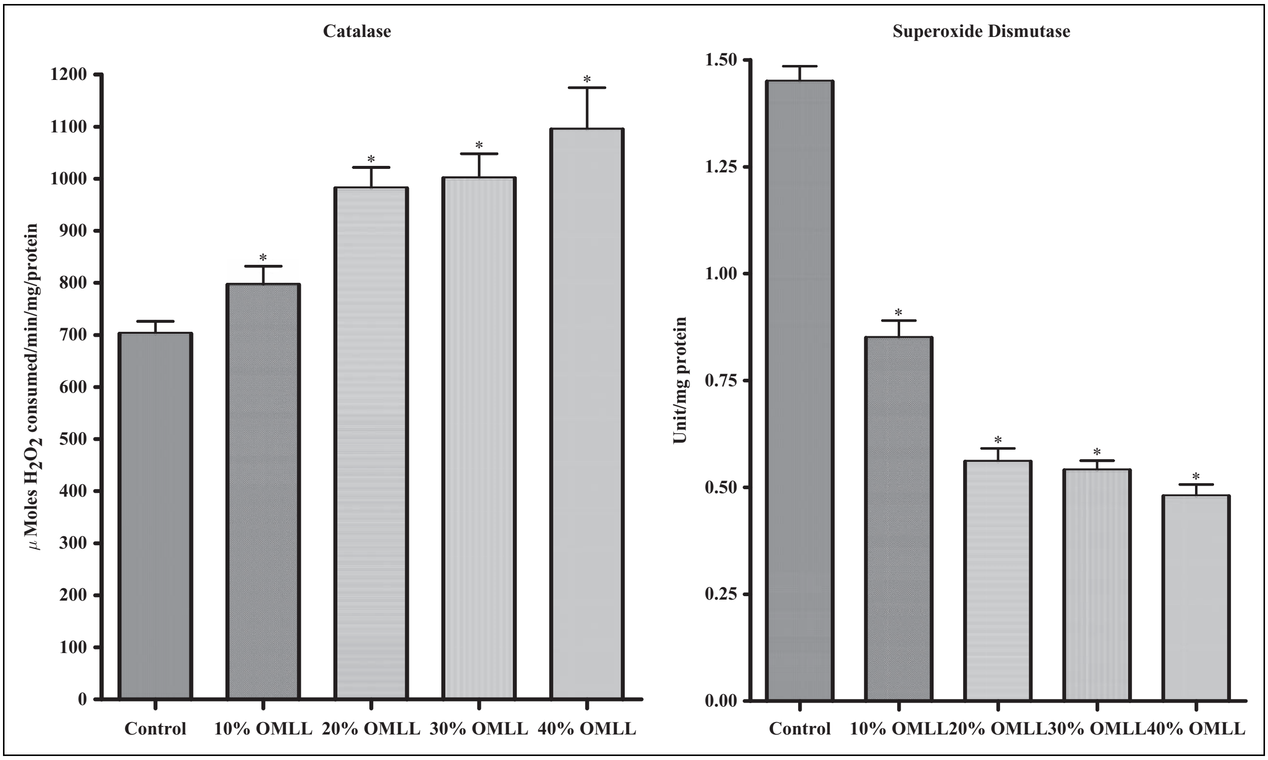


**Figure 3.** Effect of Olushosun municipal landfill leachate (OMLL) on the activities of catalase and superoxide dismutase in the livers of rats after 14 days.

When compared with the control values, the activity of CAT was elevated by 13, 40, 43 and 56%, while MDA level was elevated by 67, 310, 443 and 484% after dosing the animal with 10, 20, 30 and 40% OMLL, respectively.

## Discussion

There is a growing community concern about the general health issues and growing understanding of the role that production and industry might be playing in the causation of certain health and related environmental problems. The use of landfill to dispose of hazardous or prescribed waste is a significant contributing factor to the health risk faced by communities as a result of industrial practices. Unlined sanitary landfills have been reported to release large amounts of hazardous and deleterious chemicals to nearby groundwater and the air, via leachate and landfill gas, respectively (Christensen et al., 2001; Ikem et al., 2002). Real-life exposures to toxic substances most often involve complex mixtures of hazardous chemicals than single toxicant. Leachates consisting of mixture of many chemicals including heavy metals are a potential risk to human health (Bakare et al., 2007).

The present study showed that the physicochemical characteristics of OMLL were significantly higher than acceptable limits by regulatory authorities (FEPA, 1991; USEPA, 1996). This indicates that organics measured as BOD can cause taste and odour problems and oxygen depletion in the groundwater, thereby posing threat to aquatic organisms. In addition, the presence of high concentrations of these organics can serve as co-substrate for microorganisms, which may facilitate the conversion of hazardous chemical into more hazardous chemicals. The elevation in the physicochemical characteristics of OMLL above the normal values can cause severe degradation of groundwater quality and preclude its use for domestic water supply purposes (Lee and Jones-Lee, 1993). Moreover, the high concentration of heavy metals viz Cd, Cr, Cu, Pb, Co, As and Hg in OMLL is of great health risk. Interactions of heavy metals with the organic environmental pollutants offer an important premise of study to ecotoxicologists (Atif et al., 2005). Heavy metals have the potential to induce mutations and cancer in living cells (Fowler et al., 1994). Cd, Cu, As and Fe produce free radicals and when present in an unbound form, it produces reactive oxygen species (ROS) that can cause

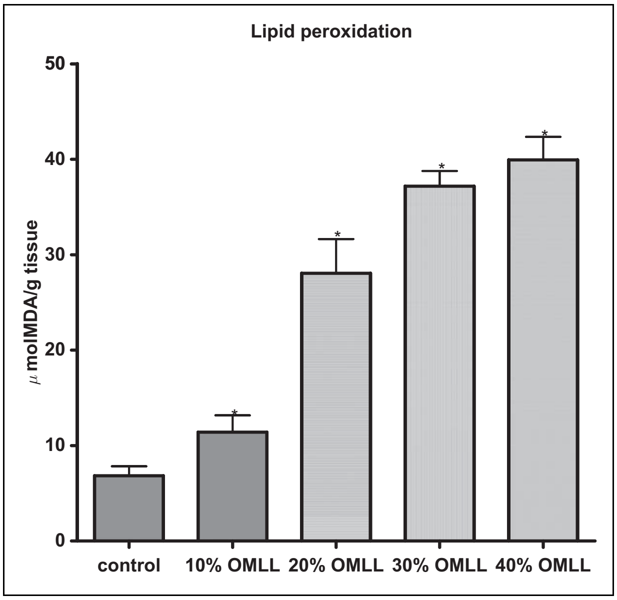


**Figure 4.** Effect of Olushosun municipal landfill leachate (OMLL) on lipid peroxidation in the livers of rats after 14 days.

DNA, protein and lipid damage (Glaris and Evangelou, 2002; Radetski et al., 2004; Sharma et al., 2009). Co is an essential trace element present in vitamin $B_{12}$. However, excessive exposure to co has been reported to increase production of ROS and cell death (Yang et al., 2004; Zou et al., 2001), which has been linked with reproductive and developmental toxicity, neurotoxicity, carcinogenicity and acute toxicity.

The present study revealed dose-dependent decrease in body weights of OMLL-treated animals compared with control. Our result is inconsistent with earlier reports of Guangke et al. (2005) and Li et al. (2006) who reported increase in body weight of mice treated with municipal landfill leachate. The discrepancy in these results may be related to leachate composition, which varies with landfill sites and season or species differences. The OMLL exposure resulted in significant increase in the activities of serum ALT and AST. These enzymes are localized in periportal hepatocytes, reflecting their role in oxidative phosphorylation and gluconeogenesis and their serum activities presumably increase as a result of cellular membrane damage and leakage (Kaplan, 1993). This underlines their use as biochemical markers for early acute hepatic damage (Adedara et al., 2010). Elevated levels of AST and ALT in circulation were indicative of a hepatic injury after OMLL exposure.

Evidence suggests that various enzymatic and nonenzymatic systems have been developed by the cell to cope with the ROS and other free radicals. However, when a condition of oxidative stress is established, the defence capacities against ROS become insufficient (Halliwell and Gutteridge, 2000). ROS attack cellular components involving polyunsaturated fatty acid residues of phospholipids, which are extremely sensitive to oxidation (Siems et al., 1995). Once formed, peroxyl radicals (ROO●) can be rearranged via a cyclization reaction to endoperoxides (precursors of MDA) with the final product of the peroxidation process being MDA (Marnett, 1999; Wang et al., 1996). Administration of OMLL resulted in dose-dependent increase in MDA level in treated animals. This result is consistent with previous study on oxidative damage induced in heart, kidney and spleen of mice treated with landfill leachate (Li et al., 2006).

The GSH plays a crucial role in protecting the cells from oxidative damage (Guoyao et al., 2004). In our study, hepatic GSH content was considerably depleted after OMLL administration. This decline in GSH content under the present experimental model suggests its overutilization to challenge the prevailing oxidative stress under the influence of ROS generated from OMLL. Low levels of GSH were observed during increased oxidative stress (Bray and Taylor, 1993). Activities of SOD and GST were markedly decreased by OMLL treatment but resulted in an increase in CAT activity. Superoxide radicals by themselves or after their transformation to hydrogen peroxide ($H_2O_2$) caused oxidation of the cysteine in the enzyme and decreased the SOD activity (Dimitrova et al., 1994). The degradation of $H_2O_2$, a potent oxidant at high cellular concentration, is effected by CAT. The rise in the activity of CAT could be due to its induction to counter the effect against increased oxidative stress. Our result is consistent with earlier report of Guangke et al. (2005). The GST is a group of multifunctional proteins that play a central role in the detoxification of electrophilic chemicals and the hepatic removal of potentially harmful hydrophobic compounds from blood (Smith et al., 1977). They could be found in the cytosol or associated with membranes (Rinaldi et al., 2002). The reduced activity of GST observed in this present study may be partly due to the lack of its substrate, GSH, and also because of oxidative modification of its protein structure.

From these findings, OMLL induces hepatic oxidative damage in a dose-dependent manner in rats that may be due to the leachate or its metabolites. Humans are exposed to heavy metals by consuming contaminated food and water, which could result in various biochemical disorders. The poisoning effects of heavy metals are due to their interference with the normal body biochemical processes. When ingested, in the acid medium of the stomach, the metals are converted into their stable oxidation states, which subsequently combine with the body's biomolecules such as proteins and enzymes to form strong and stable chemical bonds, thus, mutilating their structures and hampering their functions. Although individual metals, when consumed above the bio-recommended limits, exhibit specific signs of their toxicity, the following are general signs associated with biometal poisoning: gastrointestinal disorders, diarrhoea, stomatitis, tremor, haemoglobinuria causing a rust-red colour to stool, ataxia, paralysis, vomiting and convulsion, depression, asthma and pneumonia. The nature of effects could be toxic (acute, chronic or sub-chronic), neurotoxic, carcinogenic, mutagenic or teratogenic. The inappropriate methods employed in the waste management practices in Nigeria create the potential for a negative impact on the health of the residents as well as the entire ecosystem. Therefore, in order to safeguard the health of Nigerians, we recommend that the FEPA implement a well-coordinated management strategy to check the indiscriminate disposal of waste of large doses of toxic heavy metals into the environment.

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