BENI-SUEF UNIVERSITY JOURNAL OF BASIC AND APPLIED SCIENCES 4 (2015) 284–290

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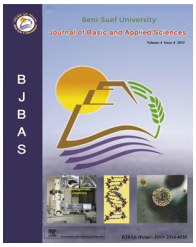

Full Length Article

# Brain dysfunctions in Wistar rats exposed to municipal landfill leachates

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ARTICLE INFO

*Article history:*
Received 24 January 2015
Accepted 19 November 2015
Available online 10 December 2015

*Keywords:*
Brain damage
Brain weight index
Histopathology
Landfill leachates
Oxidative stress
Rats

ABSTRACT

Brain damage induced by Olusosun and Aba-Eku municipal landfill leachates was investigated in Wistar rats. Male rats were orally exposed to 1–25% concentrations of the leachates for 30 days. Catalase (CAT) and superoxide dismutase (SOD) activities, and malondialdehyde (MDA) concentrations in the brain and serum of rats were evaluated; body and brain weight gain and histopathology were examined. There was significant ($p < 0.05$) decrease in body weight gain and SOD activity but increase in absolute and relative brain weight gain, MDA concentration and CAT activity in both brain and serum of treated rats. The biochemical parameters, which were more altered in the brain than serum, corroborated the neurologic lesions; neurodegeneration of purkinje cells with loss of dendrites, perineural vacuolations of the neuronal cytoplasm (spongiosis) and neuronal necrosis in the brain. The concentrations of Cr, Cu, Pb, As, Cd, Mn, Ni, sulphates, ammonia, chloride and phosphate in the leachate samples were above standard permissible limits. The interactions of the neurotoxic constituents of the leachates induced the observed brain damage in the rats via oxidative damage. This suggests health risk in wildlife and human populations.



## 1. Introduction

Population growth with subsequent industrial development is the major factor responsible for the inevitable increase in solid waste generation worldwide. Inappropriate disposal of these wastes into unsanitary landfills constitute public health risk and environmental contamination due to landfill gas and leachate production from components of the solid wastes (Alimba, 2013). Leachates contain high concentrations of toxic metals, hazardous organic and inorganic chemicals, radioactive substances, particulate matter and microorganisms; many of which are regarded as emergent environmental contaminants (Efuntoye et al., 2011; Eggen et al., 2010; Øygard and Gjengedal, 2009; Slack et al., 2005). Previously, we observed that mixture of these xenobiotics in leachates from Olusosun and Aba Eku landfills in Nigeria, induced alterations in the liver, kidney, body weight, haematological indices and erythrocyte morphology in rats (Alimba and Bakare, 2012; Alimba et al., 2012). Li et al. (2006, 2010) reported that leachate from Xingou municipal landfill in China induced lipid peroxidation, protein oxidation and disturbed the antioxidant status of liver, spleen, heart, brain and

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http://dx.doi.org/10.1016/j.bjbas.2015.11.003

kidney in exposed mice. It was suggested from these reports that the induction of systemic toxicity by landfill leachates involves free radical generation.

Among mammalian organs, the brain is the most susceptible organ to lipid peroxidation and oxidative injury from xenobiotics due to its membrane rich polyunsaturated fatty acids, low antioxidant status and high iron contents (Jayaraman et al., 2008). Evidence exists that individual chemicals in landfill leachate altered the normal functioning of the nervous system hence increasing neurotoxic diseases among human population (Neal and Guilarte, 2012; Wright et al., 2006). However, there are limited studies on the effects of landfill leachate on the functional and structural integrity of the mammalian brain. Furthermore, neurotoxic assessment is one among the health outcomes suggested by the Agency for Toxic Substances and Disease Registry (ATSDR) to be monitored during exposure to hazardous substances from solid waste disposal sites (Johnson, 1999; Schaumburg et al., 1983). In this study, a 30 day sub-chronic toxicity testing of Olusosun and Aba Eku landfill leachates in Wistar rats was carried out to assess structural alterations in the brain, antioxidant enzyme activities and lipid peroxidation status of the brain and serum, and alterations in brain weight gain. Some physico-chemical parameters and heavy metal compositions of the leachates were also analyzed.

## 2. Materials and methods

### 2.1. *Sampling site and leachate collection*

The study sites, Olusosun and Aba-Eku landfills, had been described previously (Alimba, 2013). These landfills were selected due to the high polluting status of the environment through landfill gas and leachate production, which increased public health risk through exposure to landfill chemicals and microorganisms (Alimba, 2013; Efuntoye et al., 2011). Raw leachates were collected from 20 different leachate wells on each of the landfills and thoroughly mixed to produce homogenous samples for each sampling site. The samples were transferred to the laboratory in pre-cleaned 10 litre plastic containers, where they were filtered using glass wool and Whatmann® No. 42 filter paper to remove suspended particles. They were centrifuged at 3000 rpm for 10 minutes and stored at 4 °C. The processed leachates were considered as stock samples (100%) and labelled as Aba-Eku Leachate (AEL) and Olusosun Leachate (OSL).

### 2.2. *Physical and chemical analysis of the leachate*

Physical and chemical components of the leachates were analysed according to American Public Health Association (APHA) (1998). Nitrate, ammonia, chloride, phosphate, sulphate, total hardness, total alkalinity, biochemical oxygen demand (BOD), chemical oxygen demand (COD) and total solids (TS) were determined. Also iron (Fe), lead (Pb), copper (Cu), manganese (Mn), arsenate (As), cadmium (Cd), chromium (Cr) and nickel (Ni) concentrations were determined according to United States Environmental Protection Agency (USEPA) (1996) and American Public Health Association (APHA) (1998). 100 ml each of the leachate samples was digested by heating with concentrated $HNO_3$. The resulting mixture was made up to 10 ml with 0.1N $HNO_3$ and metal concentrations determined using PerkinElmer® A3100 atomic absorption spectrophotometer.

### 2.3. *Animals and experimental design*

Male Wistar rats (mean ± SD weight; 167.64 ± 4.27 g) were obtained from the animal house unit of College of Medicine, University of Ibadan, Nigeria. They were acclimatized for 2 weeks prior to leachate treatment, and were maintained in laboratory condition of 12 hours dark and light cycle with access to drinking water and standard rodent chow (Ladokun feed Nigeria®) *ad libitum*. Rats in each group (n = 5) was gavaged 0.5 ml of 1, 2.5, 5, 10 and 25 % (leachate diluted with distilled water, v/v) concentrations, of each of the leachates for 30 consecutive days. The leachate concentrations were selected from previous sub-chronic systemic toxicity (Alimba and Bakare, 2012; Alimba et al., 2012), Similar treatment was concurrently given to the negative and positive control groups receiving distilled water and cyclophosphamide (CYP, 40 mg / kg/ bwt) respectively. The animal experiment conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23) (Gad, 2007).

### 2.4. *Clinical pathology and body and brain weight measurement*

Rats in each of the treatment groups were weighed at the beginning of the experiment using Acculab® USA, Model-vic-303 electronic analytical weighing balance. At the end of exposure, rats were fasted overnight, weighed prior to blood collection and sacrificed. Blood was collected from the orbital plexus using heparinized 70 ml micro-haematocrit capillary tubes into lithium coated serum separator tubes (Sanford, 1954). It was allowed to clot and centrifuged at 3000 rpm for 20 minutes at 4 °C to separate the serum (supernatant). Whole brain from both treated and control rats were surgically removed, rinsed with ice-cold physiological saline and blotted dry to determine the absolute and relative brain weight (brain weight / body weight x 100 g). Brain homogenate was prepared using ice-cold 10 mmol/L phosphate-buffered saline (pH 7.4) containing 0.15 M KCl (10% w/v). The homogenate was centrifuged at 6000 rpm for 30 minutes at 4 °C. Both the brain homogenate and serum (supernatants) were stored at –70 °C prior to biochemical analysis.

### 2.5. *Biochemical analysis*

The brain homogenate and serum were analysed for antioxidant enzyme activities; superoxide dismutase (SOD; E.C. 1.15.1.1) was assayed according to the method of Nebot et al. (1993), while catalase (CAT; E.C. 1.11.1.6) was in accordance with the method of Johansson and Borg (1988). Lipid peroxidation concentration in the brain homogenate and serum was by malondialdehyde (MDA) determination in accordance with the methods of Esterbauer and Cheeseman (1990). Protein concentration was measured according to the method of Lowry et al. (1951). Analytical grade reagents (Biosystems Laboratories, S.A. Costa Brava, Barcelona, Spain) were used, and the