IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402,p- ISSN: 2319-2399.Volume 14, Issue 4Ser. I (April. 2020), PP 01-04
www.iosrjournals.org

Effect of Port Harcourt (Eliozu) Landfill Leachates on Oxidative Stress Using Oxidative Stress Markers in Wistar Rats

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Abstract
Disposal of wastes into the landfills may constitutes health risk and environmental contamination due to landfill gas and leachate production from components of wastes, the dangers of possible deleterious effects on the residents around the landfills has not been adequately evaluated. This study attempts to determine the effects of Port Harcourt (Eliozu) landfill leachates on some oxidative stress markers in Wistar rats. 25 Wistar rats weighing between 180g and 250g were divided into five groups of 5 animals each. Groups are as follows: Group 1: received 1ml of table water, Group 2: recieved borehole water, Group 3: received 10% of leachate concentrations, Groups 4 and 5: received 50% and 100% of leachate concentrations every 24 hours for 90 consecutive days. On day 91, blood samples were collected for oxidative stress markers analysis. The results of serum and renal oxidative stress markers show a significant ($p<0.001$) increase in all experimental groups when compared with control groups: indicating a possible deleterious impact of the leachate on experimental animals.

Keywords: *Leachates, Eliozu, landfill, oxidative stress, deleterious.*

Date of Submission: 18-03-2020 Date of Acceptance: 03-04-2020

I. Introduction

In developing countries like Nigeria, landfilling is the most commonly practiced form of municipal solid waste (MSW) disposal due to its economic advantages [1]. However, the leachate arising from such landfills is now being recognized as a potential health risk to both surrounding ecosystems and human population [2]. Disposal of wastes into the landfills may constitutes health risk and environmental contamination due to landfill gas and leachate production from components of wastes, the dangers of possible deleterious effects on the residents around the landfills from ingestion of contaminated underground water (boreholes and wells) is enormous.

Landfill leachate may be characterized as a water-based solution of groups of contaminants like; dissolved organic matter (alcohols, acids, aldehydes, short chain sugar), Inorganic macro-components (common cations and anions including sulfate, chloride, iron, aluminum, zinc and ammonia), Heavy metals (Pb, Ni, Cu, Hg) and Xenobiotics organic compounds such as halogenated organics (PCBs, Dioxins) [3].

Leachate is any liquid that in the course of passing through matter extracts soluble or suspended solids, or any other components of material through which it has passed. When water percolates through waste, it promotes and assists the process of decomposition by bacteria and fungi [4]. These processes in turn release by-products of decomposition and rapidly use up any available oxygen, creating an anoxic environment.

The Port Harcourt (Eliozu) landfill is one of the biggest landfills in Port Harcourt metropolis. It receives deposits of both domestic and industrial wastes from Port Harcourt, these waste products are dumped in this landfill untreated, and thus may pose serious environmental risks to inhabitants in the area and entire Port Harcourt population directly or indirectly. Despite its potential hazardous nature to Port Harcourt metropolis, no study has been done on the toxicological effect of Port Harcourt (Eliozu) landfill leachate on human population around the landfill. Near-by residences around the landfill are also not aware of the contamination of water from their wells and boreholes from leachate thus, the need for the present research. This report therefore attempts to evaluate the effects of Port Harcourt (Eliozu) landfill leachate on serum and kidneys oxidative stress using oxidative stress markers in experimental animals (wistar rats) as models and is part of a more extensive assessment of the potential toxicological effects of this leachate ignored by municipal authorities in Port Harcourt, Nigeria

II. Materials and Methods

2.1 Sampling Procedures and Analysis

Raw leachate fluids were collected from leachate well on the landfill and sample was taken to laboratory in a clean and dry 5litres plastic containers. The obtained leachate fluid was filtered using glass wool and Whatmann No.42 filter paper to remove suspended particles. The filtrate was centrifuged at 3000rpm for 10 minutes and stored at 4^{o} C. The supernatant fluid obtained was considered as stock samples (100%) and labeled as Port Harcourt (Eliozu) landfill leachate (PELL).

2.2 Acute Toxicity: LD50 Determination

LD50 of the supernatant fluid was determined using the Karber's method [5] in Wistar rats. The value was estimated at 1.5ml.

2.3 Experimental Animals

25 Wistar rats weighing between 180g and 250g were obtained from the animal house unit of Faculty of Basic Medical Sciences, University of Port Harcourt, Nigeria. The animals were acclimatized for 14days before treatment and were maintained under standard laboratory conditions of 12-hour dark and light cycle with ready access to drinking water and standard rodent chow (*ad libitum*). The animals were treated according to the guide for the Care and use of laboratory animals published by United State for Laboratory and Animal Research [6].

2.4 Experimental Design

The rats were divided into five groups of five animals each. Each experimental group was treated as follows,
Group 1: received 1ml of commercially obtained bottled water
Group 2: received 1ml of water obtained from borehole 1KM from the Port Harcourt Eliozu landfill.
Group 3: received 1ml of 10% of leachate concentrations
Group 4: received 1ml of 50% of leachate concentrations
Group 5: received 1ml of 100% of leachate concentrations
All water and leachate were administered once daily using an oro-gastric cannula for 90 consecutive days. All animals had free access to rat feeds and water *ad libitum*.

2.5 Determination of oxidative stress markers

On day 91, blood was collected by direct cardiac puncture for the determination of serum oxidative stress markers. About 4ml of blood was collected from each animal and put in EDTA bottles for analysis, the following serum oxidative stress markers were determined, catalase, superoxide dismutase and malondialdehyde. The kidneys were harvested and put in a mortar with iced cubes and grinded to supernatant for the determination of kidney oxidative stress markers. Catalase was determined according to the method described by Clairbone[7] with slight modification. Superoxide dismutase activity was estimated using the method described by Mistra and Fridovich[8]. Malondialdehyde was measured by measuring formation of thiobarbituric acid reactive substance (TBARS) according to Ohkawa and Ohishi[9].

2.6 Statistical Analysis

Data obtained were subjected to statistical analysis. The analysis of variance (ANOVA) was used to compare the various groups and the significant differences were set at a p level of 0.05 and are indicated on the Tables with asterisk.

2.7 Ethical Approval

Protocols for this study was approved by the ethical committee, University of Port Harcourt. All animal used in this study were handled properly following the international, natural and institutional guidelines for care and use of laboratory animals in biomedical research as promulgated by the Canadian Council of Animal Care (1984). The procedures carried out on them were in accordance with the guidelines of Ethical Committee of the National Institutes of Health Guide for Care and use of Laboratory animals (Publication No 85-23 revised 1996).

III. Results and Discussion

3.1 Effect of Port Harcourt (Eliozu) landfill leachates on serum oxidative stress markers

Table 1 shows the effect of Port Harcourt Eliozu landfill leachate on some serum oxidative stress markers investigated. Compared to control (group 1, commercially obtained bottle water) rats, administration of Port Harcourt Eliozu landfill leachate significantly increased the values of Catalase and Superoxide dismutase in a dose dependent manner amongst Group 3, 4 and 5 rats ($p<0.001$). For instance, the value of catalase increased

from 25.80±0.95 amongst Group 3 rats to 29.96±0.84 amongst Group 4 rats to the value of 36.22±0.90 amongst Group 5 rats. These differences were found to be significant ($p<0.001$) and dose dependent.
However, value of Malondialdehyde showed a not significant increase that is not dose dependent amongst Group 3, 4 and 5 rats ($p<0.09$) compared to the control (group 1, commercial non-carbonated bottle water). For instance, the value of Malondialdehyde decreased from 65.74±6.11 amongst Group 3 rats to 50.45±2.72 amongst Group 4 rats and increased to the value of 69.16±7.40 amongst Group 5 rats. The differences were found to be not significant and not dose dependent.

Table 1. Effects of Port Harcourt (Eliozu) landfill leachates on serum antioxidative parameters

PARAMETERS	Group 1 (Commercial non-carbonated bottle water)	Group 2 (Near-by borehole water)	Group 3 (PELL 10%)	Group 4 (PELL 50%)	Group 5 (PELL 100%)
Catalase (mg/protein)	18.92±1.45 (16.5-24.5)	30.04±0.22* (29.5-30.9)	25.80±0.95* (24.0-29.4)	29.96±0.84* (26.9-31.2)	36.22±0.90* (33.7-38.1)
Superoxide Dismutase (mg/protein)	16.46±0.98 (12.8-18.5)	18.56±0.30 (18.0-19.6)	17.24±0.56 (16.1-19.1)	20.66±1.50 (16.5-24.7)	28.12±3.60* (23.5-42.0)
Malondialdehyde (mg/protein)	50.35±9.10 (29.4-83.4)	57.54±5.20 (44.4-75.7)	65.74±6.11 (46.5-83.0)	50.45±2.72 (42.9-59.9)	69.16±7.40 (47.5-89.2)

*Significant difference compared with Group 1 ($p<0.05$)

Table 2: shows results the effect of Port Harcourt Eliozu landfill leachate on renal antioxidative parameters examined. Compared to control (group 1, commercial non-carbonated bottle water) rats, administration of Port Harcourt Eliozu landfill leachate significantly increased the values of catalase, superoxide dismutase and Malondialdehyde in a dose dependent manner amongst Groups 3, 4 and 5 rats ($p<0.001$). For example, the value of Superoxide dismutase increased from 153.95±5.33 amongst Group 3 rats to 155.50±17.70 amongst Group 4 rats to the value of 233.42±6.53 amongst Group 5 rats. These differences were found to be significant ($p<0.001$) and dose dependent.

Table 2: Effects of Port Harcourt (Eliozu) landfill leachates on renal antioxidative parameters

PARAMETERS	CONTROL (Commercial non-carbonated bottle water)	GROUP 2 (Near-by borehole)	GROUP 3 (PELL 10%)	GROUP 4 (PELL 50%)	GROUP 5 (PELL 100%)
Catalase (mg/protein)	18.80±0.70 (16.3-20.5)	24.28±0.70* (22.9-26.8)	17.88±0.02 (17.8-17.9)	21.76±1.50* (16.4-25.5)	29.88±1.11* (26.3-32.9)
Superoxide Dismutase (mg/protein)	42.12±1.22 (39.6-46.3)	39.56±0.48 (38.2-41.0)	40.70±1.16 (36.9-43.9)	42.24±3.74 (34.8-56.2)	62. 88 ±4.51* (45.9-69.9)
Malondialdehyde (mg/protein)	117.11±8.60 (83.2-128.3)	152.14±11.39* (126.5-193.3)	153.95±5.33* (136.9-168.8)	155.50±17.70* (106.6-197.2)	233.42±6.53* (212.0-251.2)

*Significant difference compared with Group 1 ($p<0.05$)

3.2 DISCUSSION

The paucity of data on waste generation and its effect on health environment around Port Harcourt metropolis continue to be a pivotal problem thus inhibiting extant regulation. There is very little known about leachates, so efforts to put exposure levels to protect humans often prove very ineffective. Worthy of note is that pollution decreases the quality of life in different area and affects health and life span [10].

After analysis of the basic properties and chemical composition of leachates in PELL, it shows that landfill leachate is a mixture and contains varieties of contaminants; there are general underlying pollutants common to all landfill effluents [11].

In the present study we observed significant increase in serum CAT, SOD and statistically non-significant elevation of MDA when compared with the control and paradoxically, we observed statistically significant increase in kidney CAT, SOD and MDA.

Catalase is a tetrameric 240KDA enzyme which reacts very efficiently with H_2O_2 Ig to form water and molecular oxygen. It is known to protect cells from hydrogen peroxide generated within them [12].

Superoxide is the free radical produced during the natural pathway of oxidative phosphorylation. The enzyme SOD is an intracellular antioxidant enzyme which readily dismutase superoxide [13]. The SOD converts superoxide primarily into hydrogen peroxide. The increase recorded in SOD in serum and kidney activities in the animals exposed to leachate may be due to induction of enzyme synthesis by oxidants present in the leachate [14].

Measurement of MDA in the serum of the rats exposed to PELL and BHW increased relative to control without significance while there was a significant increase in kidney MDA. Lipid peroxidation is the systematic oxidation by free radicals fatty acids in the cell membrane. A by-product of lipid peroxidation, MDA is used to assess the level of lipid peroxidation. This is consistence with the work of [12], where there is no significant increase in the MDA of liver of Poicella Sp. when compared with the control.

The elevated peroxidation observed in the kidney of the animals exposed to PELL and BHW may be due to the presence of reactive oxygen species.

IV. Conclusion

In conclusion, this study reports that Port Harcourt (Eliozu) landfill leachate caused increased level of peroxidation whichsuggests that there were increased production of ROS and that certain substances from the leachate caused damage to membrane lipids of the cell and we recommend further studies in this regard.

Author Contribution

All authors contributed to the drafting of the research protocol. All authors have read and approved the final manuscript.

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Iyke I.Weleh,etal. "Effect of Port Harcourt (Eliozu) Landfill Leachates on Oxidative Stress Using Oxidative Stress Markers in Wistar Rats." *IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)*, 13(4), (2020): pp 01-04.