Toxicological Evaluation of Unlined Landfill Leachate on Biochemical Responses in the Tissues of *Rattus novergicus*

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***Abstract*: Effect of unlined leachate on biochemical responses in different tissues of *Rattus novergicus* was evaluated using different sub-lethal concentrations (5, 10, 15 and 20% v/v) over a period of 28 days. SOD in serum, heart, liver, stomach and colon, CAT values in heart and colon in control are significantly higher compared to Group 2,3 and 4 ($p<0.05$), SOD in stomach and colon in Group 4 was 158.15±0.09 and 195.70±0.06 while control was 192.80±0.17 and 169.96±0.40 respectively. CAT of stomach and colon in Group 3 was 0.23±0.00 and 0.22±0.00 while control was 0.29±0.00 and 0.23±0.00 respectively. Elevated Malondialdehyde (MDA) is an evidence of lipid peroxidation where MDA values in heart, liver, kidney, stomach and colon of all experimented groups are significantly higher than control ($p<0.05$), MDA of stomach and colon of rats in Group 1 was 375±175 and 339±131 while control was 114±4.36 and 123±3.00 respectively. Also, Leachate constituents interfere with protein and carbohydrate metabolisms in the liver by loss of Alanine aminotransferase (ALT) activity subjecting liver lipids to peroxidation based on Ascorbic acid (ASB) level leading to loss of membrane integrity. ALT values in heart, liver and stomach are significantly lower in all experimented groups compared to control ($p<0.05$), ALT of liver in Group 2 was 0.07±1.74E-04 while control was 0.10±2.79E-04. ASB values in kidney, stomach and colon are significantly lower in all experimented groups compared to control ($p<0.05$), ASB of stomach and colon of rats in Group 3 was 1.35±1.90E-03 and 1.22±1.17E-03 while control was 1.22±8.92E-03 and 1.23±8.64E-03 respectively.**

***Keywords*: *Unlined landfill leachate, Toxicological evaluation, Toxicity, Biochemical responses, Tissues*.**

I. INTRODUCTION

What defines waste is subjective; one person's waste might be a valuable resource for another. The creation of wastes can be both a physical and psychological activity [1]. Disposal method suitable for a particular type of waste depends on the type of waste in an area, and the collection system is dependent on the cooperation of households and individuals in various sectors of the city in providing containers for storing refuse and placing materials for collection on a regular basis. The term "waste" refers to man's undesirable items that must be discarded. Given the degree of scavenging on waste heaps in less developed nations like Nigeria by both humans and animals, it may not be widely recognized that solid wastes are "useless, unwanted, or abandoned (undesirable) items." In Nigeria, waste evacuation and disposal is a major issue, and most towns, including Warri and its metropolis, lack adequate management facilities and properly designed landfills. Lack of properly built sanitary landfill by local government authorities and the state government has allowed for the growth of open dumps that are distributed across the city and beyond.

Municipal Solid Waste (MSW) is found in companies, hospitals, schools, and private residences [2]. Glass, food waste, metals, textiles, wood, plastics, and paper make up MSW, which is a biomass waste type. It is made up of a range of biodegradable organic compounds, which is a collection of heterogeneous materials whose chemical properties are intimately linked to the chemical qualities of their constituent components. Open dumping and landfilling are the most popular techniques for managing MSW. Both methods have negative consequences such as pollution of the environment, which contributes to global warming, and labor difficulties. Leachate is water that has percolated through a solid and leached out some of the constituents. It is a by-product from dumpsites, incineration facilities, composting plants, and transfer stations from changes in municipal solid wastes. It has a high strength and toxicity [3]. It is a word common in the environmental sciences to describe a liquid that has dissolved or entrained ecologically hazardous chemicals that may later be released. Wastes are deposited in unlined landfills in many poor nations, and the leachates are released without treatment. This has environmental repercussions, particularly for species living in surface water receptors where leachates are released. Leachates are frequently evaluated using physico-chemical techniques, and the findings are compared to regulatory criteria. Because of the complexity of landfill effluents, bioassays must be employed to determine toxicity [4].

This present study evaluates the toxicity of Leachate from unlined landfill on antioxidant enzymes activities of *Rattus novergicus* (albino rats) and the biochemical reactions of the various antioxidant enzymes present in the rats' cells. It evaluates serum and enzymes antioxidants biochemical responses of organ tissues (colon, stomach, liver, kidney, and heart) orally exposed to leachate from unlined dumpsite. The enzymes antioxidants are: Alanine aminotransferase (ALT), Superoxide Dismutase (SOD), Malondialdehyde (MOD), Catalase (CAT), and Ascorbic Acid (ASB).

Elevated liver enzymes in blood is indicative of severe injury to hepatic tissues [5]. In a previous study, rats given 100 percent Port-Harcourt Eleme Landfill Leachate (PELL) for 90 days had significantly higher ALT serum activity. These enzymes are found in periportal hepatocytes, indicating that their oxidative phosphorylation and gluconeogenesis activities are increase of

cellular membrane disruption and leaking. This emphasizes their utility as biochemical markers for hepatic injury. AST and ALT in the blood indicate liver injury following PELL exposure. Even though hepatocytes have developed enzymatic and non-enzymatic mechanisms to deal with reactive free radicals, once oxidative stress is established, the defensive capabilities against ROS become negligible [6]. The enhanced liver enzyme activity matched the findings of a research on liver in distillery soil leachate-treated Swiss mice. The increased liver enzymes matched prior histological study. The end point for diagnosing organ toxicity is histopathological examination, which is effective in identifying the sorts of changes produced by xenobiotics.

Hundred (100) percent PELL treated group looked to be at danger of severe damage. Hepatic architecture was disrupted, hepatocyte necrosis was seen, and inflammatory cells were present. Toxic substances in leachate have been shown to have hematotoxic potential. In rats, raw and simulated leachates from the Olusosun dumpsite were shown to have immunotoxic potential. There were biochemical and histological alterations profile in distillery soil leachate treated Swiss Mice of 5%, 10%, and 20% leachate, according to studies. Hepatotoxicity and oxidative stress were also observed in rats in a similar research in Olusosun dumpsite leachate in Ojota, Lagos State, Nigeria. The result showed reduced SOD activity and increased MDA activity.

The particular activity of liver tissue and serum transaminases (AST and ALT) in phenol-contaminated water have also been documented. Transaminases monitor the liver's function, in the serum, and to anticipate liver disease. ALT specific activity reduction suggested that phenol-contaminated water components may be inhibiting the enzymes or causing their leaking into the serum. According to a previous study, high ALT levels in the blood showed hepatic enzyme leakage, which can severely damage liver function. Leakage of enzymes from the cell into the extracellular space is suggested by the therapy. The decrease of AST and ALT was shown to be inversely proportional to the quantity of contaminants, such as ammonia in polluted or contaminated water**.**

II. MATERIALS AND METHODS

Rattus novergicus of both sexes, weighing 200mg/kg, were obtained from the Animal House of College of Earth Science, Delta State University, Abraka, Nigeria. They were acclimatized for one (1) week prior to the commencement of exposure. 6 rats/cage/sex were randomly assigned to a concentration each of the toxicant; with distilled water as control. They were maintained in laboratory conditions of 12-hour dark and light cycle, temperature of 26 ± 2°C, relative humidity of 70 ± 20% and has access to drinking water and standard rodent chow (Ladokun feed Nigeria®) *ad libitum.* Each animal in a group was gavaged with 1000ml of 5,10, 15 and 20 % (leachate diluted with distilled water, v/v) concentrations of each of the leachates for 28 consecutive days. Similar treatment was concurrently given to the control group receiving distilled water.

The experimented animals were classified into 5 groups as follows; Group A: Control - distilled water only (1000ml). Group 1: 5% - 50ml EAL (Ehwerhe Agbarho Leachate)/ 950ml distilled water. Group 2: 10% - 100ml EAL / 900ml distilled water. Group 3: 15% - 150ml EAL / 850ml distilled water. Group 4: 20% - 200ml EAL / 800ml distilled water for 4 weeks (28 days).

The toxicant was administered to the rats morning and evening. EAL: Ehwerhe Agbarho Leachate. Doses of toxicant: 200 mg/kg body weight**.** Routes of administration: Toxicant was administered orally. Source of Toxicant: Leachate from unlined landfill at Agbarho. They were fed *ad libitum* with commercial rat chow throughout the experiment period. The experimented animals were anaesthetized by placing them in a jar containing cotton wool soaked with chloroform before being sacrificed by jugular puncture. And were quickly dissected and the whole liver and kidney were excised, freed of fat, blotted with clean tissue paper and weighed into a beaker containing ice cold 0.25M sucrose solution. The blood was obtained through cardiac puncture. A portion of the blood was collected in heparinized bottles and others in nonheparinized bottles. Serum was collected by centrifuge at 3,5000 rpm for 15 minutes and preserved at 8^{o}C. The isolated tissues were weighed and a portion of each tissue was cut out, chopped and then homogenized using pre-cooled pestle and mortar in a bowl of ice cubes. The tissue homogenates were diluted using 0.25M sucrose solution to the tune of 1 in 30 dilutions. A portion was homogenized for biochemical studies and enzyme assays. The diluted homogenates were stored at temperature of 8^{o}C until required for use.

Serum biochemical marker: organ tissues homogenates were evaluated for enzyme Alanine aminotransferase (ALT) by the method of Reitman and Frankel (1957) modified by Schmidt and Schmidt (1963). And antioxidant enzyme activities; superoxide dismutase (SOD) was assayed by the method of Misra and Fridovich (1972), while catalase (CAT) was by the method of Sinha (1971). Lipid peroxidation concentration in the organ tissues homogenate and serum was by malondialdehyde (MDA) and Ascorbic acid (ASB) determination by the methods of Varshney and Kale (1990); Roe and Kurethor (1943) respectively.

Statistical tools were applied to calculate the significance of the differences between control and experimental means. P values of 0.05 or less were considered statistically significant [7].

III. RESULTS

Specific activity of enzymes antioxidants of selected vital tissues (stomach, heart, liver, kidney and colon) of rats given water contaminated with Ehwerhe Agbarho Leachate (EAL) extracted from unlined landfill (dumpsite) are presented in Tables 1-5 below.

Table 1 presented specific activity of Superoxide Dismutase (SOD) of selected tissues of *Rattus novergicus* orally exposed to contaminated water with Leachate (EAL). Generally, in Group 1, the specific activity of SOD is significantly ($p<0.05$) lower in serum and colon but significantly ($p<0.05$) higher in heart, liver, stomach and kidney in relative to Group A (control). In Group 2, the specific activity of SOD was significantly ($p<0.05$) lower in serum and selected tissues (heart, liver, stomach and colon) and significantly ($p<0.05$) higher in kidney relative to Group A (control). In Group 3, the specific activity of SOD was significantly ($p<0.05$) lower in serum and selected tissues (heart, liver, stomach and colon) and significantly ($p<0.05$) higher in kidney relative to Group A (control). In Group 4, the specific activity of SOD was also significantly ($p<0.05$) lower in serum and selected tissues (heart, liver, stomach and colon) and significantly ($p<0.05$) higher in kidney relative to Group A (control). This showed a definite

pattern in Groups 2-4. It generally also showed a decrease in the specific activity of SOD in serum and colon with increase in level of the toxicant EAL. All selected tissues except colon showed a significantly ($p<0.05$) higher specific activity of SOD in Group 1, showing an increase in specific activity with increase in the toxicant concentration.

TABLE 1: SPECIFIC ACTIVITY (u/mg protein) OF ANTIOXIDANT SUPEROXIDE DISMUTASE (SOD) OF VITAL ORGANS OF RATS GIVEN ORAL ADMINISTRATION OF LEACHATE FROM UNLINED LANDFILL (EHWERHE DUMPSITE AGBARHO)

GROUPS	SERUM	HEART	LIVER	STOMACH	KIDNEY	COLON
A	614 ± 11.38^{a}	164 ± 0.56^{a}	201 ± 0.37^{a}	193 ± 0.17^{a}	190 ± 0.12^{a}	170 ± 0.40^{a}
1	414 ± 6.34^{b}	175 ± 0.61^{b}	215 ± 0.51^{b}	189 ± 0.21^{b}	160 ± 0.34^{b}	155 ± 0.19^{b}
2	970 ± 11.30^{c}	164 ± 0.11^{c}	184 ± 0.32^{c}	191 ± 0.15^{c}	165 ± 0.17^{c}	194 ± 0.22^{c}
3	860 ± 3.42^{d}	202 ± 0.37^{d}	198 ± 0.32^{d}	195 ± 0.06^{d}	133 ± 3.14^{d}	201 ± 0.24^{d}
4	496 ± 8.85^{e}	203 ± 0.40^{e}	198 ± 0.04^{e}	158 ± 0.09^{e}	148 ± 0.35^{e}	196 ± 0.06^{e}

Notes: Tabulated results are means (mean ± SEM) of six determinations (n=6). A=Rats placed on 1000ml distilled water (control). 1=Rats placed on water contaminated with leachate (5% - 50ml EAL / 950ml distilled water), 2=Rats placed on water contaminated with leachate (10%¼ 100ml EAL / 900ml distilled water), 3=Rats placed on water contaminated with leachate (15% - 150ml EAL / 850ml distilled water), 4=Rats placed on water contaminated with leachate (20% - 200ml EAL / 800ml distilled water).
Values on the same column carrying different superscripts are significantly different ($p < 0.05$).

Table 2 presented the specific activity of Catalase (CAT) of selected tissues of *Rattus novergicus* orally exposed to contaminated water with EAL. Generally, administration of EAL significantly ($p<0.05$) reduced activity of CAT in all experimented Groups (1-4) in relative to Group A (control). There was generally a reduced specific activity with increase in toxicant. Specifically, in Group 1, the specific activity of CAT was significantly ($p<0.05$) lower in serum, heart, liver, stomach and kidney relative to Group A (control). But the specific activity of CAT was significantly ($p<0.05$) higher in colon, though relative to Group A (control), it showed no significant difference. Generally, in Group 2 as the concentration of toxicant was increased, the specific activity of CAT in serum, heart, liver and stomach was significantly lower ($p<0.05$) and significantly ($p<0.05$) higher in kidney and colon relative to Group A (control).

Group 3 showed a definite pattern, the specific activity of CAT in serum and liver was significantly ($p<0.05$) lower and significantly ($p<0.05$) higher in heart, kidney, stomach and colon, specifically with heart showing no significance difference relative to Group A (control). In Group 4, the specific activity of CAT in liver was significantly ($p<0.05$) lower and significantly ($p<0.05$) higher in serum and other selected tissues (heart, kidney, stomach and colon) relative to Group A (control) as the level of toxicant EAL increased.

TABLE 2: SPECIFIC ACTIVITY (u/mg protein) OF ANTIOXIDANT CATALASE (CAT) OF VITAL ORGANS OF RATS GIVEN ORAL ADMINISTRATION OF LEACHATE FROM UNLINED LANDFILL (EHWERHE DUMPSITE AGBARHO)

GROUPS	SERUM	HEART	LIVER	STOMACH	KIDNEY	COLON
A	0.35 ± 0.00^{a}	0.25 ± 0.00^{a}	0.27 ± 0.00^{a}	0.29 ± 0.00^{a}	0.27 ± 0.00^{a}	0.23 ± 0.00^{a}
1	0.25 ± 0.00^{b}	0.23 ± 0.00^{b}	0.23 ± 0.00^{b}	0.23 ± 0.00^{b}	0.23 ± 0.00^{b}	0.23 ± 0.00^{a}
2	0.29 ± 0.00^{c}	0.22 ± 0.00^{c}	0.25 ± 0.00^{c}	0.42 ± 0.00^{c}	0.23 ± 0.00^{b}	0.41 ± 0.00^{b}
3	0.28 ± 0.00^{d}	0.25 ± 0.00^{a}	0.25 ± 0.00^{d}	0.23 ± 0.00^{b}	0.24 ± 0.00^{c}	0.22 ± 0.00^{c}
4	0.25 ± 0.00^{b}	0.23 ± 0.00^{b}	0.33 ± 0.00^{e}	0.37 ± 0.00^{d}	0.23 ± 0.00^{b}	0.24 ± 0.00^{d}

Notes: Tabulated results are means (mean ± SEM) of six determinations (n=6). A=Rats placed on 1000µl distilled water (control). 1=Rats placed on water contaminated with leachate (5% - 50ml EAL / 950ml distilled water), 2=Rats placed on water contaminated with leachate (10%¼ 100ml EAL / 900ml distilled water), 3=Rats placed on water contaminated with leachate (15% - 150ml EAL / 850ml distilled water), 4=Rats placed on water contaminated with leachate (20% - 200ml EAL / 800ml distilled water).
Values on the same column carrying different superscripts are significantly different ($p < 0.05$).

Table 3 presented the specific activity of Malondialdehyde (MDA). Generally, administration of EAL toxicant to *Rattus novergicus* followed a definite pattern in that the specific activity of MDA was increased as concentration of toxicant increased in serum and selected tissues of all experimented Groups (1-4) in relative to Group A (control). Generally, in Group 1, the specific activity of MDA was significantly ($p<0.05$) higher in selected tissues (heart, liver, kidney, stomach and colon) and significantly ($p<0.05$) lower in serum in relative to Group A (control). Groups 2 showed similar trend as Group 1 in which the specific activity of MDA was significantly ($p<0.05$) higher in all selected tissues (heart, liver, kidney, stomach and colon) and significantly ($p<0.05$) lower in serum relative to Group A (control). Group 3 showed a significantly ($p<0.05$) higher activity in all selected tissues but a significantly ($p<0.05$) lower activity in serum relative to Group A (control). Generally, Group 3 also showed a higher increase in significance level in activity in all groups. Group 4 also followed a similar trend in which all selected tissues had a significantly ($p<0.05$) higher activity whereas there was a significantly ($p<0.05$) lower activity in serum with the least level of significance in activity relative to Group A (control). It follows an increase in activity of MDA with toxicant EAL increase except for serum in relative to Group A (control).

TABLE 3: SPECIFIC ACTIVITY (u/mg protein) OF ANTIOXIDANT MALONDIALDEHYDE (MDA) OF VITAL ORGANS OF RATS GIVEN ORAL ADMINISTRATION OF LEACHATE FROM UNLINED LANDFILL (EHWERHE DUMPSITE AGBARHO)

GROUPS	SERUM	HEART	LIVER	STOMACH	KIDNEY	COLON
A	907±549^{a}	114E±8.56^{a}	113±12.02^{a}	111±4.36^{a}	114±5.60^{a}	123±3.00^{a}
1	381±174^{b}	353±77.34^{b}	311±139^{b}	375±175^{b}	381±103^{b}	339±131^{b}
2	377±100^{c}	322±84.93^{c}	411±33.23^{c}	412±75.32^{c}	355E±117^{c}	385±9.99^{c}
3	403±46.67^{d}	363±173^{d}	337±186^{d}	366±80.90^{d}	314E±179^{d}	399±67.64^{d}
4	424±4.27^{e}	335±326^{e}	232±131^{e}	228±51.87^{e}	228±111^{e}	227±96.41^{e}

Notes: Tabulated results are means (mean ± SEM) of six determinations (n=6). A=Rats placed on 1000µl distilled water (control). 1=Rats placed on water contaminated with leachate (5% - 50ml EAL / 950ml distilled water), 2=Rats placed on water contaminated with leachate (10%¼100ml EAL / 900ml distilled water), 3=Rats placed on water contaminated with leachate (15% - 150ml EAL / 850ml distilled water), 4=Rats placed on water contaminated with leachate (20% - 200ml EAL / 800ml distilled water).
Values on the same column carrying different superscripts are significantly different ($p < 0.05$).

Table 4 showed the specific activity of ALT concentration on serum and selected tissues of *Rattus novergicus* contaminated with toxicant EAL. Toxicant EAL generally caused a significantly ($p<0.05$) reduced specific activity of ALT in all experimented Groups (1-4) as the percentage concentration of the toxicant EAL increased. Generally, in Group 1, the specific activity of ALT was significantly ($p<0.05$) lower in serum and selected tissues (heart, kidney, stomach and colon) and no significant difference in liver relative to Group A (control). In Group 2, the specific activity of ALT was significantly ($p<0.05$) lower in serum and selected tissues (heart, kidney, stomach and colon) and significantly ($p<0.05$) higher in liver relative to Group A (control). In Group 3, the specific activity of ALT was significantly ($p<0.05$) lower in serum, heart and kidney and significantly ($p<0.05$) higher in liver, stomach and colon with colon showing a higher increase relative to Group A (control). In Group 4, the specific activity of ALT was significantly ($p<0.05$) lower in serum and kidney and significantly ($p<0.05$) higher in heart, liver, stomach (having a higher increase) and colon relative to Group A (control).

TABLE 4: SPECIFIC ACTIVITY (u/mg protein) OF ENZYME ALANINE AMINOTRANSFERASE (ALT) OF VITAL ORGANS OF RATS GIVEN ORAL ADMINISTRATION OF LEACHATE FROM UNLINED LANDFILL (EHWERHE DUMPSITE AGBARHO)

GROUPS	SERUM	HEART	LIVER	STOMACH	KIDNEY	COLON
A	0.05±0.00^{a}	0.05±0.00^{a}	0.06±0.00^{a}	0.07±0.00^{a}	0.05±0.00^{a}	0.05±0.00^{a}
1	0.10±0.00^{b}	0.10±0.00^{b}	0.10±0.00^{a}	0.09±0.00^{b}	0.10±0.00^{b}	0.09±0.00^{b}
2	0.07±0.00^{c}	0.07±0.00^{c}	0.07±0.00^{b}	0.08±0.00^{c}	0.07±0.00^{c}	0.06±0.00^{c}
3	0.08±0.00^{d}	0.08±0.00^{d}	0.08±0.00^{c}	0.08±0.00^{c}	0.09±0.00^{d}	0.09±0.00^{b}
4	0.09±0.00^{e}	0.08±0.00^{d}	0.09±0.00^{d}	0.09±0.00^{b}	0.08±0.00^{e}	0.10±0.00^{d}

Notes: Tabulated results are means (mean ± SEM) of six determinations (n=6). A=Rats placed on 1000µl distilled water (control). 1=Rats placed on water contaminated with leachate (5% - 50ml EAL / 950ml distilled water), 2=Rats placed on water contaminated with leachate (10%¼100ml EAL / 900ml distilled water), 3=Rats placed on water contaminated with leachate (15% - 150ml EAL / 850ml distilled water), 4=Rats placed on water contaminated with leachate (20% - 200ml EAL / 800ml distilled water).
Values on the same column carrying different superscripts are significantly different ($p < 0.05$).

Table 5 presented Ascorbic Acid (ASB) specific activity of serum and selected tissues (heart, liver, kidney, stomach and colon) of *Rattus novergicus* exposed to EAL toxicant. Generally, in Group 1, specific activity of ASB was significantly ($p<0.05$) higher in kidney and significantly ($p<0.05$) lower activity in serum, heart, liver, stomach (having more reduced activity) and colon relative to Group A (control). Group 2 specific activity of ASB was significantly ($p<0.05$) higher in serum and significantly ($p<0.05$) lower in heart, liver, kidney (having more reduced activity), stomach and colon relative to Group A (control). In Group 3, the specific activity of ASB was a significantly ($p<0.05$) higher in heart significantly ($p<0.05$) lower in serum, liver, kidney, stomach and colon relative to Group A (control). In Group 4, ASB was significantly ($p<0.05$) lower in serum and all selected tissues (heart, liver, kidney, stomach and colon) relative to Group A (control). Generally, EAL caused lower specific activity of ASB in all experimented Groups (1-4) as the concentration of toxicant EAL increased.

TABLE 5: SPECIFIC ACTIVITY (u/mg protein) OF ANTIOXIDANT ASCORBIC ACID (ASB) OF VITAL ORGANS OF RATS GIVEN ORAL ADMINISTRATION OF LEACHATE FROM UNLINED LANDFILL (EHWERHE DUMPSITE AGBARHO)

GROUPS	SERUM	HEART	LIVER	STOMACH	KIDNEY	COLON
A	1.07±0.00[a]	1.02±0.00[a]	1.23±0.01[a]	1.22±0.01[a]	1.27±0.01[a]	1.23±0.01[a]
1	1.09±0.00[b]	1.04±0.00[b]	1.26±0.01[b]	0.94±0.00[b]	1.85±0.02[b]	1.01±0.01[b]
2	1.25±0.01[c]	1.25±0.00[c]	1.03±0.00[c]	1.28±0.01[c]	1.26±0.00[c]	1.22±0.01[c]
3	1.05±0.00[d]	1.22±0.01[d]	1.01±0.00[d]	1.35±0.00[d]	1.01±0.00[d]	0.97±0.00[d]
4	1.04±0.00[e]	0.98±0.00[e]	1.25±0.00[e]	1.13±0.00[e]	1.08±0.00[e]	1.04±0.01[e]

Notes: Tabulated results are means (mean ± SEM) of six determinations (n=6). A=Rats placed on 1000µl distilled water (control). 1=Rats placed on water contaminated with leachate (5% - 50ml EAL / 950ml distilled water), 2=Rats placed on water contaminated with leachate (10% - 100ml EAL / 900ml distilled water), 3=Rats placed on water contaminated with leachate (15% - 150ml EAL / 850ml distilled water), 4=Rats placed on water contaminated with leachate (20% - 200ml EAL / 800ml distilled water).
Values on the same column carrying different superscripts are significantly different ($p < 0.05$).

IV. DISCUSSION

There is convincing evidence that chemicals in leachates from unlined dumpsites are capable of causing growth retardation, haematological abnormalities and altering the functioning of the biological systems [8,9]. The present study establishes the toxicological evaluation of water contaminated with major contaminants of unlined landfill leachate on the serum and biochemical parameters and the heart, kidney, liver, stomach and colon. There is growing increase in hematological abnormalities and organ diseases like cancer and tumor among the Nigerian population. Not much is known about the toxicological evaluation of dumpsite leachate on the stomach and colon of rats and this study particularly addressed that.

The findings herein showed the involvement of oxidative stress induction in the pathological alterations of Wistar rat (*Rattus novergicus*) organ tissues especially the stomach and colon exposed to unlined landfill leachates. The elevated concentrations of toxic metals and physicochemical parameters show the leachability from wastes in the unlined landfill. They are capable of inducing deleterious effects on biological systems linked to the generally specific activity increase of antioxidants CAT, SOD and MDA with concomitant decrease in specific activity of ASB and enzyme ALT of the experimented animals relative to the control. Alterations in specific activity of these given parameters suggest gastrointestinal damage to colon and stomach tissue by the leachate constituents; mostly the toxic metals. In the Niger-Delta particularly in Warri metropolis where the unlined landfill under study is located, rise in the cases of colorectal cancer which is the most common gastrointestinal cancer occurs.

Measurement of enzyme biochemical reactions in tissues/organs is a proven sensitive tool for early detection of toxicological effect of xenobiotics [8].

Specific activities of both SOD in Table 1 and CAT in Table 2 were significantly reduced in serum and liver of Leachate-treated rats in experimented Groups 1-4 and Group 1 respectively, the reason we can adduce to this is that Leachate extracted from unlined landfill (dumpsite) perhaps, induce a condition of oxidative stress in the liver. Deficiencies in antioxidant enzymes may cause liver damage. Shown from this study, in Table 2, that the specific activity of CAT in kidney was significantly higher in all contaminated Groups (2-4) except Group 1, agreeing with [10] studies which showed that increased CAT activity may lead to renal injury through the combined effect of NADPH oxidase and hydrogen perioxidase scavenging resulting in hypertension, impaired renal function and elevated oxidative stress.

CAT disproportionates hydrogen perioxide and SOD is an oxidoreductase that serves to dismutase the superoxide anion. Increase in CAT level is proportional to the increase of severity level of colorectal carcinogenesis. Table 2 presented that the specific activity of CAT in all experimented groups (1-4) in colon was significantly increased agreeing with [11] studies which showed that a main feature of cancer cells responsible for carcinogenesis is an abnormal regulation of ROS mediate adverse pathological conditions such as angiogenesis and cancer. One of these reactive oxygen species is superoxide anion (02-) that activates carcinogenetic and has adverse effect that triggers mutation in tumor suppressor gene leading to DNA damage.

Antioxidant enzymes are a primary defense system that protects biological macromolecules from oxidative damage. Superoxide Dismutase (SOD) and Catalase (CAT) are antioxidant enzymes that form part of the complex system that protects the liver from oxidative damage. SOD is a key antioxidant enzyme involved in the metabolism of oxygen free radicals. Superoxide anion is dismutated by superoxide dismutases (SODs) to H_2O_2 that is catalyzed to H_2O by catalase. Low levels of either intracellular or extracellular ROS (e.g., superoxide and H_2O_2) are indispensable in many biochemical processes, including intracellular signaling, defense against microorganisms, and cell function [12,13]. Superoxide dismutase activity prevents oxidative damage by scavenging and converting superoxide anions to hydrogen peroxide, while catalase decomposes the hydrogen peroxide to protect tissues from highly reactive hydroxyl radicals.

Significant alterations in activity of the antioxidant enzymes (Tables 1 and 2) in the selected tissues (heart, liver, kidney, stomach and colon) and serum of the experimented rats is linked to the harmful action of the leachate constituents. Alterations in the antioxidant enzymes activities along with lipid perioxidation induction in the selected tissues than in serum support the vulnerability of tissues to leachate induced gastrointestinal cancer. The metallic ions in the leachates are known to permeate organ tissues altering membrane transporters. These metallic ions caused loss of functional integrity to the tissues cell membrane of the leachate treated rats through free radical formation.

Malondialdehyde (MDA) is one of the final products of polysaturated fatty acids peroxidation in the cells. An increase in free radicals causes overproduction of MDA. Malondialdehyde level is commonly known as a biomarker of oxidative stress and the antioxidant status in cancerous patients [14]. MDA is generated in vivo via peroxidation of polyunsaturated fatty acids. MDA

interacts with proteins and is itself potentially atherogenic. From this study, in Table 3, it is seen that the specific activity of MDA in selected tissues (heart, liver, kidney, stomach and colon) was significantly higher in all experimented Groups (1-4) of the leachate-treated rats which is in collaboration with [15] findings that MDA's reaction with lysine residues generates lysine–lysine cross-links which have been identified in apolipoprotein B (apoB) fractions of oxidized low density lipoprotein (OxLDL), and have been postulated to impair the interaction between OxLDL and macrophages and thereby promote atherosclerosis [16]. MDA could directly cause myoglobin autoxidation and myofibrillar carbonylation.

Table 4 presented that the specific activity of selected tissues (heart, kidney, stomach and colon) Alanine aminotransferase (ALT) of rats (*Rattus novergicus*) given Leachate from unlined landfill (dumpsite) was significantly lower in relative to control. ALT, a key enzyme of nitrogen is often used as a biochemical indicator of stress. The changes in the specific activity of ALT would often be reflected in nitrogen metabolism and interdependent biochemical reactions [17]. ALT is an enzyme found inside liver cells.

Reduction in the ALT specific activity of serum and selected tissues (heart, kidney, stomach and colon) ALT of experimented rats in Groups 1-4 corroborates the work of [18] which observed that reduction of ALP and ACP (examples of enzymes in liver function) may be as a result of the interactions between the constituents of the contaminated water with the Leachate and the components of the plasma membrane and cytoplasm, causing the leakage of enzyme into the serum. In this case it is of the view that loss of enzyme activity is probably due to leakage into the extracellular fluid due to liver injury induced by the constituents of the leachate. This supposed liver injury may be as a result of toxic stress. From this study, there was an increase in specific activity of ALT of the liver of rats in all experimented Groups (1-4) in Table 4 which is in agreement with [18] observations which showed that an increase in serum ALP may be due to an acute damage or injury of heart or lungs (myocardial or pulmonary infarctions). It had also been reported that the impairment of enzyme functions is one of the effects of ingesting polluted water.

Also, elevated hepatic enzyme ALT occurred in liver, stomach and colon in Groups 3 and 4 which may have possible effects on colon (large intestine) and stomach leading to tumor formation (gastrointestinal cancer), collaboration with recent findings that revealed close relationship between hepatic injury (liver injury), metabolic pathways and gut microbiota [19]. Increased hepatic injury causes increased intestinal permeability and increased levels of endotoxin and tumor in the system.

Ascorbic Acid (ASB) protects against oxidative stress on various tissues [20,21]. Oxidative stress refers to conditions of imbalance between productions mechanism which include enzymatic antioxidants and non-enzymatic antioxidants (Vitamin A, C and E), proteins liker albumin, transferrin, melatonin and glutathione (GSH). Reactive species are produced when metabolism of oxygen is incomplete in the mitochondrial respiratory chain. Regarding the antioxidant functions, ascorbate acts directly to scavenge oxygen or nitrogen based radical species generated during normal cellular metabolism. The antioxidant mechanisms of ascorbic acid are based on hydrogen atom donation to lipid radicals, quenching of singlet oxygen, and removal of molecular oxygen. Scavenging aqueous radicals and regeneration of a-tocopherol from the tocopheroxyl radical species are also well known antioxidant mechanisms of ascorbic acid. From this study, Table 5 showed that the specific activity of ASB was significantly lower in serum of Groups 1, 3 and 4 as well as in the selected tissues of all experimented Groups (1 – 4) of leachate treated rats. This may account for the rapid formation of free radicals in the tissues and thereby causing lipid perioxidation.

V. CONCLUSION

Based on the results obtained from the present investigations, it is concluded that ingestion of contaminated water may possibly cause a gastrointestinal damage leading to colorectal cancer as evidenced in the alteration of specific activity of CAT and SOD in the stomach and colon of the test rats. ASB acts directly to scavenge oxygen or nitrogen based radical species generated during normal cellular metabolism. Its reduced activity which results from loss of ALT activity leads to loss of membrane integrity and loss of CAT and SOD activity. Ingestion of contaminated water also alters the specific activity of antioxidant enzyme as evidenced by the decreased activity of ALT and ASB in heart, while there is an increased activity of MDA in the serum, heart, liver and kidney as observed in the test rats which may cause cardiovascular malfunctioning by increased induction of lipid peroxidation. The liver, as the central clearing house, plays a key role in xenobiotics metabolism. It was shown that leachate induce toxic stress as evidenced by enzymes activities. We have shown that Leachate constituents interfered with protein and carbohydrate metabolisms in the liver by the reduction in specific activity of Alanine aminotransferase (ALT) causing lipid peroxidation. Reduction in specific activity of Ascorbic acid (ASB) may lead to lipid peroxidation resulting in loss of membrane integrity, as detected by loss of CAT and SOD activity. Elevated MDA is another evidence of lipid peroxidation coupled with loss of enzymic and non-enzymic antioxidants. EAL induced toxic effects in test rats via oxidative stress provoked by the toxic chemicals in the leachate. This suggests possible danger to man and wildlife population in close proximity to unlined landfill facilities with hazardous substances via surface and ground water sources, like the one under study.

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