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Toxicological and Histopathological Evaluation of Leachate-Contaminated Groundwater on Liver of Albino Rats

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Abstract
This study investigates seepage of leachate from unlined landfill into groundwater and effect of the contaminated groundwater on biochemistry and histology of rats' hepatocytes. Water from boreholes and wells located within 1.5km radius to a landfill was given to rats as sources of water over a period of 65 days. The liver relative weight was determined. Alkaline and acid phosphatases, alanine and aspartate transaminases of the liver were assayed. Histopathological examination was also conducted on the liver of the rats. Results showed that the liver relative weight of rats in the leachate group was found to be significantly lower ($p<0.05$) relative to the rats in the tap water (control) group. Specific activity of ALP in liver of rats in tap water group was found to be 3 folds that of leachate group, 2 folds that of well (1km) group of rats and significantly higher than those of rats in the other groups. ACP activity of liver of leachate group was about 1/3 that of tap water and that of well (1km) group was found to be about 1/2 that of tap water group of rats. Generally, the specific activity of ALT and AST of liver of rats in other groups was found to be significantly lower ($p<0.05$) than that of rats in tap water group. Increasing body of evidence revealed that consumption of leachate-contaminated groundwater could result to damage to liver cells and loss of hepatic function.

Keyword: Leachate; groundwater; histopathological; toxicological; liver

Introduction
Groundwater constitutes by far the largest reserve of potable water in the habitable lands of the world. In arid region and areas remote from streams or lakes or other surface water, millions of people obtain groundwater from wells. Groundwater is the safest and most reliable source of available fresh water. Only 3% of earth's fresh water is located in streams, lakes and reservoirs. The remaining 97% of fresh water is groundwater. It can be utilized for energy production in the form of geothermal energy as well as energy conservation by using heat pumps (1). Since groundwater ultimately comes from the surface of the earth, contamination can be introduced from a wide variety of sources. With few exceptions, most pollution stems from human activities (2). Circulatory groundwater can leach elements such as arsenic, selenium, mercury and other toxic metal out of the minerals occurring naturally within rock and soil and consequently raising their concentration to harmful levels within the water (3).

Generally, groundwater pollution arises from two major sources; point and non-point sources. Point sources are factories, and other industrial and commercial installations that release toxic substances into the water. Non-point sources represent a much harder problem. They include landfills and underground septic tanks (4). Groundwater pollution whether from point or non-point sources is especially insidious because it is not visible and often goes undetected for sometimes.

Lagos has often been referred to as one of the dirtiest cities in the world as all water bodies in Lagos, both surface and groundwater are polluted. The quality of water has been adversely affected by the increase in population which leads to increase water use which in turn increases generation of wastewater. The water table in Lagos is very high, in some places it is about three meters from the surface. The soil is loose and easily permeable allowing infiltration of contaminants. In Nigeria, groundwater contamination is one of the least recognised environmental problems. This may be due to lack of awareness because groundwater problems are not readily detected and pathways for contamination are not as noticeable as those affecting surface water (5).

It had earlier been reported that leachate from Odo Iya Alaro landfill seeps into groundwater sited within 3km radius to it (5). The leachate therefore pollutes this groundwater sources and due to inadequate public awareness cum ignorance, people use this water for domestic purposes posing various health challenges. Earlier study also showed that consumption of leachate-contaminated groundwater could hinder growth in children and lead to abnormal haematological changes (6). A separate study also revealed that consumption of leachate-contaminated groundwater could lead to tissue oxidative damage (7). Damage to kidney and impairment of kidney function had equally been

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linked to consumption of leachate-contaminated groundwater (8). The present study however, investigated the effect of leachate-contaminated groundwater on liver of rats as an addition to the increasing body of evidence on effect of leachate-contaminated groundwater on public health.

Materials and Methods

Chemicals and solvents are of analytical grade and are the products of Sigma-Aldrich, inc., St. Louis, USA.

The experimental water for the study was collected from the residential areas located within the vicinity of Odo Iya Alaro landfill in Ojota, Lagos, Nigeria. The experimental water samples were obtained from two different wells located at about 1 and 1.5 km, respectively, from the landfill; and two boreholes located at about 1 and 1.5 km, respectively, from the landfill. The water samples were collected using stainless steel buckets. Leachate simulation was carried out following the ASTM method (9). Physicochemical characteristics of the experimental water samples were done and reported (5).

Sixty (60) Albino rats (Rattus novergicus) were obtained from the Animal Holding of the Department of Biochemistry University of Ilorin, Ilorin, Nigeria. These rats were fed ad libitum with commercial feeds obtained from Livinco feeds, Jubilee road, Ikare Akoko, Ondo State, Nigeria. The experimental animals were kept inside a wooden cage assigned into six (6) groups of ten (10) animals each. The first two groups of rats were placed on tap water and simulated leachate samples, respectively. The third and fourth groups were placed on water samples obtained from wells located at a distance of about 1 and 1.5 km, respectively, from Odo Iya Alaro landfill. The remaining two groups of rats were placed on water samples obtained from boreholes located at a distance of about 1 and 1.5 km, respectively, from the landfill. The feeding exercise lasted over a period of 65 days, a long term standard for rats, preceded by 10 days acclimatization period. The experimental animals were handled in accordance with the guidelines for handling laboratory animals as stipulated by the relevant agency. The rats were anaesthetized by placing them in a jar containing cotton wool soaked with chloroform before being sacrificed by jugular puncture. The liver of the animals were removed into a beaker containing ice cold 0.25 M sucrose solution. The isolated liver was weighed and a portion of it was cut out, chopped into very small pieces and then homogenized using a pre-cooled pestle and mortar in a bowl of ice cubes. The tissue homogenates were diluted using 0.25 M sucrose solution to the tune of 1 in 30 dilutions. The diluted homogenates were stored at a temperature of 81C until required for use.

Protein concentration was determined by the biuret reaction described (10). In this method, copper ion (blue) is made to react with the peptide bond of protein to give a purple-coloured complex, the intensity of which was measured spectrophotometrically at 540 nm. The method of Bessey et al. (11) as modified by Wright et al. (12) was employed in the determination of alkaline and acid phosphatases. The amount of phosphate ester that is split within a given period of time is a measure of the phosphatase enzymes. Activities of aspartate transaminase (AST) and alanine transaminase (ALT) were determined using the method described (13). The method measures spectrophotometrically the intensity of the red coloured hydrazone formed from the reaction of pyruvate with 2,4-dinitrophenylhydrazine at 546 nm. Histopathological study on the kidney obtained from experimental rats was carried out following the method described by Drury and Wallington (14).

Statistical analysis

Analysis of variance ANOVA; Duncan's multiple range test (DMRT) was the statistical analysis used (15). $P<0.05$ was regarded as significance.

Results

Result of the liver relative weight is presented in Figure 1. The liver relative weight of rats in the leachate group was found to be significantly lower ($p<0.05$) relative to the rats in the tap water (control) group. The relative liver weight of rats in well and borehole groups were found not to be significantly ($p>0.05$) different from the tap water group of rats. Specific activity of ALP in liver of rats in tap water group was found to be 3 folds that of leachate group, 2 folds that of well (1km) group of rats and significantly higher than those of rats in the other groups. This result is presented in Figure 2.

Figure 3 presents the specific activity of ACP of liver of rats placed on leachate-contaminated groundwater over a period of 65 days. It was found that the ACP activity of liver of rats in other groups were significantly lower ($p<0.05$) than that of rats in tap water group. Specifically, enzyme activity of liver of leachate group was about 1/3 that of tap water, that of well (1km) group was found to be about 1/2 that of tap water group of rats.

Liver AST specific activity of rats placed on leachate-contaminated water over a period of 65 days is presented in Figure 4. Generally, the specific activity of AST of liver of rats in other groups was found to be significantly lower ($p<0.05$) than that of rats in tap water group. Specifically, relative to rats tap water group, AST activity of liver of rats in leachate group was about 29%, that of well (1km) was about 38%, that of well (1.5km) is about 54%, that of borehole (1km) is about 66% and that of borehole (1.5km) is about 75%.

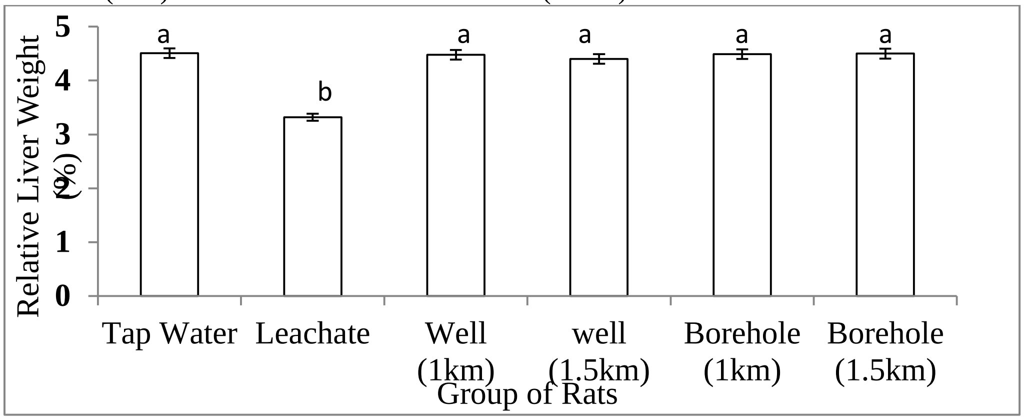

Figure 1: Relative liver weight of rats placed on leachate-contaminated groundwater over a period of 65days. Plotted results are means of 10 determinations ± SEM. Values carrying different notations are significantly different (p<0.05).

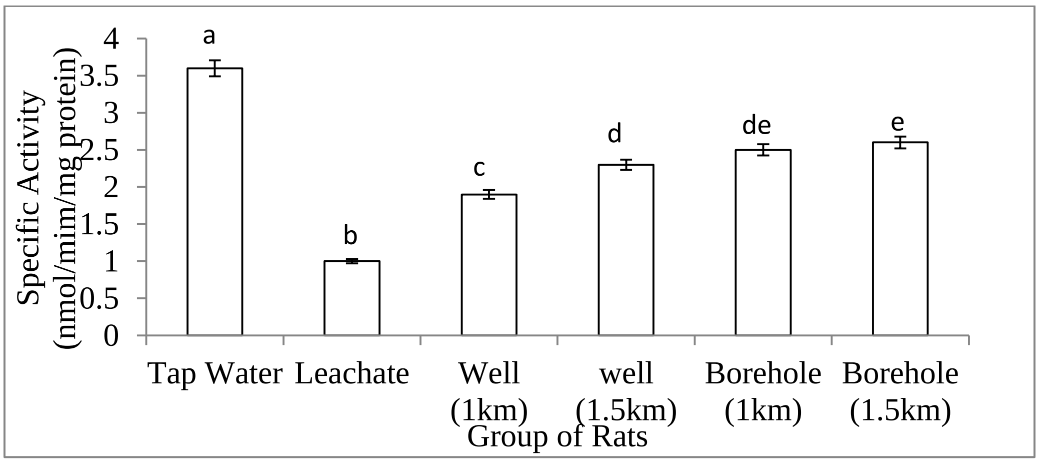

Figure 2: Specific activity of alkaline phosphatase of liver of rats placed on leachate-contaminated groundwater over a period of 65 days. Plotted results are means of 10 determinations ± SEM. Values carrying different notations are significantly different (p<0.05).

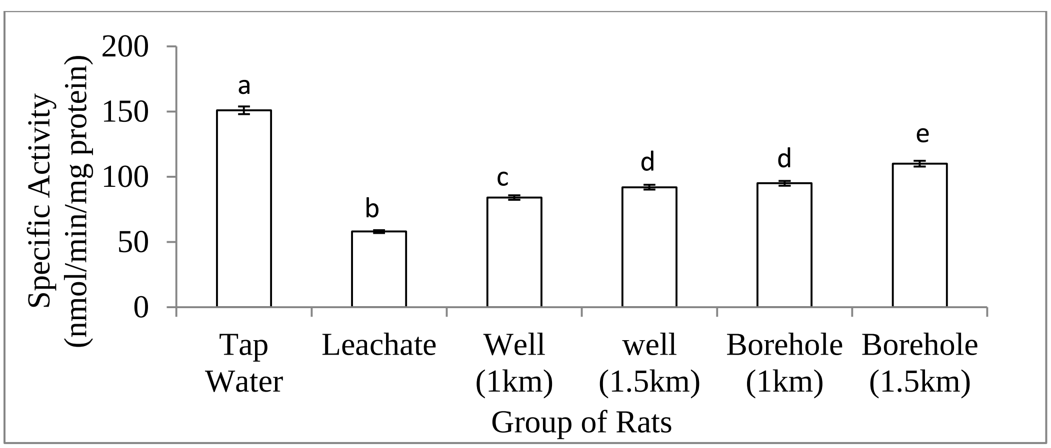

Figure 3: Specific activity of acid phosphatase in liver of rats placed on leachate-contaminated groundwater over a period of 65 days. Plotted results are means of 10 determinations ± SEM. Values carrying different notations are significantly different (p<0.05).

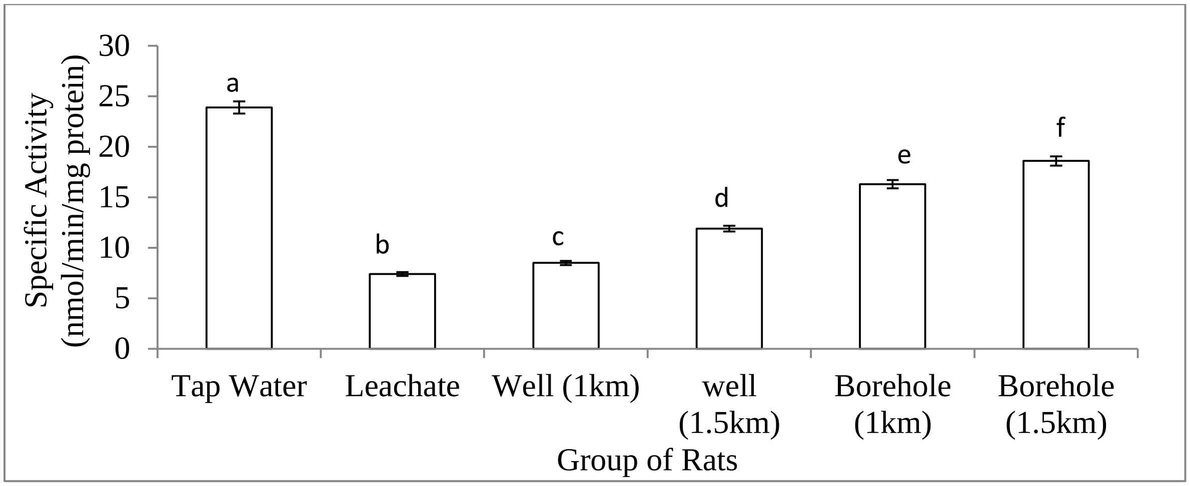

Figure 4: Specific activity of aspartate transaminase of liver of rats placed on leachate-contaminated groundwater over a period of 65 days. Plotted results are means of 10 determinations ± SEM. Values carrying different notations are significantly different (p<0.05).

Figure 5 shows the result of specific activity of ALT of liver of rats placed on leachate-contaminated groundwater over a period of 65 days. A general significant ($p<0.05$) decrease of specific activity of liver ALT of rats in other groups relative to the rats in tap water group was found. Noteworthy at this point is that the loss of activity increases as the distance of water source from the landfill decreases, in other words, the closer the water source to the landfill, the more significant the loss of enzyme activity. This observation is true, to a certain extent, for other results presented in this study.

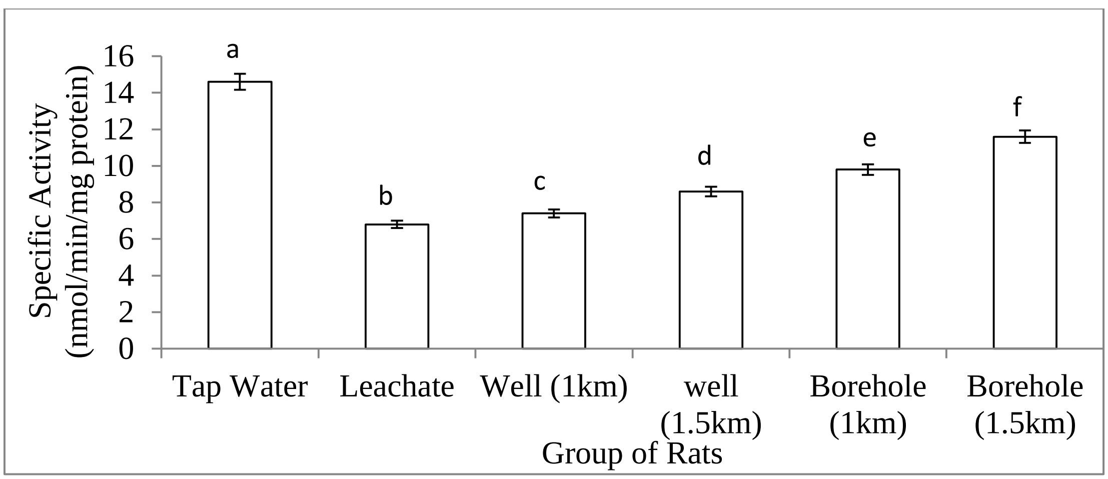

Figure 5: Specific activity of alanine transaminase of liver of rats placed on leachate-contaminated groundwater over a period of 65 days. Plotted results are means of 10 determinations ± SEM. Values carrying different notations are significantly different (p<0.05).

Histology micrographs of liver of experimental rats are presented in plates 1-6. Results revealed no visible lesion in the hepatocytes of rats in tap water group. However, effects observed in the liver of rats in other groups ranges from severe haemorrhage to mild congestion of the portal vessels.

Plate 1: Light micrograph (x400) of liver of rats placed on tapwater over a period of 65 days. No visible lesion.

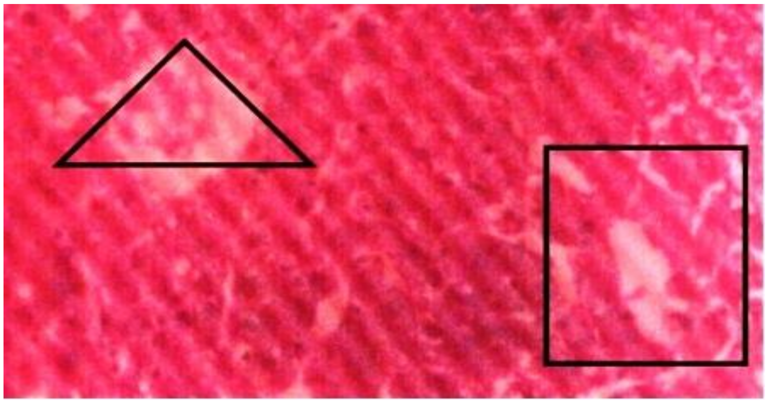

Plate 2: Light micrograph (x400) of liver rats placed on simulated leachate over a period of 65 days. Multiple foci of severe haemorrhage. Muscle fibre necrosis and mild cellular infiltration by macrophage.

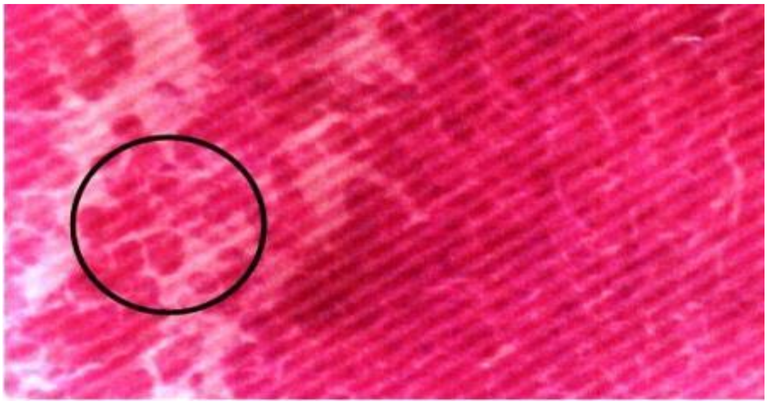

Plate 3: Light micrograph (x400) of liver of rats placed on well water (1km from landfill) over a period of 65 days. The portal vessels are markedly congested. The bile duct appears thickened. Few areas of haemorrhage.

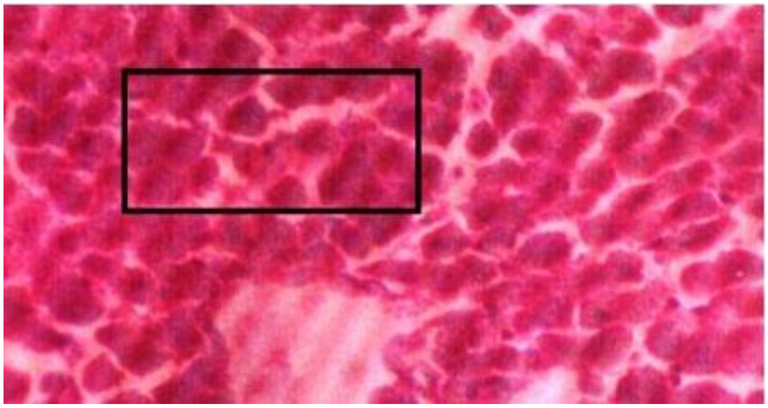

Plate 4: Light micrograph (x400) of liver rats placed well water (1.5km from landfill) over a period of 65 days. The portal vessels are congested.

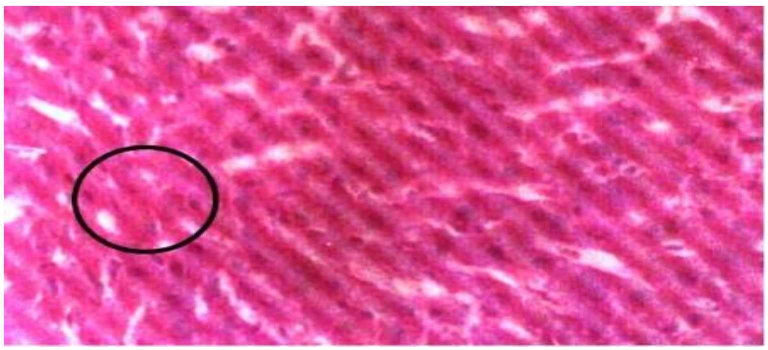

Plate 5: Light micrograph (x400) of liver of rats placed on borehole water (1km from landfill) over a period of 65 days. The sinusoids are mildly congested.

Plate 6: Light micrograph (x400) of liver of rats placed on borehole water (1.5km from landfill) over a period of 65 days. The portal vessels are mildly congested

Discussion

The present study is significant as it presents both biochemical and histopathological evidence of risk posed by leachate-contaminated groundwater on the liver. Groundwater is believed to have excellent natural quality, usually free from pathogens, color, turbidity, and can be consumed directly without treatment. This misconception made many consume untreated groundwater which had also led to several pathological conditions. This study reveals that groundwater is losing natural purity.

Comparison of relative organ weights between treated and untreated groups of animals have conventionally been used to evaluate the toxic effect of substances (16). Although no reference values has been established for relative organ weight but use of relative organ weight obtained for the untreated group as reference values at each testing facility for laboratory animals has become a standard practice (17). In this study, no significant difference was observed among the relative organ weight of the rats in other groups except that of the leachate group that was observed to be significantly lower ($p<0.05$) relative to the tap water group. This observation perhaps, showed that the leachate was indeed the source of contamination.

Alkaline phosphatase (ALP) is a marker enzyme for the plasma membrane. Two abnormal levels of alkaline phosphatase have been proposed (18) increased or decrease in normal levels. Damage to biological membrane may lead to any of the two abnormalities. The observation presented in Figure 2 suggests possible damage to the plasma membrane of the liver of experimental rats relative to the tap water. Acid phosphatase (ACP) is needed to trigger specific chemical reactions and therefore, it is used to monitor carcinogenicity (18). Liver ACP of rats as presented in Figure 3 is suggestive of hepatic cell rupture leading to the release of hydrolytic enzymes which may induce damage to adjacent cells.

Abnormal values of tissue ALT and AST have been observed for hepatic diseases (19). Increased ALT/AST ratio may be indicative of the extent of cellular damage (19). Loss of AST and ALT activity in tissues may also be interpreted as a compromise of the tissues integrity. Although ALT and AST are "marker" enzymes for the liver, it is believed that any alteration at the subcellular level may affect the activity of these enzymes in other tissues (19). The decrease in the ALT and AST activity in the liver as observed in this study (Figures 4 and 5) suggests that pollutants of the leachate-contaminated groundwater possibly inhibit ALT and AST activity. The experimental data revealed that pollutants of the leachate-contaminated groundwater may therefore alter protein metabolism, amongst others, at the subcellular level and this may be indicative of the impairment of the function of the liver.

Photomicrograph of liver of experimental rats (Plates 1-6) lend credence to the results obtained from the enzyme assay as it revealed normal cellular architecture for the rats in tap water group while cellular architecture distortion observed for the other groups of animals varies depending on the source of groundwater (well or borehole) and distance from landfill (1km or 1.5km). Notably too, is that liver micrograph of rats in leachate group revealed severe cellular inflammation.

Conclusion

Experimental evidence from this study showed that water from well are more contaminated than the borehole water sited within 1km radius to Odo Iya Alaro landfill and that the farther the distance from the landfill the less the contamination of the groundwater. It was also revealed that consumption of leachate-contaminated groundwater could result to damage to liver cells and loss of hepatic function. It is the authors' view therefore, that public enlightenment on the danger posed by Odo Iya Alaro landfill to public health should be aired to prevent impending catastrophe that may arise if the consumption of such leachate-contaminated groundwater is not discouraged.

Conflict of Interest

The authors declare no conflict of interest.

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