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Waste leachate induces histological alterations in *Limicolaria aurora* (Jay, 1839) digestive gland

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Unsanitary open dumping and burning of wastes in dumpsites results to constant leaching of waste pollutants into the surrounding environment with attendant harmful consequences on wildlife and public health. Here, we investigated the histological effects of Onitsha municipal waste leachate on the Giant African land snail, *Limicolaria aurora* (Jay 1839). The histopathological effects of waste leachate on the digestive cells of the snails were examined following standard protocols. Snails were exposed to different concentrations (0, 6.25, 12.5, 25.0 and 50.0%) of the leachate for a total duration of 21 days. Histological results obtained showed that waste leachate caused dose and duration dependent alterations in the digestive glands of *L. aurora.* Obvious histological variations were observed in snails exposed to 50% concentrations of the leachate compared to other doses. Observed histological changes were mainly degeneration of the digestive tubules, fragmentation and disappearance of the digestive cells, marked increase in excretory cells, necrosis and epithelia sloughing. This study therefore highlights the potential toxicity of waste leachate of causing mild to severe damage in the tissues of organisms. Reinforcing the fact, that waste leachate contains a mix of toxic substances that could pose a severe injury to the biological system of surrounding biota, including humans.

Keywords: Environmental pollution, Giant African land snail, Waste management

Urbanization comes with increase in waste generation with serious implication when the rapid growing waste rates are not efficiently managed. Poor and unscientific management of these generated wastes has resulted in serious environmental hazard[1]. The volume of solid waste generated in Nigeria continues to increase at an alarming rate than the capacity of the responsible waste agencies to improve on the waste management, as some major roads and markets experience accumulated waste from commercial and industrial activities[1]. At current population growth rate of over 210 million[2] and economic growth, waste generation is projected to increase over the next few years. Open dumping and burning of waste remain the usual unhygienic method of waste management in Nigeria[3]. Generation of toxic leachates has been a major problem arising from this uncontrolled waste dumping with serious public health concern[4].

Leachates constitute a wide range of xenobiotic organic compounds, macro-inorganic compounds, heavy metals and microorganisms, which cause severe human health problems[5]. Small amounts of leachates can pollute large volume of ground and surface water ecosystem[6]. Studies have shown that pollutants present in leachates have damaging effects on living cells[3,5]. Waste leachate has been associated with increases in cancer incidence, diseases outbreaks, reproduction and developmental problems among human populations residing close to landfill sites[7]. Owing to the grave consequences of waste leachate exposure resulting from inappropriate waste management, studies have focused on biomonitoring the contaminants in leachates from dumpsites[8-10].

Land snails are the most species-rich group of terrestrial mollusks with enormous ecological importance[11]. Giant African land snails (*Limicolaria aurora* Jay) are regular delicacy in diets of some rural communities in Nigeria. They form cheap source of protein to the local populace and plays important ecological roles. These snails have been recognized as a suitable bioindicator of environmental stress due to their ecological distribution, easy sampling, tolerance to stress and ability to bio accumulate compounds[12]. In snails, the digestive gland plays an important role in accumulation, biotransformation and detoxification of various substances, making it the target organ in environmental pollution evaluation[13]. Therefore, histological alterations in the digestive gland of snail is a significant bioindicator of environmental pollution[13].

Thus, in this study, we did histological analysis of the digestive gland of giant African land snail *Limicolaria aurora* to understand the potential tissue damaging effect of waste leachate from Onitsha municipal waste dumpsite.

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Materials and Methods

Description of study area

Onitsha, one of the major cities in Anambra State of eastern Nigeria is one of the most important commercial centers in sub-Sahara Africa with significant human population. More than 90% of solid waste generated in Onitsha metropolis is deposited in open dumpsite without any initial source separation. Onitsha municipal waste dumpsite is located along Onitsha-Owerri road Obosi, Ogbaru Local Government (latitude 06º 6′N and longitude 06º 48′E) Anambra State, Nigeria. This is the main dumping sites receiving most of the solid waste from Onitsha metropolis for over 10 years, with an average of 125,000 tonnage of wastes per month[3]. The dumpsite is not equipped with a leachate collection and treatment system; thus, leachate produced is freely discharged and possibly pollute the surrounding aquatic and terrestrial environment.

Leachate collection

Raw leachates were collected from 15 leachate wells (holes in the ground), at different point on the dumpsite and thoroughly mixed to provide a homogenous and composite representative sample. The leachate was transported to the laboratory and examined for some physicochemical parameters as described in our previous study[2]

Experimental procedure

Live samples of adult *Limicolaria aurora* (average weight of 10.47+0.42 g) were commercially obtained from a snail farm in Nsukka, Enugu State. The specimens collected were identified according to Crowley & Pain[14] and were transported to the animal house of Department of Zoology and Environmental Biology, University of Nigeria, Nsukka. Snails were acclimatized for six days according to the methods of Thompson & Cheney[15] and Ebenso *et al.*[16]. Snails were reared in plastic boxes 12×12×60 cm^3 with lids perforated for ventilation, under constant conditions with a temperature cycle of 27±2°C. They were fed *ad libitum* with freshly chopped green pawpaw (*Carica papaya*) leaves during the period of acclimatization. The use of these snails was in accordance with the Ethics and Biosafety Committee of Faculty of Biological Sciences, University of Nigeria Nsukka (Reference number – UNN/FBS/EC/1015). The experiment was carried out under the natural photoperiod prevalent at Nsukka (Nigeria) in the month of August.

Five concentrations (0, 6.25, 12.5, 25.0 and 50.0%) of the leachate sample (v/v; leachate: dechlorinated tap water) were prepared. Sterilized Loamy soil was moistened with the different treatment solutions, placed in their respective boxes up to 2 cm deep. This serves as substrate and source of exposure. Ten (10) snails each were randomly allotted to each plastic boxes of each concentration and the control. Oral application of leachate samples was also given to the snails; pawpaw (*C. papaya*) leaves were presented to snails at different concentrations. These pawpaw leaves were contaminated by completely dipping them in their respective treatment concentrations, and dried at 27°C for 5 min before feeding it to the snails. Snails were fed and watered at 1700 h each day. During the 21-day exposure, three snails from each group was randomly selected at interval (day 7, 14 and 21) for the histopathology analyses.

Histopathological analysis

Slices of the digestive gland from exposed and control snails were processed as described by Lance *et al.*[17]. Tissues were fixed in 10% phosphate buffered formalin for 48 h and were subsequently trimmed, dehydrated in 4 grades of alcohol (70%, 80%, 90% and absolute alcohol), cleared in xylene and embedded in molten wax. On solidifying, the blocks were sectioned, 5 µm thick with a rotary microtome, floated in water bathe and incubated at 60°C for 30 min. The 5 µm thick sectioned tissues were subsequently cleared in 3 of xylene and rehydrated in 3 grades of alcohol (90, 80 and 70%). The sections were then stained with Hematoxylin for 15 min. Blueing was done with ammonium chloride. Differentiation was done with 1% acid alcohol before counterstaining with Eosin. Permanent mounts were made on degreased glass slides using a mountant; DPX. The prepared slides were examined with a Motic™ compound light microscope using 4X, 10X and 40X objective lenses. The photomicrographs were taken using a Motic™ 5.0 megapixels microscope camera at 160X and 400X magnification.

Statistical analysis

Biological assessment was done to identify histopathological alterations in the digestive glands of the snails. Pathological changes observed in the digestive glands were classified in four degrees of occurrence: absent (0), rare (1), few (2), common (3) and very common (4) from each of the three snails per exposure.

Result and Discussion

Physicochemical characteristics of leachate

Table 1 shows the physicochemical parameters and heavy metals analyzed in the waste leachate sample. The values of COD, BOD, TDS, hardness, alkalinity, chlorides, phosphate, sulphate, sodium, nitrate, ammonia, and the heavy metal analysed were high compared to standard permissible limits.

Histological Observation of the Digestive gland

The histological changes of digestive gland exposed to different concentrations of the waste leachate for 21 days are shown in Figs 1-3 and Table 2.

Table 1 — Physical and chemical characteristics of Onitsha Municipal Waste leachate

Parameters+	OWL	USEPA	NESREA
pH	8.20±0.1	6.5-8.5	6.0-9.0
Chemical Oxygen Demand (COD)	464±74.9	410	90
Biochemical Oxygen Demand (BOD)	263±52.8	-	50
Total Dissolved Solids (TDS)	1328.73±69.3	500	500
Hardness	259±173.9	0-75	-
Alkalinity	387±116.4	20	150
Conductivity	383±50.6	-	125
Chloride	951±164.9	250	250
Ammonia	16.82±5.2	0.02	1.0
Phosphate	288.39±10.9	-	2.0
Nitrate	14.03±1.5	10	10
Sulphate	379.02±16.4	250	250
Sodium	47.92±1.2	-	0.50
Potassium	55.18±0.6	-	100
Calcium	38.04±3.2	-	50
Lead	0.73±0.2	0.015	0.05
Cadmium	0.47±0.2	0.05	0.2
Copper	1.93±0.5	1.0	0.5
Iron	2.05±0.2	0.3	-
Zinc	14.74±4.5	-	6.0-9.0

[NESREA, National Environmental Standards and Regulations Enforcement Agency[18]; USEPA, United State Environmental Protection Agency (2006). (www.epa.gov/safewater/mcl.html). Source: Ugokwe *et al.*[3]]

Observation in control groups

The digestive gland sections of snails of the control group showed normal microscopic anatomy of the digestive glands (Fig. 1A). The gland showed numerous variably sized tubules surrounded by thin circular fibro-muscular layer. Each tubule is lined by a single layer of columnar to irregular shaped cells consisting of predominantly digestive cells (dc); with less excretory cells (ec) and calcium cells (cc) (Fig. 1B).

Digestive cells constitute the major cellular component of the digestive gland tubule epithelium. They are columnar with flattened or slightly rounded apical surfaces bearing well-developed brush border. The basally located nuclei of digestive cells are rounded or oval in outline with condensed chromatin and a single nucleolus. Calcium cells are fewer than digestive cells and they occur either singly or in pairs in the tubules. They have pyramidal shape with narrow distal end and a marked broad base. The excretory cells are ovoid, containing a large cytoplasmic vacuole with golden brown excretory granules. The excretory products are accumulated in the vacuole often in the form of a large brown body. The nucleus is small and pressed flat against the cell base.

Treatment groups at Day 7

The treatment groups at day 7 showed a deviation from its normal microscopic architecture compared to the control. In the group exposed to 6.25 and 12.5% of the leachate, the tubules were mostly hypertrophic and showed increase in the population of excretory cells and calcium cells in relation to the digestive cells (Fig. 2A). In the groups exposed to higher concentrations, the tubules appear hypertrophied and show necrosis with accentuation of the interlobular connective tissues (Fig. 2B). Sloughing of the epithelia tubules with expanded lumen was also observed (Fig. 2C)

Treatment groups at Day 14 and 21

The treatment groups at days 14 and 21 showed a severe deviation from its normal microscopic

Table 2 — Frequency and severity of histopathological alterations in digestive gland of snails exposed to Onitsha municipal waste leachate

Duration	Day 7					Day 14					Day 21				
Conc. (%)	0	6.25	12.50	25.00	50.00	0	6.25	12.50	25.00	50.00	0	6.25	12.50	25.00	50.00
Histological changes															
Increase Excretory cell	0	2	3	3	3	1	4	3	3	4	1	4	4	4	4
Necrosis	0	0	1	2	2	0	0	2	2	1	0	1	2	2	3
Hypertrophic tubules	0	1	1	1	1	0	1	3	2	2	0	2	3	3	3
Congestion	0	1	0	1	0	0	1	1	1	3	0	3	4	3	4
Expanded lumen	0	0	0	1	1	0	0	0	2	4	0	2	2	3	4
Vacuolation	0	0	0	1	2	0	1	1	3	3	0	4	4	4	4
Degeneration	0	0	1	1	1	0	0	1	2	3	0	3	3	4	4

[Severity of snail digestive gland histological changes were assessed using the following scale: 0 = a change that was either absent or sporadic in all animals of a group; 1= a change that was rare in all animals of a group; 2= a change that was found in a few animals of a group; 3 = a change that was relatively common in all animals of a group; and 4= a change that was very often found in all animals of a group] architecture compared to the control. Marked increase in the population of excretory cells (ec) were observed in these groups (Fig. 3). Necrosis of tubules (N) with accentuation of the interlobular connective tissues (I) were mainly observed at 6.25% concentration (Fig. 3B). At higher concentrations of 12.5 and 25.0%, severe widespread necrosis and sloughing of the tubular epithelia (Fig. 3 A & B) were observed. The sloughed cells appear in clusters within the expanded lumen of the tubules while distribution of the constituent cells of the tubules was not affected. The entire epithelial cells show marked vacuolar degenerative changes (Fig. 3C) at the highest concentration (50%). The affected cells appear swollen, containing multiple tiny or single large clear cytoplasmic vacuoles (Fig. 3D).

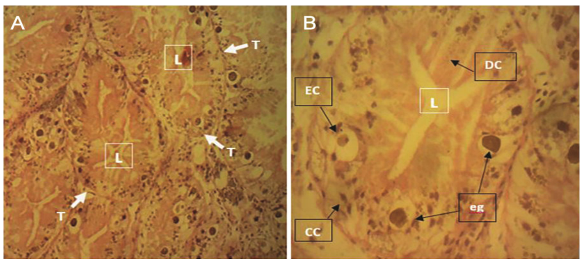

Fig. 1 — (A) Digestive gland; and (B) Digestive tubules of snails exposed to 0.0% concentration of the leachate. [T = tubules; DC = digestive cells; EC = excretory cells; CC = calcium cells; L = lumen; eg = excretory granule. H & E 160X; 400X]

Waste leachate is a major source of pollution in aquatic and terrestrial environment with antecedent threats to biota. These leachates contains a hazardous cocktail of organic and inorganic compounds that interplays and possibly induce cascades of biological damages[19]. This study investigated the histological effects of municipal waste leachate on the digestive gland of land snails. Histological changes in digestive gland are useful biomarkers of hazardous exposure[20]. The digestive gland is responsible for digestive enzyme production, nutrient absorption, endocytosis of certain food ingredients, food storage and excretion[21]. It is comparable to the liver in humans and most target organ in toxicological findings due to its role in excretion of xenobiotics, biotransformation and detoxification. Therefore, it is most susceptible organ in mollusk to hazardous waste chemicals.

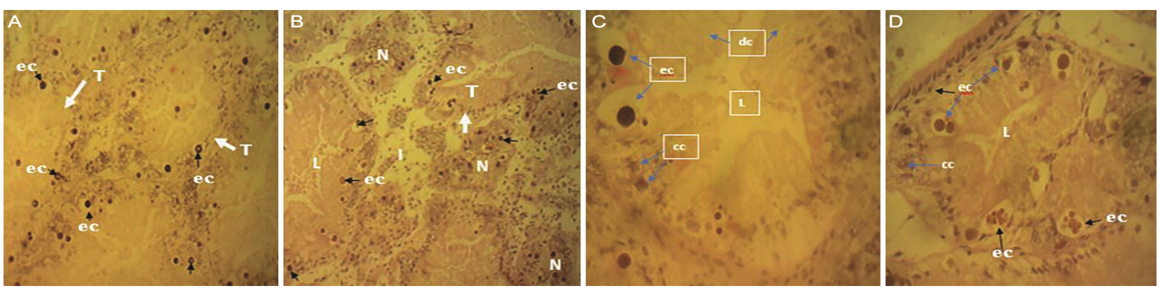

Fig. 2 — Histological sections of digestive gland of exposed snails showing (A) remarkable increase in the number of excretory cells and partial degeneration of some digestive cells; (B) Necrosis of tubules with accentuation of the interlobular connective tissues and increased excretory granules; (C) Degeneration of digestive cells with expanded Lumen; and (D) Sloughed tubule with increased excretory granules. [T = tubules; N = necrosis; I = interlobular connective tissues; dc = digestive cells; ec = excretory cells; cc = calcium cells; L = lumen. H&E 160X; 400X]

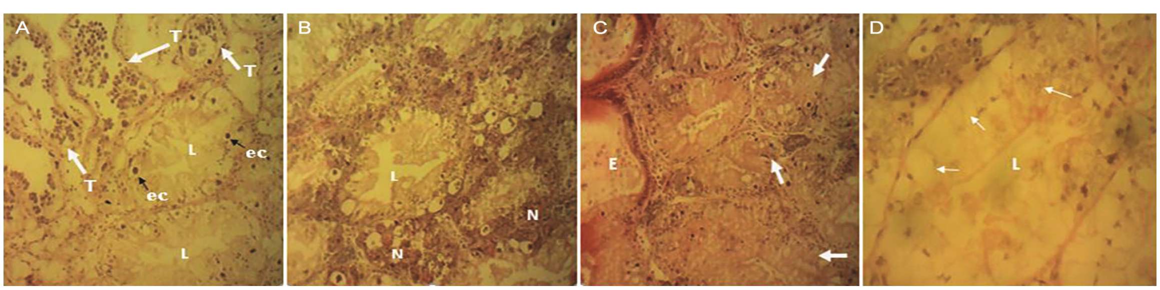

Fig. 3 — Histological sections of digestive gland of exposed snails showing; (A) widespread necrosis and sloughing of the tubular epithelia (T). The sloughed cells appear in clusters within the lumen of the tubules and excretory granules (ec); (B) Necrosis and sloughing of the epithelia in tubules (N), the entire epithelial cells show varying degrees of vacuolar degenerative changes; (C) Marked hypertrophy of the tubules with vacuolar degeneration of the epithelia (arrow), Esophagus (E); and (D) Entire epithelial cells show marked vacuolar degenerative changes. The affected cells appear swollen, containing multiple tiny or single large clear cytoplasmic vacuoles (arrow). [H&E 160X; 400X].

The results observed indicated that the alterations in digestive glands were dose and duration dependent. The investigation at day 7 and 14 showed degeneration of the digestive tubules, disappearance of the digestive cells and changes in the basement membrane in a dose-duration-dependent manner, resulting to a marked deterioration of the tissues at the highest concentrations. These results are in accordance with the findings of Radwan *et al.*[22] whom stated that the structural changes and the loss of digestive cells in snails are general responses after an exposure to pollutants. Valavanidis *et al.*[23] also detailed that the deterioration of the digestive cells induced changes in the digestive process which can be attributed to the ingestion of chemical pollutants.

At day 21, this study recorded marked tissue changes in the digestive gland of *L. aurora* as shown by the visible structural changes in snails exposed at different doses. These marked changes were obvious in snails exposed to waste leachate at 25.0 and 50.0% concentrations. The histopathological responses observed when exposed to lower concentrations were mainly changes in epithelial hyperplasia, hypertrophied tubules, disappearance of digestive cells and marked increase of excretory cells. At higher concentrations, accentuation of the interlobular connective tissues, necrosis and sloughing of the epithelia with varying degrees of vacuolar degenerative changes were observed. Further histological examination at high concentrations revealed clustered sloughed cells, hemolymphatic spaces between the tubules with a more expanded lumen and desquamation of epithelial cells, accompanied by hypertrophy and swelling. This result suggested that the waste leachate contain mixture of hazardous compounds that synergistically capable of causing severe tissue damages in *L. aurora*. This finding is congruent with that of Radwan *et al.*[22]. Observed damage in the cells could lead to the disruption in intercellular exchange and fluidity, which might often result to cellular necrosis[22]. Similar findings are reported in the adults of *Helix aspersa* fed with copper-contaminated diet which exhibited significant effects of copper dose on the hepatopancreatic epithelium cells and digestive glandular epithelium area[20]. Gust *et al.*[24] also reported histological lesions in the digestive gland of New Zealand mudsnail (*Potamopyrgus antipodarum*), which hypertrophy of cells and vacuolization of digestive cells were observed.

The digestive cells of *L. aurora* exposed to different concentrations of leachates showed different degrees of morphological alterations, which could be attributed to the differences in their different phases of activity. This is in accordance with the earlier report by Moore and Halton[25] who reported phases of absorption, digestion and fragmentation in the cycle of the digestive cells of *L. truncatula*. Dimitriadis and Konstantinidou[26] also reported that the cell changes observed in the digestive gland epithelium of *H. lucorum* were regarded as different phases of digestive cell activity. Contrast to the control, there were decrease digestive cells and irregular cell structures of *L. aurora* treated with varying concentrations of waste leachate. This might be due to the fact that digestion and absorption of nutrients mainly occur in the digestive cells, and absorption of pollutants might alter their structure and abundance. Additionally, it was also observed that waste leachate exposure might result in extensive digestive-cell breakdown and release of cytoplasmic content into the digestive gland lumen of *L. aurora*. This collaborated with the findings of Bowen and Davies[27] and Oxford and Fish[28] in *Arion hortensis* and *Cepaea nemoralis*, where release of enzymes from the cytoplasm of digestive cells was associated to high rate of cell autolysis. Comparable findings were reported in the digestive cells of *Littorina littorea* exposed to organic pollutants[29] and in digestive cells of *Mytillus galloprovincialis* after exposure to heavy metals[30].

Increased calcium and excretory cells in the digestive glands were observed, suggesting that these cells are specialized for accumulation and elimination of toxic compounds[31,32]. These results agreed with the findings of Hamed *et al.*[33] who found that methomyl-treatment increased the number and size of excretory granules after 5 and 7 days exposure to *E. vermiculata*. Similarly, Aioub *et al.*[34] observed an increase in the number of excretory cells in the digestive gland of *E. vermiculata* after exposure to oxamyl. Additionally, Chabicovsky *et al.*[35] showed that heavy metal pollutants, such as Cd, Cu and Zn, induce increased number of calcium and excretory cells at the cost of digestive cell numbers. They suggested that dying cells are phagocytosed by calcium cells. In addition, calcium shows multiple roles in gastropods and other mollusks in which they are regarded as a component concerned in detoxification of heavy metals[31,36].

Conclusion

In this study, we observed histopathological alterations in the digestive gland of *Limicolaria aurora* induced by the waste leachate in a concentration and duration dependent manner. It highlights the potential toxicity of waste leachate of causing mild to severe damage in the tissues of organisms. These observed tissue damage might be the endpoint of cascade of physiological alterations caused by the waste leachate in an organism. The findings suggest that the waste leachate may contain a mix of toxic substances that could pose a severe injury to the biological system of surrounding biota, including humans. This study further advocated the use of histopathological investigation and the sensitivity of the giant African land snail in biomonitoring the toxicity of environmental pollutants that may pose public health concern.

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Conflict of Interest

Authors declare no competing interests

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