

Induction of Genetic Alterations and Oxidative Stress in Giant African Land Snail (*Limicolaria aurora*) Exposed to Municipal Waste Leachate

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Abstract: Uncontrolled waste disposal has continuously threatened the health of the surrounding environment through the leaching of hazardous xenobiotics. Systemic toxicity and genotoxicity potential of waste leachates from Onitsha municipal dumpsite were investigated in giant African land snail (*Limicolaria aurora*) through oxidative stress biomarker and micronucleus test assessment respectively. Physicochemical indices were evaluated in the leachate following standard protocols. Snails were exposed to different concentrations (0, 6.25, 12.5, 25.0 and 50.0%) of waste leachate for 21 days; oxidative stress biomarkers and micronucleus analysis performed on snail digestive gland and hemocyte respectively. The leachate induced dose-dependent increase ($P < 0.05$) in Superoxide dismutase, Catalase, malondialdehyde and Glutathione peroxidase levels with associated decrease in total protein concentrations in the exposed snails compared to the control. Similarly, the frequency of micronucleus and other nuclear abnormalities shows concentration dependent increase ($P < 0.05$) in treated groups. This observed genotoxic effect might be induced by the oxidative stress, via the production of reactive oxygen species. This shows that waste leachate contains hazardous and genotoxic compounds capable of inducing oxidative stress and DNA damage. Therefore, continuous exposure of waste leachate into the environment could pose a grave health risk to the surrounding biota, humans included.

Keywords: Biochemical, Giant African Land Snail, Micronucleus, Nigeria, Waste Pollution.

Introduction

Uncontrolled urbanization and high population growth results in waste generation increase, with serious environmental implications when the rapid growing waste rates are not effectively disposed. Management of municipal solid waste has become a grave problem in most developing countries. In Nigeria, per day, more than 12 million tons of solid wastes is generated, of which less than 28% are efficiently managed, with landfilling and/or open dumping common methods for these waste disposals (Adetoro *et al.*, 2018). This method of waste management is a known source of environmental pollution, which can cause severe impact on human and environmental health (Gupta *et al.*, 2019). Improperly disposed wastes in landfills/dump sites undergo series of biological and chemical changes that results in the formation of toxic leachate (Ghosh *et al.*, 2017).

Waste leachate is generated due to filtration of water through waste and/or moisture containing by-products of biochemical interactions that occur in the dumpsite (Ghosh *et al.*, 2017). Leachate transports organic and inorganic compounds, in downward and outward movement, conveying contaminants from the landfill (Zin *et al.*, 2012). This has become a major source of pollution for underground and immediate surface water ecosystems (Li *et al.*, 2017). Waste leachates are highly complex in nature; consisting of numerous toxic compounds such as macro-inorganic compounds, phenols, heavy metals, pesticides and pathogens. Combined, these compounds can bio-accumulate in aquatic organisms, eventually reaching to animals and humans through the food chain (Zhao *et al.*, 2017). Studies have strongly suggested that pollutants present in leachates have harmful effects on some living cells (Adetoro *et al.*, 2018; Gupta *et al.*, 2019). This poses concerns on the health safety of the environment, animals and humans, hence, it has

become imperative to monitor and evaluate waste leachate toxicity.

Generally, leachate assessment is based on identification of pollutants through chemical analyses which can only detect a slight amount of the toxic compounds present in leachate samples (Ghosh *et al.*, 2017), however, evaluation of leachate can also be assessed on the interactions with biota (Tsarpali *et al.*, 2012). Bioassay toxicity tests can evaluate effects on organisms and can detect toxicity even when the pollutants are not detected by chemical analysis. It integrates the effect of all contaminants and provides indication on their bioavailability (Tsarpali *et al.*, 2012).

Animal use in evaluating the toxicity of waste leachates has become a well-established practice. Land snails, have an ecological role and importance as the most species-rich group of terrestrial mollusks and are broadly acknowledged as being an effective bioindicator of environmental stress. They are easily used in evaluating terrestrial pollution (Vega *et al.*, 2012), due to their wide distribution, easy sampling and capability to bio accumulate pollutants (Anim *et al.*, 2011).

Onitsha, one of the major cities in Anambra State, southeastern Nigeria, is one of the most important commercial hubs in sub-Saharan Africa with significant human population. It has the biggest municipal waste dump, situated along Onitsha-Owerri road, Obosi, and receive most of the solid waste from Onitsha metropolis. Leachate collection and treatment system is lacking in this dumpsite; thus, leachates generated from the waste are easily discharged into the surrounding aquatic and terrestrial environmental media. Hence, this study aimed at evaluating the systemic toxicity and genotoxicity potential of waste leachate from Onitsha municipal waste dumpsite in giant African land snail (*Limicolaria aurora*) via oxidative stress and micronucleus test assessment respectively.

Materials and Methods

Leachate collection

Obosi dumpsite situated at Onitsha-Owerri road, Ogbaru Local Government (latitude 6°06' 12"N and longitude 6°48' 43"E) Anambra State, was chosen as the collection site for leachate sample. This site is the primary municipal dumping site of Onitsha and its metropolitan area for over 10 years. Dumping of waste at this site is unrestricted and contains mixtures of both organic and inorganic waste materials, including industrial, agricultural, domestic and medical wastes. Raw leachates were collected from different leachate wells in the dumpsite, thoroughly mixed to provide a representative leachate sample. The leachate was taken to the laboratory in pre-washed 5-liter plastic container and stored at 4°C until use.

Leachate characterization

Leachate analysis was done by measuring the physical and chemical components of the leachate. Parameters such as pH, electrical conductivity, total dissolved solid (TDS) and Dissolved oxygen (DO), were determined in-situ with a digital Multimeter (TOPAC INSTRUMENTS INC). Chloride, phosphate, Nitrate, ammonia, sulphate, total hardness, total alkalinity, biochemical oxygen demand (BOD) and chemical oxygen demand (COD) were determined according to APHA (2012). The concentrations of heavy metals; Iron (Fe), Lead (Pb), Copper (Cu), Zinc (Zn), Cadmium (Cd) and Chromium (Cr) were carried out using Buck Scientific Atomic absorption

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spectrophotometer, after digestion with a mixture of HClO₃ and HNO₃ (ratio of 1:2), according to USEPA (1996) and APHA (2012).

Biotoxicity Test

Adult giant African land snails (*Limicolaria aurora*) (average weight; 10.47±0.42g) were used as test organism to assess the toxicity of the collected waste leachate. These land snails are delicacy in diets of rural communities (Ebenso *et al.*, 2004b), they play an important ecological roles and have been recognized as a suitable bioindicator of environmental stress due to their ability to bio accumulate compounds (Ebenso *et al.*, 2004a,b; Ebenso *et al.*, 2005; Anim *et al.*, 2011). The snails were commercially acquired from a snail farm in Nsukka, Enugu State, and transported to the animal house of the Department of Zoology and Environmental Biology, University of Nigeria, Nsukka. They were kept in plastic boxes 12 x 12 x 60cm³ with lids punctured for ventilation, under constant conditions with a temperature cycle of 27 ± 2°C. They were acclimatized for six (6) days according to the method described by Ebenso *et al.* (2004a) and fed *ad libitum* with fresh green pawpaw (*Carica papaya*) leaves. The use of these snails was in accordance with the guidelines on the use of animals for research as stated by the institutional animal care committee of University of Nigeria Nsukka. The experiment was conducted under the natural photoperiod prevalent at Nsukka (Nigeria) in the month of August.

The treatment groups comprises of five different concentrations (0, 6.25, 12.5, 25.0 and 50.0%) of the leachate sample (v/v; leachate: dechlorinated tap water). Sterile Loamy soil was placed at base of different boxes of each group (each in triplicate) up to 2cm deep, and moistened with the different treatment solutions respectively. This acted as both substrate and source of exposure. Ten (10) snails each were randomly allotted to each plastic box of each concentration. Oral application of different leachate samples was also given to the snails using chopped pawpaw (*C. papaya*) leaves. This was done by immersing the pawpaw leaves in their respective leachate concentrations before feeding it to the snails. Snails were fed and watered twice a week. During the 21-day exposure period, three (3) snails from each group were randomly selected at interval (day 1, 7, 14 and 21).

Haemolymph was collected through the hemal pore according to the methods of Sminia and Barendsen (1980). Briefly, the foot of the snail was stimulated with the tip of micropipette; extruded hemolymph was collected with micropipette for micronucleus test after the snail deeply retract into its shell. The snails were later euthanized and the digestive gland dissected out for evaluation of oxidative stress parameters.

Biochemical Assays

Estimation of Lipid Peroxidation: Lipid peroxidation was estimated by determining the concentration of malondialdehyde (MDA) equivalents produced by reaction with 0.6% thiobarbituric acid (El-Moshaty *et al.*, 1993). Absorbance was determined at 532 nm and the result expressed in µmol/mg proteins.

Estimation of Superoxide dismutase (SOD): Superoxide dismutase activity was measured by Nitro Bluetetrazolium (NBT) spectrophotometry as described by the method of Zhu *et al.* (1990). Xanthine and xanthine oxidase generated superoxide anion radicals, which react with 2-(4-indophenyl)-3-(4-nitro-phenyl)-5-phenyl tetrazolium chloride (NBT) to form a red formazan dye. Inhibition of 50% of the reaction was defined as one unit of superoxide dismutase enzyme. Absorbance was determined at 560 nm and the result expressed as units/mg proteins.

Estimation of Catalase: Catalase (CAT) activity was determined spectrophotometrically according to the method of Zhang *et al.* (2010), by measuring the decrease of absorbance at 240 nm in a reaction mixture containing 0.3 ml H₂O₂ (0.1 M) and 0.1 ml extract. The result was expressed as µmol/mg of proteins.

Estimation of Glutathione Peroxidase: The level of Glutathione peroxidase (GPx) was measured in a cuvette with a solution consisting of 2 µg protein homogenate, 50 mM potassium phosphate buffer, 0.4mM EDTA, 0.15 mMβ-NADPH, 1unit glutathione reductase, and 1 mM glutathione (reduced) according to the method of Livingstone *et al.* (1992). The change in absorbance was determined based on the decrease of NADPH at 340 nm for 1 min and was expressed as µmol/mg of proteins.

Estimation of Total proteins: The protein concentration was determined according to Bradford (1976) method, based on the reaction of proteins with Coomassie Brilliant Blue G-250 dye. The calibration curve was prepared with bovine serum albumin (BSA) and absorbance read in a spectrophotometer at 595 nm.

Micronucleus assay

Micronucleus test was evaluated on snail hemocytes according to the methods of Villela *et al.* (2007). The collected hemolymph was added to 1ml of 0.075 M potassium chloride in a tube and maintained under incubator at 34°C for 1hr. Samples were centrifuged at 550×g for 10 min; supernatants were removed and drop of cell suspensions smeared on clean microscopic slides, and then allowed to air-dry at room temperature. The cells were fixed in absolute methanol for 5 min, allowed to air-dry and subsequently stained with 5% Giemsa for 10 min. Total of 2000 hemocytes were scored per sample under a light microscope (1000× magnification) and results recorded as micronucleus frequency (MN frequency). The mean frequencies of each category were estimated and expressed per 1000 cells (%).

Statistical Analysis

Two-factor analysis of variance (ANOVA) was used to examine the effects of leachate concentrations and exposure durations on the level of oxidative stress parameters, and frequency of nuclear abnormalities in snails. The means were separated using Turkey's test at 0.05% significance level. Results with *P* < 0.05 were considered statistically significant. The analysis was done using R statistical software, version 3.5.3.

Result

Physicochemical characteristics of leachate

The values of phosphate, sulphate, COD, sodium, BOD, EC alkalinity, TDS, nitrate, ammonia, and chloride ion analysed were higher than regulatory safe limits (Table 1). Equally, the leachate concentrations of heavy metals; lead, cadmium, Chromium, copper, iron and zinc were higher compared to standard permissible limits (Table 1).

Effects of waste leachate exposure on oxidative stress parameters in Limcoraria aurora

Concentration and duration of waste leachate exposures influenced the levels of oxidative stress indices in *Limicolaria aurora* (Table 2). The mean level of SOD and CAT was significantly (*P* < 0.001) higher in those exposed to the different concentrations of leachate compared to those of the control group (Figures 1 and 2). With respect to the duration of exposure, the mean levels of SOD observed on days 14 and 21 was significantly higher than those of days 1 and 7. No significant difference was observed between day 1 and 7, nor between day 14 and 21 in the mean SOD levels (Figure 1). The highest level of CAT was also observed on day 14 and 21, which was significantly different from those of day 7 and 1 (Figures 2).

Comparing snails exposed to leachate treatment to the control, the levels of malondialdehyde (MDA) and Glutathione Peroxidase (GPx) were observed to be significantly higher in the snails treated with leachate, compared to the control. There was, however, no significant difference in the levels of MDA and GPx for the different concentrations of

Table 1. Physicochemical characteristics of Onitsha Municipal Waste leachate.

Physicochemical Parameters ⁺	Leachate Sample	Standards	
		NESREA	USEPA
pH	8.20 ± 0.1	6.0 – 9.0	6.5 – 8.5
COD (mg/l)	464 ± 74.9	90	410
BOD (mg/l)	263 ± 52.8	50	-
TDS (mg/l)	1328.73 ± 69.3	500	500
Hardness (mg/l)	259 ± 173.9	-	0-75
Alkalinity (mg/l)	387 ± 116.4	150	20
EC (µs/cm)	383 ± 50.6	125	-
Chloride (mg/l)	951 ± 164.9	250	250
Ammonia (mg/l)	16.82 ± 5.2	1.0	0.02
Phosphate (mg/l)	288.39 ± 10.9	2.0	-
Nitrate (mg/l)	14.03 ± 1.5	10	10
Sulphate (mg/l)	379.02 ± 16.4	250	250
Sodium (mg/l)	47.92 ± 1.2	0.50	-
Potassium (mg/l)	55.18 ± 0.6	100	-
Calcium (mg/l)	38.04 ± 3.2	50	-
Lead (mg/l)	0.73 ± 0.2	0.05	0.015
Cadmium (mg/l)	0.47 ± 0.2	0.2	0.05
Chromium	1.93 ± 0.5	0.05	0.01
Copper (mg/l)	2.05 ± 0.2	0.5	1.0
Iron (mg/l)	14.74 ± 4.5	-	0.3
Zinc (mg/l)	7.32 ± 2.7	6.0 – 9.0	-

Key: NESREA=National Environmental Standards and Regulations Enforcement Agency, USEPA=United State Environmental Protection Agency, COD= Chemical Oxygen Demand, BOD =Biological Oxygen Demand, TDS = Total Dissolved Solids, EC=Electrical Conductivity. Values are mean of three replicate determination (n = 3) ± standard deviations

Table 2. Influence of leachates treatment effects on oxidative stress indices in *L. aurora*.

Parameters	SOD	CAT	MDA	GPx	TP
Concentration	<	<	<	<	< 0.001
Duration	0.001	0.001	0.001	0.001	< 0.001
Concentration x Duration	Ns	0.001	0.001	0.001	ns

ns = not significant

leachate (Figures 3 and 4). With respect to the duration of exposure to leachate, a significant increase in the mean level of MDA was observed on day 7 of exposure. This increase was not significantly different in days 14 and 21 (Figure 3). On the other hand, a significant increase in the mean level of GPx was observed on day 14 of exposure. This increase was not significantly different in day 21 (Figure 4).

The mean level of Total protein (TP) was observed to be significantly lower in snails treated with leachate, compared to the control snails. There was, however, no significant difference in the level of TP for the different concentrations of leachate. Upon exposure to waste leachate for different duration of time, the mean level of TP was observed to be highest on day 1 and lowest on day 21 (Figure 5).

Effects of waste leachate concentration and duration on the frequency of micronuclei (MN) and other nuclear abnormalities (NA)

The frequency of micronuclei (MN) in snails exposed to leachates was significantly influenced by both concentration and duration of exposure, whereas the frequency of other nuclear abnormalities (NA) was significantly influenced by only the concentration of the leachates.

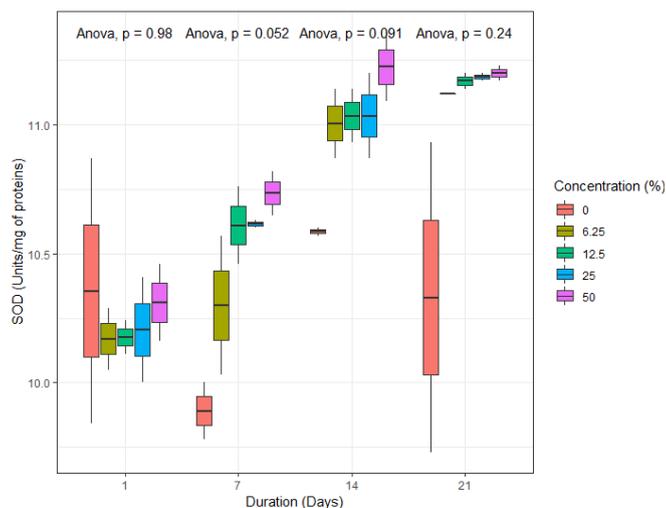


Figure 1: Boxplot of the mean levels of SOD in snails exposed to different concentrations of leachate, grouped by their duration of exposure.

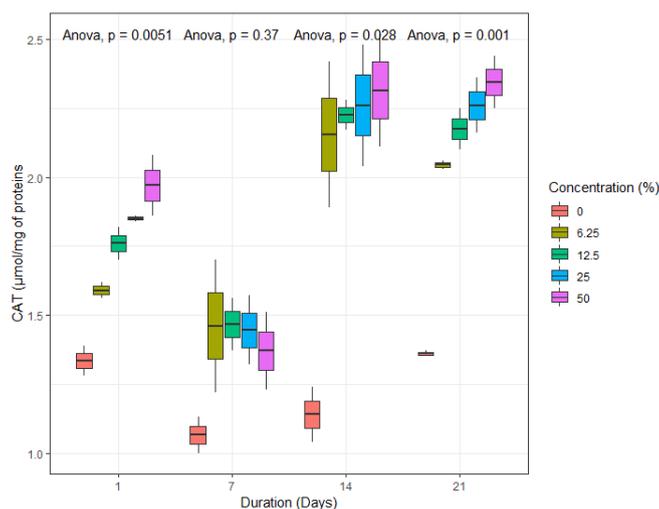


Figure 2: Boxplot of the mean levels of CAT in snails exposed to different concentrations of leachate, grouped by their duration of exposure.

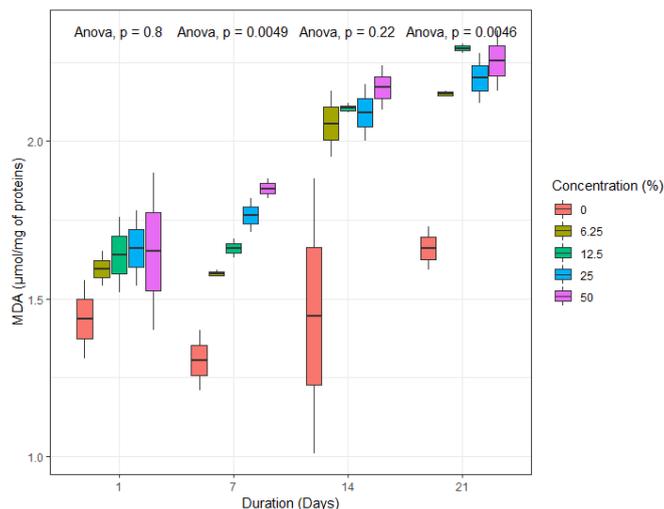


Figure 3: Boxplot of the mean levels of MDA in snails exposed to different concentrations of leachate, grouped by their duration of exposure.

(Table 3). The mean frequency of MN in snails exposed to leachate treatment was significantly higher than those of the control snails. A change in the leachate concentration, however, had no effect on the frequency of MN in those snails exposed to leachate treatment. With

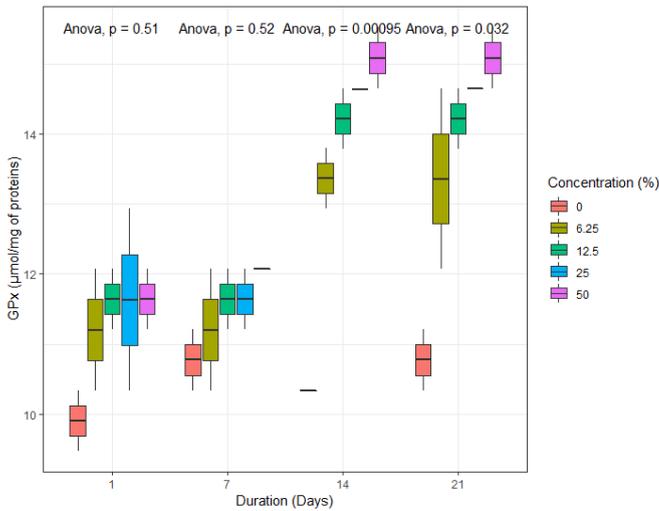


Figure 4: Boxplot of the mean levels of GPx in snails exposed to different concentrations of leachate, grouped by their duration of exposure.

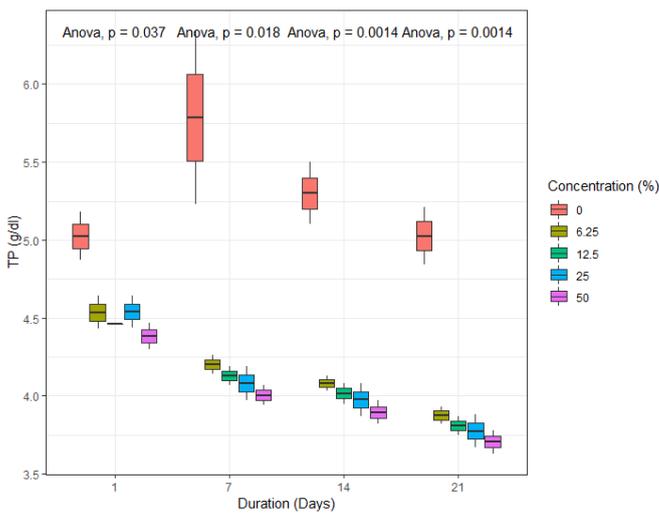


Figure 5: Boxplot of the mean levels of TP in snails exposed to different concentrations of leachate, grouped by their duration of exposure.

Table 3. Influence of treatment effects on the frequencies of micronucleus (MN) and nuclear abnormalities (NA) for leachates.

Parameters	Micronuclei (MN)	Nuclear abnormalities (NA)
Concentration	< 0.001	< 0.001
Duration	0.005	ns
Concentration x Duration	Ns	ns

ns = not significant.

respect to duration of exposure to leachate, significant increase in the mean frequency of MN was observed on day 14, and was not significantly different from that of day 21 (Figure 6). In comparison with the control, a significantly higher frequency of other NA was observed in snails exposed to leachate concentrations of 12.5%, 25% and 50%. No significant difference was observed between the control snails and those exposed to leachate concentration of 6.25% (Figure 7).

Discussion

Waste leachates constitute major source of environmental pollution and contains hazardous compounds such as organic and inorganic compounds. These toxic compounds induce physiological dysfunction and DNA damage in most biota (Alimba and Bakare, 2016), and have become a serious health issue, due to their ability to bond with DNA molecules and induce cascades of biological damages. The application

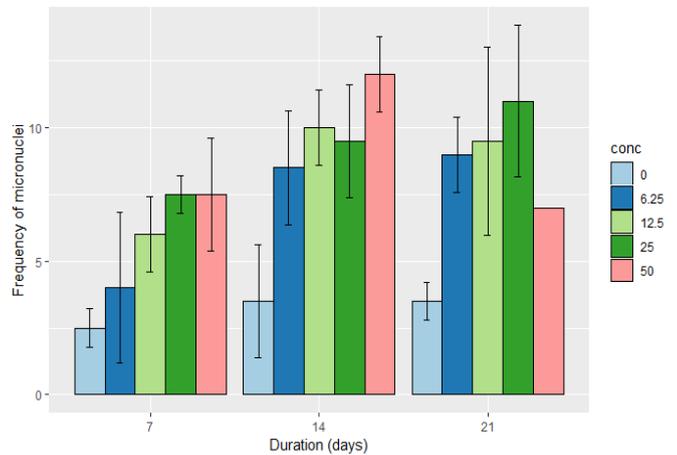


Figure 6: Mean and SE for frequency of MN in snails exposed to different concentrations of leachate, grouped by their duration of exposure.

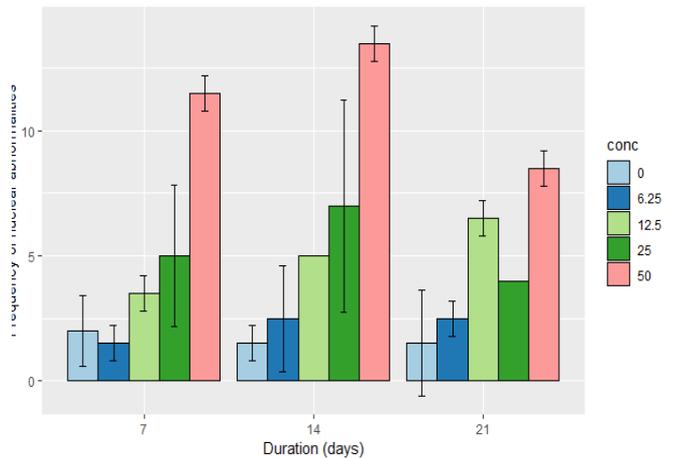


Figure 7: Mean and SE for frequency of NA in snails exposed to different concentrations of leachate, grouped by their duration of exposure.

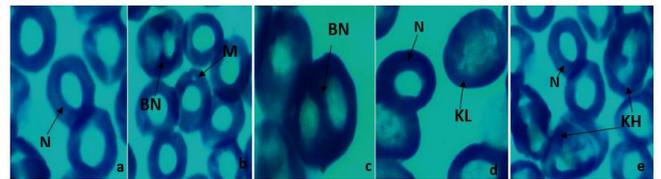


Figure 8: Hemocytes of *L. aurora* exposed to waste leachate showing, Normal hemocyte (N), micronucleated cell (M), binucleated cell (BN), Karyolytic cell (KL) and Karyorrhetic cell (KH) (×1000)

of ecologically relevant species like land snails offers a chance to perform early tests on ecosystem health in relation to exposure to these contaminants (Jha, 2004).

Oxidative stress is one of the most reported biological response observed in snails treated with several chemical compounds. Digestive glands in mollusks are known to be one of the important sites of multiple oxidative reactions and maximal free radical generation (Ali *et al.*, 2012). The increase or decrease in antioxidant activity in organisms results to an oxidative stress (Ojha *et al.*, 2011). Oxidative stress, therefore, occur as an imbalance in the biological oxidant to antioxidant ratio; which results to lipid, proteins, carbohydrates and nucleic acids impairment. In most cases, is the high production of reactive oxygen species (ROS), which results to severe damage to cell structure, indicative of oxidative damage (El-Demerdash, 2007). High production of ROS and its direct interaction with biological tissues are the major mechanisms exerted by pollutants in biological systems (Ma *et al.*, 2013).

The elevation of the antioxidant enzymes observed in the present study

suggests that waste leachate have oxidative stress inducing effects in the exposed snails. SOD and CAT are the initial line of defense in antioxidant systems due to their significant function against oxidative stress. In this study, the significant increase in the activities of SOD and CAT observed in the digestive gland of *L. aurora* exposed to waste leachate is an indicative of cellular damage that results in release of antioxidant enzymes to inhibit the accumulation of reactive oxygen intermediates. Wu and Yi (2015) reported that exposure to pollutants increases the antioxidant enzymes like SOD and CAT to neutralize the impact of ROS. Increase in SOD and CAT activity was dose-duration dependent and was observed in snails exposed to highest concentrations (25% and 50%) of leachate over longer durations (days 14 and 21). This might have occurred to compensate for increase in ROS caused by high leachate concentrations, resulting to oxidative stress. It has been suggested that toxicants may induce different antioxidant/prooxidant responses depending on their ability to produce ROS (Barata *et al.*, 2005). Similar to this study, Fahmy *et al.* (2014) reported the toxicity of zinc oxide nano-particles, which include inhibition and stimulation of both SOD and CAT activities, depending on their concentrations in tissues of freshwater snail *Biomphalaria alexandrina*. They suggested that the observed effects could be due to ROS formation and increase in antioxidant defense system dysfunction.

Lipids are one of the principal targets of oxidative stress. Lipid peroxidation results to generation of highly damaging intermediates, which consequently increase the production of ROS in biological system. Malondialdehyde (MDA) is the main biomarkers of lipid peroxidation and its quantification is a direct indicator of oxidative stress (Tao *et al.*, 2013). In this study, increase in MDA concentration was observed in *L. aurora* exposed to waste leachate compared to the control. Free radicals generation by pollutants in biological system has been reported to result to increased levels in MDA (Belhaouchet *et al.*, 2012). This result is comparable with the reports of Siwela *et al.* (2010) that observed high increase in MDA level in *Lymnaea natalensis*, exposed to environmental pollutants. In addition, Fahmy *et al.* (2014) recorded a significant increase in MDA level in the tissues of *biomphalaria alexandrina* exposed to zinc oxide nanoparticles. Giarratano *et al.* (2014) suggested that the increase free radical production might be associated with the high content of iron (Fe) in digestive gland tissue of snails. Through Fenton type reactions, Fe can induce the production of toxic radicals and aggravates peroxidation of lipids; therefore, the high increase of MDA content observed in this study might also be due to the high concentration of Fe in the leachate. This is in consonance with the findings of Radwan *et al.* (2010) which demonstrated positive correlation of MDA with heavy metals, and suggested that the presence of ionic metals in the digestive gland of snails catalyzes the Fenton reaction and increase the risk of cell injury.

Glutathione peroxidase (GPx) is known as the most significant peroxidase that detoxify the peroxide and hydroperoxides to water and hydroxyl compounds, respectively (Pinto *et al.*, 2003) thus it constitutes an essential part of the antioxidant system. In this study, significant increases in GPx activity in *L. aurora* exposed to varying concentrations of leachate were recorded compared to the control. This might be due to the formation of free radicals in the cells, since ROS is the main substrate for GPx activation (Hermes-Lima, 2004). GPx stimulation shows dose and duration dependent of leachate exposures. This could be associated to its compensatory role in the defense system of the antioxidant mechanism as toxicant in the system increases. Ali *et al.* (2012) observed an increase GPx activity in the digestive gland of *Lymnaea luteola* and suggested the cause to be overproduction of ROS after ZnO nanoparticles exposure. The reported increase is also in consistent with the findings of El-Shenawy *et al.* (2012) who observed elevated GPx levels in snails exposed to cadmium, lead, copper and iron.

Proteins are one of the major groups of biological materials comprising the chief nitrogenous elements of the body tissues. The concentration of free amino acids in mollusk varies with pollution levels (Bishop *et*

al., 1983). In this study, significant decrease in Total Protein levels was observed in snail treatment groups compared to the controls. Singh *et al.* (1996) reported that the quantity of proteins depends on the rate of synthesis or its degradation. The decrease in the reported levels of total protein may be partly resulted from imbalance between the rate of synthesis and the rate of degradation. Depletion in the concentration of protein might be due to either suppressed incorporation of protein synthesis or increased breakdown of proteins in to amino acids, which further diffused out of cells (Gaur, 2011). In addition, the harmful effect of the waste leachate could cause increase in energy utilization and damage to cell organelles of exposed snails leading to inhibition of protein synthesis (Eissa *et al.*, 2002). The present study is in consonance with the observations of Radwan *et al.* (2008), that methiocarb caused significant decrease in total proteins in the tissues of *E. vermiculata*.

Micronucleus (MN) test has been a common method in environmental genotoxicity assessment. MN are formed in actively dividing cell as a result of mitotic spindle dysfunction or lagging chromosomes that failed to incorporate in the daughter nuclei during cell division (Krishna *et al.*, 2000). Bakare *et al.* (2012) opined that the formation of micronucleus is an indication of DNA damage. Haemocytes are frequently used in cytogenetic evaluation due to their sensitivity to genotoxic pollutant, relatively non-invasive, easily sampled, and play significant role in immune defense and detoxification (Magni *et al.*, 2006). In the present study, micronuclei (MN) and other nuclear abnormalities (NA) were investigated in hemocyte of *Limicolaria aurora* exposed to different concentrations of waste leachate. The study revealed increase of micronuclei and other nuclear abnormalities on snails exposed to leachate compared to the control. This result suggested that the waste leachate contain xenobiotics, mostly toxic metals and organic compounds, which are clastogenic and or aneugenic and are capable of causing chromosomal damage in *L. aurora*. This result is in accordance with the findings of Khalil (2016) who reported increase formation of MN in land snail (*Eobania vermiculata*) exposed to sublethal doses of methomyl lannate.

Frequency of micronuclei observed in treated snails was also significantly influenced by the duration of exposure to leachate. Observed increase in MN frequencies on days 7 and 14 indicate increased genetic alterations, which might enhance cell mutation and cancer formation, and possibly, leading to decreased embryonic viability, genetic disorders and biodiversity loss of biota (Russo *et al.*, 2004). These findings were in congruence with the report of Alimba and Bakare (2016) wherein they observed significant increase in MN formations in catfish, quail and rat exposed to landfill leachates.

Aside the formation of micronuclei, increased frequencies in other nuclear abnormalities in hemocytes of treated snails were observed and their presence complement the scoring of MN and further accentuate the genotoxic effects of the leachate. These nuclear abnormalities are indicators of cellular dysfunction in snail and can lead to genetic damage (Rodilla, 1993). The occurrence of binucleated cells is the result of the inhibition of cytokinesis during M phase of the cell cycle leading to the formation of a cell with two nuclei (Fenech *et al.*, 2003). Ali *et al.* (2008) and Alimba and Bakare (2016) reported similar observations. The presence of Karyorrhectic cells might be associated with the chromatin disorder caused by xenobiotics in the waste leachate, resulting to dense network of nucleochromatin elements that lead to fragmentation and disintegration of the nucleus. Karyolytic cells appears to have no nuclei. This indicates cell injury, which are associated to the late stage of cell death process.

The main impact of oxidative stress is chromosomal damage (Radetski *et al.*, 2004), and elevated levels of oxidative enzymes was earlier observed in this study. Induction of DNA damage due to oxidative stress have been reported in waste leachate exposures (Bakare *et al.*, 2013). This occurrence might be due to oxidative biotransformation that produces high DNA-reactive specie (Woodhead *et al.*, 1999). This postulation reinforced the reports of Bakare *et al.* (2012), wherein they showed that MN induction by landfill leachate in mouse might be due

to oxidative damage caused by chemicals present in the leachate.

Furthermore, heavy metals can form a bond with phosphate and DNA bases, interfering with protein structure and function to cause DNA damage (Resit *et al.*, 1998). High concentrations of heavy metals above the standard permissible limits observed in the leachate might also be accountable for these observations. Heavy metals are known mutagenic and studies have linked the genotoxic potential of heavy metals to accumulation of free radicals and DNA damage actions in some organisms (Andreikėnaitė *et al.*, 2007). In addition, the elevated levels in conductivity, TDS, COD, BOD and anions levels suggested that the waste leachate contain high proportion of inorganic pollutants, which can also contributed to the oxidative damage and genotoxic effects observed in this study.

Conclusion

In this study, observed induction of oxidative stress and genotoxicity in concentration and duration dependent manner was stimulated by the sampled waste leachate in *L. aurora*. The genotoxic effect observed was likely induced through the production of reactive oxygen species (ROS) which also resulted in oxidative stress. This reinforced that waste leachate consists of hazardous compounds capable of posing a public health risk to the environment, surrounding biota and humans included. This study also demonstrate the sensitivity of giant African land snail to environmental pollutants and therefore, recommends its use in environmental monitoring studies.

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Conflict of interest

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Reference

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