

ECOTOXICOLOGICAL ASSESSMENT OF LEACHATE FROM AMILEGBE DUMPSITE, ILORIN, NIGERIA USING *Clarias gariepinus* (Burchell 1822) AND *Allium cepa*

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ABSTRACT

*Indiscriminate discharge of solid waste materials from anthropogenic activities has become a major environmental problem in Nigeria. Leachate samples were collected from Amilegbe dumpsite in Ilorin. The physico-chemical qualities of the leachate as well as heavy metals content were analysed using standard methods. Different concentrations (3.625 %, 6.25 %, 12.5 %, 25.0 %, 50.0 % and 100.0 % (v/v leachate/distilled water)) of leachate samples were prepared. The 96–h LC₅₀ of leachate samples was determined for *Clarias gariepinus* fingerlings using Probit method. *Allium cepa* bulbs were also exposed to the different concentrations of the leachate and the root length inhibition and chromosomal aberration were investigated. The results showed certain sample-constituents of the leachate (e.g. pH, BOD, COD, heavy metals) to be at concentrations beyond the maximum permissible limits set by National Environmental Standards and Regulatory Enforcement Agency NESREA. The 96–h LC₅₀ of leachate to *Clarias gariepinus* fingerlings was 20.26%. Prior to the mortalities at various concentrations, symptoms of toxicity such as rapid and erratic swimming, uncoordinated movement and prolonged gaping of jaws were observed. These observations as well as mortality records were concentration–dependent. The root lengths' mean of *A. cepa* exposed to different concentrations of the Amilegbe leachate when compared to the control, were statistically significantly different ($p < 0.05$) with concentration dependent. The leachate concentrations were observed to induce different chromosomal aberrations with mitotic indices decreasing as the concentration rises. Leachates from the dumpsite have detrimental effects on both *Clarias gariepinus* and *Allium cepa*.*

Keywords: Leachate, *Clarias gariepinus*, LC₅₀, *Allium cepa*, Chromosomal aberration, Mitotic index.

INTRODUCTION

Solid wastes are generated by almost every activity of man and the amount varies by source, season, geography and time (Robert, 1999). One of the most important by-products of an urban lifestyle - municipal solid waste, is growing at a faster rate than the rate of urbanization (Hoornweg and Bhada-Tata, 2012). In Nigeria, municipal solid waste generated was estimated to be 44 pounds (20 kg) per capita per year (Olafusi, 2004). Disposal of solid waste is a major environmental problem faced by Nigerian cities and towns. The most common forms of waste disposal in the country are dumping in open spaces, uncontrolled burning and disposal in surface water bodies (Adewoyin, 2016). There are also government designated landfills. The landfills in some developed and developing nations are unsanitary without liner, covers and leachate collecting systems (Bakare *et al.*, 2013). Through these disposal methods, hazardous substances are being released into the environment and these can adversely affect the survival of living organisms including human (Alimba, 2013). Leachate is a mixture of dissolved organic matter, inorganic macro-components, heavy metals, xenobiotic organic compounds and microbes that are opportunistic pathogens (Oshode *et al.*, 2008; Bialowiec, 2011). It is generated due to the infiltration of water/precipitation through the waste mass and the wastes biodegradation (Bakare *et al.*, 2013).

Domestic and industrial wastes gradually find their ways into the aquatic environment (Ajala *et al.*, 2015), therefore adversely affecting aquatic organisms. Fishes are a key unit in many natural aquatic food webs and they can also serve as environmental indicators of polluted water (APHA, AWWA, WEF, 1998). Fish protein makes up complete protein sources in many people's diets around the world (Oyelere, *et al.*, 2016). *Clarias gariepinus* is of commercial importance in aquaculture due to its positive attributes like resistance to diseases, high fecundity and ease of larval production (Hogendoorn, 1980; Haylor, 1991; Kestemont *et al.*, 2007). However, in the larval stages of catfish, there is high mortality attributed to infectious diseases caused by parasites (Adewoyin, 2016). The effects of toxicity of organic compounds on the aquatic organisms can range from mortality to hepatotoxicity, immunotoxicity, carcinogenicity, and metabolic alterations that can lead to a decrease in the rates of reproduction, predation, and decomposition (Araujo *et al.*, 2006; Dave and Nilsson, 2005). Behavioural responses of *Clarias gariepinus* exposed to leachate include uncoordinated movement, rapid and erratic swimming, up and down darting with occasional jumpy movement, alternate swimming on the lateral and ventral sides and rapid opercula movement; all these were as a result of bioaccumulation in the gill filaments that lead to hyperactivity in fish (Oshode *et al.*, 2008; Aderemi *et al.*, 2012; Olujimi *et al.*, 2016; Kosemani and Edward, 2017).

Onions form essential part of the traditional daily diet since they are consumed in different ways by different people (Mohammed *et al.*, 2013). Due to the sensitivity and good correlation

of *Allium cepa* test with mammalian test systems (Grant, 1994; Rank and Nielson, 1997), it is one of the best established test systems routinely used to evaluate the genotoxicity potential of environmental chemicals (Sarvesh, 2009). Many researchers have used *Allium cepa* as bioindicator of environmental pollution; the rooted bulbs were employed as standard short-term assays in monitoring the environment and as well as tool for evaluating and ranking chemical with reference to their toxicity (Mishra, 1993; Leme and Marin-Morales, 2009). Cytologically, mutagens can be detected through cellular inhibition, disruption in metaphase; induction of chromosomal aberrations, numerical and structural, ranging from chromosomal fragmentation to the disorganization of mitotic spindle and consequently of all, subsequent dependent mitotic phase (Tedesco and Laughinghouse IV, 2012).

Leachate was highly toxic to *Allium cepa*; it caused 95% toxic effect (Grażyna and Dorota 2008). Different types of chromosomal aberrations were induced (sticky chromosome, disturbed spindle, anaphase bridge, vagrants, fragments etc.) (Bakare *et al.*, 2000; Iwegbue *et al.*, 2007; Sarvesh, 2009; Olorunfemi *et al.*, 2011). Mohammed *et al.* (2013) observed lower mitotic indices, increase in cytological damages with increase in raw leachate concentrations and also chromosomal aberrations in *Allium cepa* exposed to dumpsites leachates. Information on the toxicity of leachate from Amilegbe dumpsite is scanty. Therefore, this study investigated the mortalities of *Clarias gariepinus*' fingerlings and growth inhibition and chromosomal aberrations of *Allium cepa* bulbs exposed to different concentrations of the leachate collected from a dumpsite located within Ilorin, Nigeria.

MATERIALS AND METHODS

Study Area, Sample Collection and Analysis

Amilegbe dumpsite is located on longitude 4°35'E and latitude 8°30'N. It is located in Ilorin East local government area, Kwara state, Nigeria. Collection of leachate samples were from 20 leachate wells. The samples were mixed thoroughly to make a composite sample. A total volume of about 10 L was transferred in prewashed plastic containers to the laboratory. Filtering of the samples was done to remove debris and stored in the refrigerator at 4°C prior to analysis. Samples for biochemical oxygen demand (BOD) were not refrigerated; they were collected in 250 ml amber bottles. In situ measurement of pH, dissolved oxygen (DO) and total dissolved solids (TDS) were carried out by the use of pH meter model UNISCOPE ATpH-6, DO meter model Acorn DO and TDS meter model Exstik EC 400 respectively. Standard procedures in the methods described by the American Public Health Association (APHA, 1998) were used to determine BOD, Chemical oxygen demand (COD), total alkalinity, nitrate, sulphate, ammonia and chloride.

Samples for the analysis of metals (Copper (Cu), Manganese (Mn), Cadmium (Cd), Lead (Pb), Iron (Fe), Mercury (Hg) and Arsenic (As)) were digested using the nitric acid digestion

method (APHA, 1998) and concentrations measured using the BWF atomic absorption spectrophotometer.

Acute toxicity test using *Clarias gariepinus* fingerlings

Clarias gariepinus fingerlings (9.58 ± 2.05 g) were obtained from Kwara State fish farm located in Ilorin. International guidelines on the use of fishes for research described by the American Fisheries Society (2004) were followed. The fish were fed on commercial fish feed (coppens-2 mm with about 40 % crude protein) twice daily at 4 % body weight (Meyer *et al.* 1993). They were acclimatized in the Fishery laboratory of Zoology Department, University of Ilorin, using chlorine-free bore-hole water for 14 days. The feeding of the fish was stopped 24 hrs before the commencement of the experiment and they were not fed throughout the 96 hrs of the experimental period (USEPA, 1996). The acute toxicity test was carried out by exposing fingerlings of *Clarias gariepinus* (n=210) into seven different aquaria containing the control (dechlorinated water) and varying concentrations (3.625%, 6.25%, 12.5%, 25%, 50% and 100% v/v) of leachate. Ten (10) fish were introduced into 30 litre capacity circular plastic aquarium each and the experiment was set in triplicate. Mortality of the fingerlings was monitored and recorded every 24 hrs for a period of 96 hrs following the OECD Test Guidelines for testing chemicals. The estimation of the median lethal concentrations (LC₅₀) was calculated using the mortality data subjected to EPA Probit Analysis Program, version 1.5 (USEPA, 1997).

***Allium cepa* assay**

The *Allium cepa* assay was carried out according to the standard procedures described by Bakare (2009). The assay has two parts: Growth inhibition and chromosomal aberration assessment.

Onions (n=70) were obtained from Ipata market, Ilorin and sun dried for three weeks. The ring of root primordial was left intact while the outer scale and the brownish bottom plates were removed. The onions were then cleaned with fresh water. Different concentrations of the test liquid were prepared, the bulbs were placed in them and these were left in the dark for 72 hrs. The liquid were being changed every 24 hrs. The root tips of 2 of the growing bulbs were cut at 48 hrs and these were fixed in methanol: glacial acetic acid in the ratio of 3:1(v/v) for 24 hrs.

The root lengths of the remaining 2 bulbs were measured at 72 hrs and the mean lengths were calculated and recorded for growth inhibition assessment.

The fixed root tips were hydrolysed in 1N HCL at 60° for 5 mins for chromosomal aberration analysis. Rinsing of the root tips were done thrice in distilled water to remove all acid. Teasing of root tips were done on microscope slides and they were stained with acetocarmine for 10 mins. Filter papers were used to remove excess stain and the slides were covered with cover

slips. The slides were tapped gently after covering and wrapped with tissue paper to ensure that no air bubbles are in the slides. The edges of the cover slips were then sealed with nail polish and allowed to dry. These slides were then viewed under the microscope at 1000X magnification to check for chromosomal aberration.

Statistical Analysis

$$\text{Mitotic Index} = \frac{\text{No. of dividing cells}}{\text{Total No. of cells}}$$

Microsoft Excel 2010 and Statistical Package for Social Sciences (SPSS) version 20.0 were used for all statistical analyses. Median lethal concentration (LC₅₀) was calculated using the EPA Probit Analysis Program, version 1.5 (USEPA, 1997). One-way analysis of variance (ANOVA) was used to compare means of the root inhibition and chromosomal aberrations. Results are presented as mean $\pm$ standard error for 10 onion bulbs per concentration. Tukey post-hoc test at the 0.05 probability level was used to determine the statistical significant differences between control and the different concentrations of the leachates.

RESULTS

Physico-chemical analysis

The results of the physico-chemical parameters and heavy metals analysed are presented in Table 1. The pH of the water sample, BOD, ammonia and other physicochemical parameters as well as all the heavy metals analysed were above the limits stipulated by National Environmental Standards and Regulatory Enforcement Agency (NESREA, 2011).

Clarias gariepinus Mortality

The percentage mortality of *Clarias gariepinus* exposed to varying serial dilution of leachate was recorded in Table 2. The lowest percentage mortality (10 %) was observed in 6.25 % concentration of leachate, while the highest percentage mortality (100%) of *Clarias gariepinus* was observed in the group exposed to 100% (highest) concentration of leachate after 96 hrs. The percentage mortality of *Clarias gariepinus* recorded was directly proportional to increase in the leachate concentration. This showed a concentration-dependent mortality. The 96 hrs LC₅₀ value of 20.26 % of leachate concentration was obtained for *Clarias gariepinus*.

Allium cepa Assay

With increase in leachate concentration, the root length of *A. cepa* decreases (Table 3). Thus, with increase in leachate concentration, growth inhibition of *A. cepa* root length increases with 100% having the highest growth inhibition due to highly concentrated leachate composition.

Table 4 shows the concentration dependent decrease in mitotic index (MI), thus, the higher the concentrations of leachate, the lower the mitotic index.

DISCUSSION

Leachate, complex mixture of organic, inorganic and many unidentified toxicants, may pose risk of unknown magnitude to aquatic life (Nwabueze, 2011). There are serious health implications due to the presence of illegal waste dumpsites in public places and lack of proper solid waste management (Duh *et al.*, 2017). The flow of leachate from waste dumpsites into nearby water bodies and also percolation into soil may have adverse effect on aquatic organisms as well as plant growths.

The 96 hrs LC₅₀ value of 20.26% recorded in this study is in contrast with the report of Shosaku *et al.* (2006) who recorded 19.2 % as the 96 h LC₅₀ values of leachate in larvae of *Oryzias latipes* and Emmenike *et al.* (2012) who recorded 32 % as LC₅₀ value in *Pangasius sutchi*. The difference in toxicity of leachate to *Clarias gariepinus* could be attributed to differences in susceptibility and tolerance related to metabolic pathways that could result to different biotransformation and excretion (Alkahem, 1994).

Allium cepa test is a useful bio-indicator for the detection of genotoxicity (Cabuga Jr *et al.*, 2017) and thus it was utilized in this study to test Amilegbe leachate. Chromosome aberrations were shown in the four phases of cell division (prophase, metaphase, anaphase and telophase) of the *Allium cepa*, thus indicating chromosomal abnormalities. Chromosome aberrations analyses allow estimation of genotoxic effects and evaluation of clastogenic and aneugenic actions (Rank and Nielsen, 1998). The mitotic index analysed in this study showed the genotoxic effect of leachate on *A. cepa* because low mitotic index reflects direct genotoxic effects of a toxicant. With increasing concentration of the leachate, decreases in mitotic index are shown by the cells of *A. cepa* root tips. This suppression of mitotic activity is often used in tracing cytotoxicity (Smaka-kinel *et al.*, 1996). The abnormal conditions of the cells might have been induced by the exposure of the toxicant. The abnormalities of chromosomes could have been caused by the blockage of DNA synthesis or inhibition of spindle formation. Suppression of mitotic activity and occurrence of chromosomal aberration accompanied root wilting.

This study provides evidence that growth inhibition and chromosomal aberration in *A. cepa* were caused by the exposure to leachate. The presence of some heavy metals and physico-chemical properties of the water body which goes beyond the standard values given by NESREA (2011) may have caused inhibition. Growth inhibition was most seen at 100% concentration. Growth inhibition decreased with a decrease in concentration of leachate. The maximum growth was recorded in the control.

Rate of chromosomal aberration also increases as leachate concentration increases. According to Odeigah *et al.* (1997), high toxicity is indicated by sticky chromosomes and it is an irreversible effect that leads to cell death. Chromosome stickiness causes immediate reaction with DNA during inhibition periods, causing DNA-DNA or DNA protein cross linking (Amin, 2002). Therefore, the anomalies observed in the *Allium cepa* root meristem could be as a result of heavy metals such as Cd, Pb, Mn, Ar and other interaction in the leachate sample. Also, plant (onion) roots are extremely useful in biological testing of different pollutants since the root tips are the first to be exposed to toxicants dispersed in soil or in water (Fiskesjo, 1988; (Cabuga Jr *et al.*, 2017)).

CONCLUSION AND RECOMMENDATION

This study shows that leachate reduces the physico-chemical qualities of any water body which leads to mortalities in fish and chromosomal aberration, mitotic disturbances and growth inhibition in *Allium cepa*. The LC₅₀ values determined in this study provides the baseline for toxicity test of Amilegbe leachate. Therefore, leachate from illegal dumpsites in Nigeria and other developing countries may contaminate the environment and negatively impact on human populations and other living organisms. It is important that appropriate regulatory authorities implement sustainable methods of managing wastes so as to protect human and environmental health.

Table 1: Physico-Chemical properties of the Amilegbe Leachate

PARAMETERS*	AL	NESREA LIMIT
pH	7.3	6.5-8.5
BOD	59.2	6
COD	14.4	30
TDS	296	
Nitrate	18.50	40
Ammonia	20.20	2
Sulphate	56.778	500
Total alkalinity	0.6	
Chloride	1.75	350
Lead	1.11	0.1
Cadmium	0.14	0.01
Manganese	0.42	
Copper	0.19	0.01
Iron	0.78	0.5
Mercury	0.02	0.0005
Arsenic	0.06	0.05

*All values are in mg/L, except pH that has no unit. AL: Amilegbe Leachate; BOD: Biochemical Oxygen Demand; COD: Chemical Oxygen Demand; TDS: Total Dissolved Solid; NESREA: National Environmental Standards and Regulations Enforcement Agency.

Table 2: Mortality rate of *Clarias gariepinus* fingerlings exposed to leachate for the period of 96 hours

Concentration (%)	Number of fish	24 h	48 h	72 h	96 h	% Mortality
Control (0.00)	10	0	0	0	0	0
3.625	10	0	0	0	0	0
6.25	10	0	0	0	1	10
12.5	10	0	0	1	2	30
25	10	0	1	2	3	60
50	10	0	3	2	3	80
100	10	3	4	3	0	100

Table 3: Mean root length and growth inhibition observed in *Allium cepa* exposed to Amilegbe leachate

Concentration (%)	Mean root length (cm)	Growth inhibition
0.00	4.8 ±1.15	-
3.625	3.7±1.05	1.1
6.25	3.4±1.01	1.4
12.5	2.9±0.13*	1.9
25	2.7±0.13*	2.1
50	1.7±0.74*	3.1
100	0.8±0.02*	4.0

*Statistically significant $p < 0.05$

Table 4: Cytological effects of the Amilegbe Leachate on *Allium cepa* root cells

Concentration (%)	Mean number of cells	Mean number of dividing cells	Mitotic index (%)	Number of aberrant cells	% of aberrant cells
0.00	1119	300	0.26	0	0
3.625	857	102	0.11	8	4.91
6.25	693	64	0.09	13	7.98
12.5	613	42	0.06	19	11.66
25	372	20	0.05	30	18.40
50	315	12	0.04	45	27.61
100	150	5	0.03	48	29.45

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