PRINT ISSN 1119-8362
Electronic ISSN 2659-1502

Full-text Available Online at
https://www.ajol.info/index.php/jasem
http://www.bioline.org.br/ja

J. Appl. Sci. Environ. Manage.
Vol. 27 (8) 1633-1638 August 2023

Effect of Raw Leachate on Gills and Liver of Juvenile African catfish (*Clarias gariepinus*)

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ABSTRACT: The effect of toxicity of raw leachate obtained from dumpsites on *Clarias gariepinus* (C. gariepinus) (n = 6) was studied under laboratory conditions with the aim of establishing its effect on the gills and liver of C. gariepinus. The physico-parameters (pH, conductivity, total dissolved solid, salinity, temperature, dissolved oxygen, chemical oxygen demand, biological oxygen demand) and heavy metals (lead, cadmium, chromium, copper and nickel) were also analyzed using standard methods. The results of physico-chemical analyses of leachate showed contamination of organics, salts and heavy metals. Furthermore, lethal concentration (LC_{50}) of leachate effluent on C. gariepinus was determined using probit method to ascertain fish mortality. Result revealed that C. gariepinus exposed to different concentration of raw leachate recorded increasing mortality of 0, 0, 1, 4 and 5 at 0%, 5%, 10%, 25% and 75% concentration respectively for a period of 96 hours. The colour and behavioral responses were normal in the control experiment. But there were abnormal behavior including erratic swimming and quick sudden movements in concentrated samples. Also, results of the histopathological analysis showed alterations in the gills and liver. The study concluded that raw leachate is toxic to C. gariepinus, and therefore recommends for adjusting factors enhancing anaerobic biodegradation that lead to leachate stabilization as well as treatment and monitoring of effluent released into water bodies.

DOI: https://dx.doi.org/10.4314/jasem.v27i8.4

Cite this paper as: ODUH, O; WILFRED, O; OKURE, V. U. (2023). Effect of Raw Leachate on Gills and Liver of Juvenile African catfish (*Clarias gariepinus*). *J. Appl. Sci. Environ. Manage.* 27 (8) 1633-1638

Dates: Received: 23 July 2023; Revised: 21 August 2023; Accepted: 26 August 2023 Published: 30 August 2023

Keywords: *Clarias gariepinus;* leachate; fish gills; fish liver; African catfish

Nigeria is faced with several environmental challenges including lack of judicious and prudent management of solid wastes. The animal generation of municipal solid wastes (MSW) in Nigeria is about 29.78×10^9 kg yearly (Ojolo and Bamgboye, 2005), and this has increased due to rapid urbanization and population growth rate. Wastes in developing nations are commonly disposed in uncontrolled and unbind landfills, and improperly sited open dumps located in public places and wetlands or other areas with seasonally high water. The disposal sites are capable of releasing large amounts of harmful chemicals to nearby water sources and air via leachate and landfill gases respectively (Christensen, *et al.*, 2006). Pollutants from solid wastes most often penetrate towards the lower soil horizons as leachates, and subsequently polluting the ground water at varying degrees (Adedosu *et al.*, 2013). The bio-effect of leachate from waste drop sites leaching into nearby water bodies is alarming, especially on aquatic life, though this effect is minimized depending on the distance from the source of generation (Olujimi *et al.*, 2016). Additionally, chemical compounds in leachates pose serious risks to the ecosystem and human health when the chemicals migrate to surface water and drinking water wells (Sawyerr *et al.*, 2017) or accumulated in the body of fishes consumed by man. Such risks are known to be very high when the landfill site lacks an impermeable liner of leachate collection system which allows for the direct flow of leachate into ground water (Sawyerr *et al*., 2017). Fish is a bio-indicator species and plays an increasing important role in the monitoring of water pollution because it responds with great sensitivity to the changes in the aquatic environment, hence, as one of the consumer sources of protein, it is imperative to critically evaluate the degree of contamination of water bodies caused by leachate. The realization of the polluting effect of leachate has become a public health concern, therefore prompting a number of studies on its toxicity on aquatic organism. The objective of this study was to evaluate the effect of raw leachate on the gills and liver of juvenile African catfish (*Clarias gariepinus*).

MATERIALS AND METHODS

A bioassay was performed for 96 hours using fingerlings of C. gariepinus as the test organism to examine the toxicity of different concentrations of leachate on fish.

Study area: Leachate samples were collected and analyzed to assess their characteristics and stability. Two locations were considered for this study, Choba market dumpsite and Abuja campus of UNIPORT dumpsite. The Choba dumpsite is located near the new Calabar River in Obio/Akpor Local Government Area of Rivers State in Nigeria. In terms of size, the Choba market dumpsite is considered one of the biggest dumpsites in the region. Choba is an area occupied by fishermen, farmers, students as well as industries. The dumpsite is connected to the new Calabar River where leachate from the dumpsite washes down into the sloppy structure to the river.

Coordinate of the dumpsite: Latitude North: 0.4’53’20.2”; Longitude East: 006’53’54.4”; Elevation: 10 m; Distance: 39.6 m

The Abuja campus (UNIPORT) dumpsite is also located in Choba, Abuja campus, near the swimming pool and behind the medical hostel. Also, several business activities take place within the area.

The coordinate of the dumpsite: Latitude North: 04’53’48.3”; Longitude East: 006’55’17.5”; Elevation: 386 m; Distance: 112.9 m

Sample collection and fish model: The leachate effluent was collected from three stations in both locations (Choba market dumpsite and Uniport - Abuja campus dumpsite). The dumpsites were dug with a shovel and a machete to a depth of 1 metre to collect the leachate and was transferred into a 10-liter plastic container previously washed with non-ionic detergent, rinsed with tap water and later soaked with 10% HNO_3 for 24 hours, then finally rinsed with de-ionized water prior to usage. During sampling, bottles were rinsed with sample leachate three times and then filled to the brim. The samples were taken to the Toxicity laboratory, Animal and Environmental Biology Department, University of Port Harcourt for preservation during the period of experiment. Thirty-six (36) C. gariepinus (n = 6) were obtained from the African Regional Aquaculture Centre (ARAC), Aluu-Port Harcourt Nigeria. Healthy C. gariepinus with average weight of 4.78 g and length 5.30 cm were conditioned and acclimatized in a non-chlorinated water in plastic tank aquarium measuring 30 cm by 25 cm by 25 cm for two (2) weeks under laboratory conditions to allow them to adapt to experimental temperature (27 ± 2 ºC) and fed *ad libitum* with commercial feed at 5% body weight. C. gariepinus was used because it can withstand stress and serves as high commercial value in Nigeria.

Determination of physico-chemical parameters and trace metals in leachate: Physicochemical parameters (hydrogen ion concentration (pH), temperature, conductivity, total dissolved solid (TDS), dissolved oxygen (DO), salinity, chemical oxygen demand (COD) and biological oxygen demand (BOD) of the leachate sample from the two dumpsites were analyzed according to the Standard Methods for the Examination of Water and Wastewater (Eaton and Franson, 2005). Heavy metals including lead, (Pb), cadmium, (Cd), chromium, (Cr), nickel (Ni), and copper (Cu) were also analyzed using Atomic Absorption Spectrophotometer Schimadzu model AA-6650 (Thomas Scientific, Swedesboro, USA) as described by Eaton and Franson (2005) at Diamond Standard Integrated Service Limited, Rumuodara, Port Harcourt.

LC_{50} determination, Behavioral and histological assessment: Range finding test was done to ascertain the concentration of the test solution needed to induce mortality within 96 hours (Reish and Oshida, 1980). Toxicants were prepared by adding appropriate volume of water effluent. Five concentrations were made: 5%, 10%, 25%, 50% and 75% for leachate effluent. Six C. gariepinus were exposed to the five different concentrations of the leachate in each container. Behavioral pattern of C. gariepinus and any associated changes were accordingly observed. Dead C. gariepinus were identified by the lack of locomotion, and were discarded immediately. The number of dead C. gariepinus per group was recorded against time. LC_{50} values of the fish for 96 hours was

then calculated using the probit analysis. At the end of the 96 hours, the gills and liver of C. gariepinus were removed and prepared for histological examination. First, the organs were fixed in bouin's fluid for 24 hours, washed with 70% ethanol and were embedded in paraffin. About 5 um thickness was sectioned and stained with haematoxylin-eosin and thereafter qualitatively analyzed under a light microscope.

Statistical Analysis: Data obtained on mortality was analyzed using probit analysis method (Finney 1971). All the results were expressed as mean ± S.E.M. and analyzed using analysis of variance (ANOVA) statistical package. Mean was compared for significance and p-values ≤ 0.05 were considered to be statistically significant.

RESULTS AND DISCUSSION

Results of the toxicity test of C. gariepinus exposed to raw leachate are shown in Table 1. There was no mortality of C. gariepinus through the 96 hours of the experiment in the control and the 5% concentration. Mortality however increased generally with increasing time and concentration, except in the 50% solution which recorded zero mortality throughout the duration of the experiment. The 10% concentration recorded the lowest mortality of 1, whereas the highest mortality (5) was recorded in the 75% concentrated sample. Based on the probit values from Table 2, the lethal concentration of the samples required to kill 50% of the test organism (LC_{50}) after 96 hours was found to be 4365.16 ml/L.

Table 1. Mortality C. gariepinus exposed to different concentration of raw leachate (n=6).

Conc. Ml/L	Log of Conc.	Total	24hrs	48hrs	Probit	72hrs	Probit	96hrs	Probit
0	0	6	0	0	0	0	0	0	0
5	1.70	6	0	0	0.00	0	0.00	0	0.00
10	2.00	6	0	1	3.72	1	3.72	1	3.72
25	2.39	6	1	2	4.16	2	4.16	4	4.16
50	2.70	6	0	0	0.00	0	0.00	0	0.00
75	2.88	6	0	2	4.16	2	4.16	5	5.00

The mean values of physical and chemical analyses of the leachate samples are presented in Table 3. pH and temperature values were 8.23 ± 0.25 and 26.50 ± 0.25 and falls within the WHO standard range. Except for conductivity which recorded 5998 ± 35, TDS and DO showed values below the WHO standard (52.75 ± 0.88 and 3.700 ± 3.1 respectively). The values for COD and BOD were beyond the limit (3201 ± 25 and 215 ± 13.0 respectively). Table 4 shows mean concentrations of heavy metals in leachate samples obtained from Choba market dumpsite and UNIPORT dumpsite, Nigeria. The concentrations of Pb, Cd, Cr, Ni and Cu are 0.21 ± 0.12 mg/L, 0.09 ± 0.10 mg/L, 0.01 ± 0.10 mg/L, 0.01 ± 0.10 mg/L, and 0.01 ± 0.16 mg/l, respectively. Cd showed a slightly higher value above the standard.

Table 2. LC_{50} values for the different concentrations

Time (Hrs)	Regression equation	LC_{50}
24	-	-
48	y= -0.78+ 1.37x	16218.1ml/l
72	y= -0.78+ 1.37x	16218.1ml/l
96	y= -1.74+1.85x	4365.16ml/l

Table 3. Physicochemical analyses of leachate samples collected from two locations

Parameters	Mean values from the two dumpsites	WHO standard
pH	8.23 ± 0.25	6.5 – 8.5
Conductivity (µs/cm)	5998 ± 35	5000
TDS (mg/L)	52.75 ± 0.88	2000
Temperature (ºC)	26.50 ± 0.25	Ambient
DO (mg/L)	3.700 ± 3.1	7.50
COD (mg/L)	3201 ± 25	400
BOD (mg/L)	215 ± 13.0	20

Table 4. Mean concentration of heavy metals of leachate samples collected from two locations

Metals	Mean metal conc., mg/L	WHO Standard, mg/L
Lead	0.21 ± 0.12	0.05
Cadmium	0.09 ± 0.10	0.01
Chromium	0.01 ± 0.10	0.05
Nickel	0.01 ± 0.10	0.20
Copper	0.01 ± 0.16	1.00

Histopathological findings: Gills from the control had prominent and well-defined gill rakers, gill lamella, intact arches and normal histology of the primary lamella filament (Figure 2A). However, those extracted from the 75% (highest) concentrations showed abnormalities relative to concentration and time, and showed progressive eroding of the gill filaments, inner lamellae filled with chloride cells (mucoid metaplasia), epithelial hyperplasia cells of immune system and alteration of normal structure of primary lamella (Figure 2B) after 96 hours. Similarly, the liver from the control showed no visible pathological lesion, only hepatocytes (Figure 2C). But liver from the 75% (highest) concentrations showed karyolysis, dilation of sinunoids, congestion, vacuolar degeneration, cloudy swelling of hepatocytes and focal necrosis after 96 hours (Figure 2D).

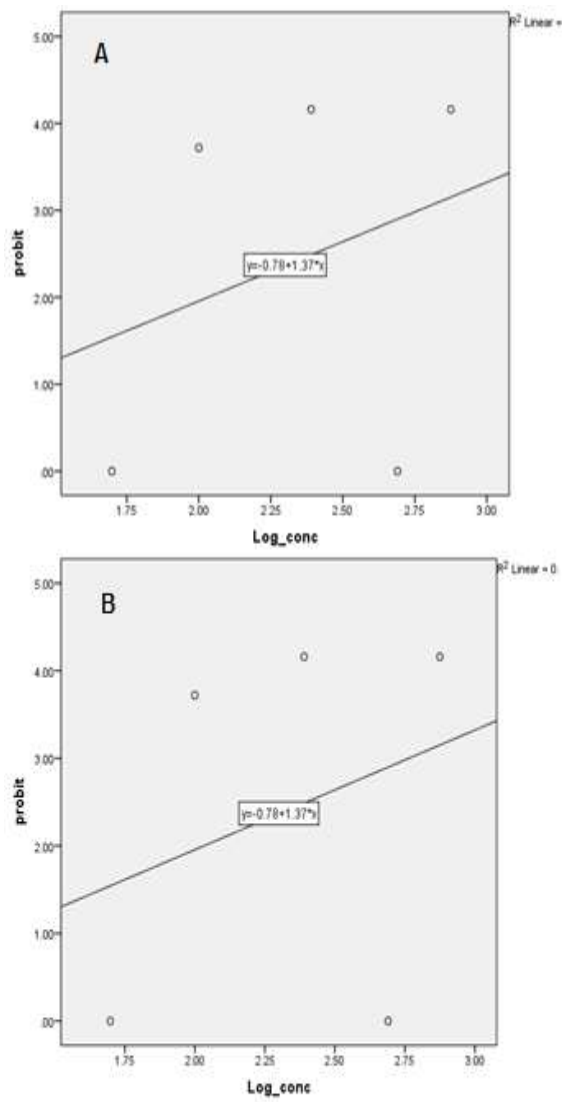

Fig 1. Graph of mortality in probit values against log of concentrations (A) after 48hrs and 72hrs (B) after 96hours.

Behavoural observations: After 96 hours, the test organism from different concentrations were compared with those of the control. The colour and behavioral response was normal in the control experiment. However, in the different concentrations, there were abnormal behavior including erratic swimming and quick sudden movements. In the tanks with high concentrations, most of the fishes were weak and settled at the bottom of the tanks. The present study tested the effect of raw leachate on C. gariepinus, which appears to be overwhelming and significant, indicating that raw leachate is toxic to aquatic organisms in general. Though C. gariepinus recorded no mortality after the 96 hour period in the control experiment and in the 5% concentration, it is because the control had no traces of leachate, and the 5% concentration contained insignificant level of leachate concentration to induce mortality. However, in higher concentrations, the toxic effect of the leachate resulted in increased mortality, except in the 50% sample which did not record any mortality as a result of contamination. The study further revealed that the 96 hour LC_{50} value was 4365.16 ml/L for C. gariepinus fingerlings. This study is the first of its kind as no previous study has reported similar figures on LC_{50} of the fishes within the geographical area studied. Again, within the different concentrations, there were abnormal behaviors including erratic swimming and quick sudden movements, with some of the fishes settling at the bottom of the tanks with higher concentrations, which were similar observations of Omitoyin *et al.,* (2006) and Abowei and Sikoki (2005), indicating that higher concentration of leachate contains toxins which are capable of inducing mortality of the fishes. The histopathological changes in the gills and liver of C. gariepinus have further revealed the toxic effect of raw leachate. The gills extracted from the 75% concentrated sample showed progressive eroding of the gill filaments, inner lamellae filled with chloride cells (mucoid metaplasia), epithelial hyperplasia cells of immune system, and alteration of normal structure of primary lamella, suggesting that the damage done to the gills increased with increasing leachate concentration. Similarly, in the healthy liver, there were no visible pathological lesion, only hepatocytes (Figure 2C), however, liver section from the 75% (highest) concentrated sample showed karyolysis, dilation of sinunoids, congestion, vacuolar degeneration, cloudy swelling of hepatocytes and focal necrosis after 96 hours (Figure 2D), which is consistent with Reiser *et al.,* (2010), thereby implicating the toxic effect of chemicals from the leachate on C. gariepinus which is prone to damage by the toxic chemicals due to its role as a vital organ in the metabolism of chemicals (Doherty *et al*., 2016). The levels of the physiochemical parameters have also been reported. Here, the pH (8.23 ± 0.25) was alkaline, and falls within the WHO standard range (6.5 – 9.5). The alkaline nature of the leachate means that the leachate has over time experienced washing away of the complex varieties of inorganic soluble substances (Oygard *et al.,* 2007). Hence, the older the dumpsite, the higher the pH. Moving on, conductivity of leachate serves as an important indicator of its saline or salt content. The study further reported high values for COD, conductivity and BOD in the leachates (above WHO guidelines) and could be as a result of high level of pollutants and increased volume of dissolved organic materials (Aluko *et al.,* 2002).

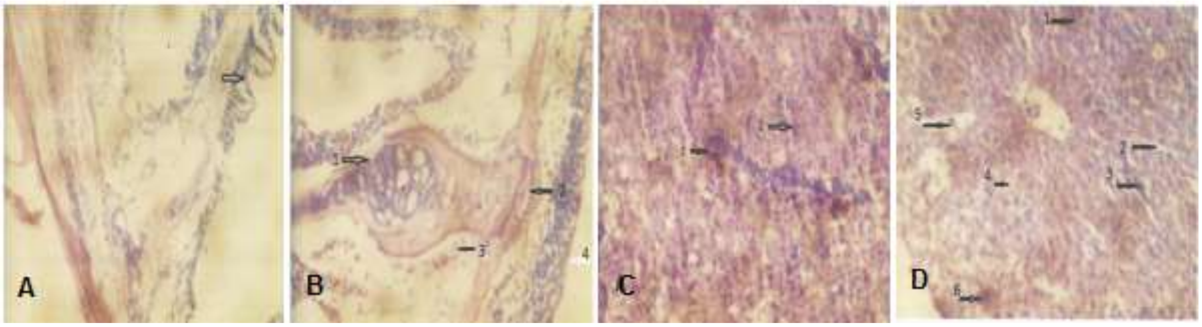

Fig 2. Histological section of A. Gill filament (control) showing normal histology of primary lamellae. Arrow shows well defined gill rakers. **B**. Gill filament from 75% conc. Arrows indicate (1) inner lamellae filled with chloride cells (mucoid metaplasia), (2) epithelial hyperplasia, (3) cells of immune system, and (4) alteration of normal structure of primary lamella. **C.** Liver from control showing normal hepatocytes (1) and sinusoids (2). **D**. Liver from 75% conc. showing (1) karyolysis, (2) dilation of sinusoids, (3) congestion, (4) vacuolar degeneration, (5) cloudy swelling of hepatocytes, (6) focal necrosis.

However, DO (3.700 ± 3.1) showed a lower value compared to WHO's standard (7.50 which is essential for aquatic life sustenance). Chapman, (1997) earlier opined that a concentration lower than 7.50 could be detrimental to aquatic life, providing the basis for the high mortality of C. gariepinus studied. Coincidentally, concentrations of Cr, Ni and Cu (with exception of Pb and Cd) were below the WHO standard values, hence they cannot be seen to be of threat to aquatic life as they are less affected by these metals (Tótha *et al.*, 2016). Raw leachate showed significant effect on C. gariepinus, suggesting that it is toxic to aquatic lives in general. Its effect on lower biota could even be far more devastating. The LC_{50} values and result of the histopathological study obtained imply that raw leachate is toxic to aquatic lives, therefore, monitoring the behavioral, morphological, physiological responses and histological changes in C. gariepinus would be beneficial as it will assist in evaluating aquatic pollution, fish health and fish quality in rivers and other water bodies receiving waste effluent.

Conclusion: The outcome of the study will provide a basis for monitoring the behavioral, morphological, physiological responses and histological changes in C. gariepinus as it will assist in assessing aquatic pollution, fish health and fish quality in water bodies receiving waste effluent. Furthermore, policies and regulations contributing to the environmental release of effluent and other toxic substances into water bodies should be reviewed to reduce emission, discharge and disposal of waste from the known sources including but not limited to household, hospitals markets, and industries. Finally, all necessary precautionary measures should be taken to mitigate practices resulting to environmental degradation due to the release of effluent and toxic substances into aquatic environments.

REFERENCE

Abowei, JFN; Sikoki, FD (2005). Water pollution management and control. Double Trust Publication Co., Port Harcourt.

Adedosu, HO; Adewuyi, GO; Adie, GU (2013). Assessment of heavy metals in soil, leachate and underground water samples collected from the vicinity of Olusosun landfill in Ojota, Lagos, Nigeria. *Trans. J. Sci. Technol.* 3(6): 73-86.

Aluko, OO; Sridhar, MKC; Oluwande, PA (2002). Characterization of leachates from municipal solid waste landfill site in Ibadan Nigeria. *J. Environ. Health Res.* 2(1) 32-37.

Chapman, D (1997). Water Quality Assessment. A Guide to the use of Biota Sediments and Water in Environmental Monitoring. E & FN Spon, London.

Christensen, TH; Kjeldsen, P; Bjerg, PL; Jensen, DL; Christensen, JB; Baun, A; Albrechtsen, H JC (2001). Biogeochemistry of landfill leachate plumes. *Appl. Geochem* 16:659-243.

Doherty, VF; Ladipo, MK; Aneyo, IA; Adeola, A; Odulele, WY (2016). Histopathological alterations, biochemical responses and acetylcholinesterase levels in Clarias gariepinus as biomarkers of exposure organophosphatases. *Environ. Monitor. Assess.* 185(5): 312.

Eaton, AD; Franson, MAH (2005). American Water Works Association, Water Environment Federation: Standard method for the examination of water and wastewater. American Public Health Association. Washington.

Finny, DJ (1971). Probit analysis. Cambridge University Press, Cambridge.

Ojolo, S; Bamgboye, AI (2005). Thermochemical conversion of municipal solid waste to produce fuel and reduce waste. *Agric. Eng. Int.: the CIGR Ejournal* 7:1-8.

Olujimi, OO; Ajayi, OL; Oputu, OU (2016). Toxicity assessment of Olusosun and Igando leachates using the African Catfish (Clarias gariepinus) as bioindicator species part I. *Ife J. Sci* 18(3): 693-704.

Omitoyin, BO; Ajani, EK; Adeshina, BT; Okuagu, CNF (2006). Toxicity of Lindane (GammaHexachloro-Cyclohexane) to Clarias gariepinus (Burchell 1822). *Wld. J. Zool.* 1: 57-63.

Oygard, JK; Gjengedal, E; Royset, O (2007). Size Charge Fraction of Metals in Municipal Solid Waste and Landfill Leachate. *Wat Res*. 41(1): 47-54.

Reiser, S; Schroeder, JP; Wuertz, S; Kloas, W; Handel, R (2010). Histological and physiological alterations in Juvenile turbot (Psetta Maxima, L.) exposed to sublethal concentrations of ozone-produced oxidants in ozonated seawater. *Aquaculture* 307(1-2):157-164.

Reish, DL; Osjida, OS (1987). Manual of methods in aquatic environment research. Part 10. Short term static bioassays. *FAO Fisheries Technical Paper* 247:1-62.

Sawyerr, HO; Adeolu, AT; Afolabi, SA; Salami, OO; Badmos, BK (2017). Impact of dumpsites on the quality of soil and groundwater in satellite towns of the Federal Capital Territory, Abuja, Nigeria. *J. Health Pollut.* 7(14): 15-22.

Tótha, G; Hermann, T; Da Silvac, MR; Montanarella, L (2016). Heavy metals in agricultural soils of the European Union with implications for food safety. *Environ. Int. J*. 88:299–309.