

Toxicity Assessment Using *Clarias gariepinus* and Microbial Characterization of Leachate from Akomo Municipal Solid Waste Landfill, Igbemo - Ekiti, Ekiti State, Nigeria

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Abstract This study aimed at investigating the toxic effect of raw leachates on freshwater and organisms inhabiting it, most especially fish. Samples of leachates and some associated microorganisms were obtained from Akomo landfill, Igbemo Ekiti, Ekiti State, Nigeria. Physico-chemical analysis showed that the leachate contained toxic elements in high concentration including some heavy metals namely Pb, Cd, Fe, Mn, Cu and Zn. The 96hrsLC50 (95% confidence interval) on *Clarias gariepinus* was 21.5% (14.82 - 31.2), haematocrit percent, erythrocyte number, haemoglobin concentration, leukocyte number was found to increase with increase in leukocyte concentration. Potential pathogen and toxin-producing microorganisms identified include *Pseudomonas fluorescences*, *Staphylococcus aureus*, *Bacillus cereus*, and *Penicillium oxidicum*. These observations are of prime health concern because there is no containment or treatment system for the leachate generated from the study site. This finding would therefore be of importance in assessment from landfill discharge into the aquatic environment in Ekiti State.

Keywords: leachate pollution, microbial characterization, toxicity, waste landfill, Igbemo – Ekiti

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1. Introduction

The intensity of man’s activities has led to increasing volume of solid waste worldwide despite the current level of technology advancement and industrialization [1]. Explosive population growth and improved life style pattern and poor law enforcement in terms of waste disposal are some of the factors responsible for increased municipal solid waste (MSW) [2]. Landfills being the most common means of waste disposal in developing nations may however pose serious threat to the quality of the environment when incorrectly secured and improperly operated [2]. Landfills pose a great threat to surface and ground water which could be deleterious if not checked on time, the scale of this threat depend on the composition and quality of leachate and the distance of a landfill from water sources [3]. The annual generation of municipal solid waste (MSW) in Nigeria is about 29.78×10^9 kg [3] and this may increase due to rapid urbanization and population growth rate. Although the effect of leachate is weakened with distance from the source of generation, it can still cause pollution of surface and groundwater. Leachate organic compounds affect odor and taste of groundwater and increase nitrogen compounds leading to eutrophication in surface water, high nitrate in drinking water, toxic heavy

metals in ground and surface waters [4]. Fish should be a major test organism in ecotoxicological studies because of their link to man in the food chain. They are particularly useful for assessment of water-borne and sediment-deposited toxins where they may provide advance warnings of the potential danger of new chemicals and possibility of environmental pollution [5]. In addition, fish health is essential to the success of aquaculture industry, and the industry is of growing importance in protein production for humans [6]. However, little information is available on the toxicity of MSW landfill leachate in Ekiti State, Nigeria and such a database would be useful in evaluating treatment options and possibilities of reuse. In Nigeria fish is regarded as one of the commonest source of protein, thus contamination of water bodies where fish resides deserves great attention. This work was designed to assess the impact of Akomo MSW landfill leachates and the associated microbes on *Clarias gariepinus*.

2. Materials and Method

Study Area: The study site used for this work was Akomo landfill, located in Igbemo Ekiti, Irepodun/ Ifelodun Local Government of Ekiti State, Nigeria, which was created in the year 1964 and has been opened to the public for municipal waste disposal and managed by both

public and private waste management operations. This landfill was not equipped with leachate collection and treatment system, the leachate produced is freely discharged into the surrounding aquatic and terrestrial environment media.

Sample collection: Raw leachate sample was collected in 500ml plastic bottle from leachate well (not less than 20grabs to a composite sample). It was transferred to the laboratory in pre-washed plastic container (10L capacity) which was filtered to remove debris and it was stored at 40°C until use, 48hrs later. This was considered as the stock solution and designated Akomo Raw Leachate (ARL). Serial dilutions were prepared in accordance with the standard procedure for short-term static bioassay [7]. The physical and chemical properties of the leachate were determined including, chemical oxygen demand (COD), biochemical oxygen demand (BOD), dissolved oxygen (DO), conductivity, chloride, sulphate, ammonia, and nitrate. The concentration of six heavy metals which includes lead (Pb), cadmium (Cd), manganese (Mn), zinc (Zn) and chromium (Cr) were estimated in the leachate using atomic absorption spectrophotometer (Buck AAS models, United Kingdom). 180 mg/L of leachate were collected in sterile plastic containers from three randomly selected points at the study site for microbiological analysis during the month of June to November 2010 (raining season) and November to March 2011 (dry season). The samples were analyzed for microorganisms using standard microbiological procedure [8].

Test organism: Fingerlings of *Clarias gariepinus* were obtained from Ekiti State Ministry of Agriculture, Fisheries Department, Ado Ekiti. The fishes were acclimatized and maintained in plastic tanks (40L capacity) at 26 °C for 14 days, the fishes were fed with commercial fish pellets (Coppens). Average weight of fish was 3.005±0.58g. Five serial concentrations (5%, 15%, 25%, 35 % and 45%) of leachate and control group were prepared. These concentrations of leachate were utilized randomly for the acute toxicity. Twenty fishes per tank were used to

conduct this assay with three replicates for each concentration and control. The setup was observed for 96hr and the test fishes were observed hourly for behavioral and physiological changes and mortality. The changes in hematology of the test fish was examined in the acute toxicity study. Three concentrations (3.08%, 6.17% and 12.33%) and a control group in duplicates were set up. Ten fishes in each group were fed at 5% body weight once daily, each concentration and the control were renewed at intervals. The blood samples of fishes were collected on the 7th, 14th and 21st day from the start of the experiment from the caudal vein of 5 fishes which was randomly selected from each group. Total erythrocyte and leucocyte count, packed cell volume (PVC) and hemoglobin estimation were carried out in the laboratory. The data collected for the study were subjected to appropriate descriptive and inferential statistics.

3. Result

The results in Table 1 revealed that the pH values of the three samples of the leachate used in this study for toxicity test are slightly acidic. The temperature of the samples was between 30.5-33.5°C. The BOD, COD and conductivity are relatively high. All values of BOD, Pb and Cr were higher than standard set by international regulatory body [11]. Table 2 showed that total coliform counts ranged from 0.8x10⁵ to 2.4x10⁹ and it also shows that total fungi counts ranged from 0.4 x 10⁹ to 1.2x10⁹, while total viable counts of microorganism in the raw leachate samples ranged from 1.4 x 10⁹ to 9.6 x 10⁹. The samples analyzed had positive potential pathogenic species which are toxin-producing organisms and included *Bacillus cereus*, *Pseudomonas spp*, *Staphylococcus aureus*, *Clostridium cordalli*, *Clostridium perfringens*, and *Micrococcus florescence* (see Table 3). The fungi isolated in the samples were also shown in the table which included *Aspergillus niger*, *Aspergillus terreus*, *Penicillium spp* etc. (Table 3).

Table 1. The Physical and Chemical Characteristics of Akomo Raw Leachate

Parameters	Sample A	Sample B	Sample C	WHO (2003)
Temperature °C	32.5	30.5	33.5	28.6-35.5
pH	6.7	6.5	6.4	6 – 9
Dissolved Oxygen(mg/L)	5.5	4.8	6.5	5.0
COD (mg/L)	33.2	34.1	28.5	-
BOD (mg/L)	75.17	58.5	65.8	50
Conductivity (µsmc ^l)	950	910	914	300
Nitrate (mg/L)	18.5	15.4	12.8	10
Ammonia(mg/L)	3.88	8.55	8.58	1.5
Sulphate (mg/L)	22.5	28.4	25.3	20
Chloride (mg/L)	42.2	48.5	42.8	250
Pb (mg/L)	0.07	0.07	0.07	0.05
Cu (mg/L)	0.05	0.05	0.05	1.0
Zn (mg/L)	3.52	3.51	3.32	5.0
Cr (mg/L)	2.50	2.48	2.50	0.05

Table 2. Means of Akomo Raw Leachate samples compared with International Regulatory Standards.

Parameters	ARL	USEPA ^a	FEPA ^b	WHO ^c
Temperature °C	32.17	30.0- 35.5	30.0– 35.5	30.0 - 35.5
pH	6.53	6.5-8.5	6.5 – 8.5	6.5 -8.5
Dissolved Oxygen(mg/L)	5.60	5.0	5.0	5.0
COD (mg/L)	31.93	410	-	-
BOD (mg/L)	66.49	-	-	50*
Conductivity (µsmc ^l)	924.67	-	-	1400
NO ₃ (mg/L)	15.57	10	-	44
NH ₄ (mg/L)	7.00	0.02	0.01	1.5*
SO ₄ (mg/L)	25.40	250	20	40
Cl (mg/L)	44.50	250	-	200
Pb (mg/L)	0.07	0.015	0.01	0.05
Cu (mg/L)	0.05	-	1.0	0.01
Zn (mg/L)	3.45	5.0	5.0	5.0*
Cr (mg/L)	2.49	0.10	0.05	0.10

^a (www.epa.gov/safewater/mcl.html)

^b Federal Environmental Protection Agency (2001).

^c World Health Organization 2006/2010.

* World Health Organization 2003.

Table 3. Microbiological Analysis of Akomo Raw Leachate Samples

Sample Code	Total Viable Counts	Total Coliform Counts µmL ⁻¹	Total Fungi Counts
Sample A	1.4x10 ⁹	0.8x10 ⁵	0.4x10 ⁹
Sample B	7.8x10 ⁴	1.6x10 ⁶	1.0x10 ⁶
Sample C	9.6x10 ⁷	2.4x10 ⁹	1.2x10 ⁹

Table 4. The Isolated Microorganism in the Leachate Samples Collated from Akomo Landfill

Sample A	Sample B	Sample C
<i>Bacillus cereus</i>	<i>Pseudomonas florescence</i>	<i>Staphylococcus aureus</i>
<i>Pseudomonas florescence</i>	<i>Micrococcus lites</i>	<i>Streptococcus facium</i>
<i>Pseudomonas aeruginosa</i>	<i>Serratia marscescense</i>	<i>Pseudomonas aeruginosa</i>
<i>Serratia marscescense</i>	<i>Bacillus cereus</i>	<i>Pseudomonas florescence</i>
<i>Bacillus marcerand</i>	<i>Proteus morgani</i>	<i>Micrococcus acidophilus</i>
<i>Staphylococcus aureus</i>	<i>Bacillus marceraus</i>	<i>Micrococcus lites</i>
<i>Klebsilla acrogens</i>	<i>Pseudomonas aeruginosa</i>	<i>Bacillus cereus</i>
<i>Clostridium cordalli</i>	<i>Staphylococcus aureus</i>	<i>Serratia marscescense</i>
<i>Proteus morgani</i>	<i>Salmonella typhimurinum</i>	<i>Salmonella typhimurinum</i>
<i>Aspergillus niger</i>	<i>Salmonella orizonae</i>	<i>Escherichia coli</i>
<i>Aspergillus terreus</i>	<i>Klebsilla acrogens</i>	<i>Klebsilla acrogens</i>
<i>Fusarium oxyporum</i>	<i>Escherichia coli</i>	<i>Aerobacter acrogens</i>
<i>Penicillium oxalicum</i>	<i>Aspergillus niger</i>	<i>Staphylococcus prievomric</i>
<i>Escherichia coli</i>	<i>Staphylococcus facium</i>	<i>Klebsilla pneumoniae</i>
<i>Micrococcus lites</i>	<i>Penicillium oxalicum</i>	<i>Proteus morganni</i>
<i>Salmonella typhimurinum</i>	<i>Saccharomyces onei</i>	<i>Xanthomonas spp</i>
<i>Numia spp</i>	<i>Fusarium oxysporum</i>	<i>Dostidium botulinum</i>
<i>Staphylococcus facium</i>	<i>Acrobacter acrogens</i>	<i>Aspergillus terrus</i>
	<i>Flavobacterium spp</i>	

Table 4 revealed that mortality increases with increase in leachate concentration in the replicates while there was no mortality in the control. It was also observed that

toxicity increased with increased concentration. The colour of the exposed fishes were darker with increasing concentration, so also was mortality rate increased with

increased concentration. Table 5 presented the haematological changes observed in the test fish when the leachate was introduced into the experimental culture tanks. There were various behavioural changes like uncoordinated movement, darting up and down, rapid and erratic swimming, occasional jumpy movement, and negative thigmotropsism with prolonged gapping of jaws. The mortality rate was high too as it increased from 25% to 45%. Results further showed that haematocrit percentages, erythrocyte number and haemoglobin concentration

increased with increased concentrations compared with control where no change was observed in all these values. There was considerable increase in leucocyte and lymphocytes number when compared with the controls of the exposed fishes Table 6. The result also showed that there was an increase in total differential blood count (lymphocyte monocyte eosinophils). Also, in 14th and 21st day, it was observed that there was no significant difference between the values obtained for in 12.33%, 6.17% and 3.08%.

Table 5. 96 hr percent mortality of *Clarias geriepinus* Exposed to Akomo Raw Leachate (ARL)

Test (%) Concentration	No of Fishes	1 st Replicate	2 nd Replicate	3 rd Replicate
Control	20	0	0	0
5	20	25	20	25
15	20	25	30	60
25	20	55	65	60
35	20	70	75	75
45	20	90	95	90

Table 6. Haematological changes in fish exposed to Akomo raw leachate

Days	Concentrations (%)	PCV	RBC	Hb	WBC
7 th	Control	29.6±0.33 ^a	20.33±0.03 ^b	3.00±0.01 ^b	14.33±0.03 ^b
	12.33%	26.33±0.33 ^a	15.33±0.33 ^a	3.33±0.06 ^b	14.00±0.58 ^b
	6.17%	25.67±1.20 ^a	12.6±0.08 ^a	2.87±0.08 ^a	14.33±0.03 ^b
	3.08%	26.30±0.66 ^a	14.33±0.33 ^a	3.17±0.14 ^b	13.33±0.00 ^a
14 th	Control	29.50±0.035 ^a	20.5±0.12 ^a	3.11±0.29 ^a	16.33±0.35 ^a
	12.33%	28.37±0.35 ^a	15.45±0.25 ^a	3.45±0.05 ^a	16.00±0.57 ^a
	6.17%	25.67±1.20 ^b	12.56±0.57 ^a	3.00±0.01 ^a	15.00±0.57 ^b
	3.08%	29.50±0.35 ^a	15.35±0.35 ^a	3.26±0.06 ^a	14.33±0.35 ^b
21 st	Control	29.50±0.12 ^b	22.38±0.41 ^b	4.00±0.24 ^b	16.35±0.25 ^a
	12.33%	30.0±0.45 ^b	16.05±0.45 ^a	3.10±0.20 ^a	16.00±0.78 ^a
	6.17%	26.0±2.30 ^a	14.33±0.78 ^a	3.00±0.14 ^a	16.05±0.07 ^a
	3.08%	28.5±0.45 ^a	15.05±0.78 ^a	3.00±0.16 ^a	15.35±0.40 ^b

Values are mean ± standard error.

Means with same uperscript along the same column within sampling day boxes are not significantly different. P < 0.05 = Level of statistical significance.

Table 7. MCV and MCHC and Total Different Blood Counts of the Fish Exposed to Akomo Raw Leachate Total Differential.

Days	Concentrations (%)	MCV	MCHC	Leucocyte	Lymphocyte	Monocyte	Eosinophils
7 th	Control	29.6±0.33 ^a	20.33±0.03 ^b	3.00±0.01 ^b	14.33±0.03 ^b	10.33±0.03 ^a	1.33±0.33 ^b
	12.33%	26.33±0.33 ^a	15.33±0.33 ^a	3.33±0.06 ^b	14.00±0.58 ^b	13.0±0.57 ^b	10.6±0.67 ^a
	6.17%	25.67±1.20 ^a	12.6±0.08 ^a	2.87±0.08 ^a	14.33±0.03 ^b	15.33±0.67 ^{bc}	13.33±0.67 ^a
	3.08%	26.30±0.66 ^a	14.33±0.33 ^a	3.17±0.14 ^b	13.33±0.00 ^a	10.67±0.67 ^a	14.67±0.67 ^a
14 th	Control	29.50±0.035 ^a	20.5±0.12	3.11±0.29 ^a	16.33±0.35 ^a	10.55±0.23 ^a	12.33±0.35 ^a
	12.33%	28.37±0.35 ^a	15.45±0.25 ^a	3.45±0.05 ^a	16.00±0.57 ^a	12.55±0.58 ^a	11.57±0.52 ^a
	6.17%	25.67±1.20 ^b	12.56±0.57 ^a	3.00±0.01 ^a	15.00±0.57 ^b	12.05±0.45 ^a	12.55±0.58 ^a
	3.08%	29.50±0.35 ^a	15.35±0.35 ^a	3.26±0.06 ^a	14.33±0.35 ^b	14.35±0.58 ^b	14.67±0.55 ^a
21 st	Control	29.50±0.12 ^b	22.38±0.41 ^b	4.00±0.24 ^b	16.35±0.25 ^a	15.52±0.45 ^a	12.05±0.45 ^a
	12.33%	30.0±0.45 ^b	16.05±0.45 ^a	3.10±0.20 ^a	16.00±0.78 ^a	15.52±0.43 ^a	12.05±0.45 ^a
	6.17%	26.0±2.30 ^a	14.33±0.78 ^a	3.00±0.14 ^a	16.05±0.07 ^a	14.26±0.78 ^a	14.22±0.45 ^a
	3.08%	28.5±0.45 ^a	15.05±0.78 ^a	3.00±0.16 ^a	15.35±0.40 ^b	16.88±0.58 ^a	14.67±0.78 ^a

Values are mean ± standard error.

Means with same uperscript along the same column within sampling day boxes are not significantly different. P < 0.05 = Level of statistical significance.

4. Discussion

The results of this study indicated that Akomo raw leachate is toxic and contained opportunistic pathogens. Physico-chemical and heavy metal analysis of the leachate samples showed the presence of the constituents at different concentrations. Some of these concentrations (including DO, BOD, NO₃, NH₄, Pb and Cr) were higher than the values that are suitable for aquatic life and limits set by International Regulatory Authorities (Table 1). The high DO and BOD indicates high organic strength in the leachates of the landfill site and the presence of ammonia nitrogen is probably due to the deamination of amino acid during the decomposition of organic compounds as suggested by [9]. The presence of trace amounts of Pb, Cr and other metals indicate the disposal of Pb batteries, fluorescent tubes and other metal – laden wastes which are often non-biodegradable. Also, food wastes as well as burning of tyres, poly vinyl materials dry cell batteries, steel scraps and paint cans could be possible sources of trace and heavy metals in the leachate and eventually water samples. The observations made in this study corroborates the findings of [1,10,11,12].

In this study, an attempt was made to investigate the concentration of some of the chemical constituents of Akomo raw leachate samples, it should be noted however, that these chemicals might not represent all or even the majority of chemical species in the leachate. Leachates usually contain a complex mixture of organic and inorganic chemicals, and many unidentified toxicants known as non-conventional pollutants (NCPs), which may pose risks of unknown magnitude to aquatic biota. Chemicals such as benzene, naphthalene, persistent organic pollutants, dioxins, polychlorinated biphenyls, polycyclic aromatic hydrocarbon and alkylating agents were reported to be present in leachates [13,14,15]. Alkylating agents, for example, were reported to be electrophilic compounds with affinity for nucleophilic centers in organic macromolecules [16]. Hence the cause of toxicity of leachate assayed in this study could have been due to one component or a combination of known and unknown constituents.

The high values of total differential count observed in this study are common when compared with reports on fishes exposed to different toxicants [12]. The increase in leukocyte and lymphocyte counts observed during the study may be attributed to the immune response of *Clarias gariepinus* to toxicants. Nussey [17] worked on *O. mossambicus* exposed to copper. Their results showed an increase in eosinophil values which is similar to the increase in eosinophils found in *Clarias gariepinus* exposed to leachate in this study. The potential for chemicals to cause damage to immune system is of considerable public health significance as alterations in immune function can lead to increased incidence of hypersensitivity, disorders in autoimmune system and infectious diseases. Oshode *et al.* [14] reported increase in hematocrit count as indication of a stress response causing RBC swelling. White blood cell (WBC) plays an important role in the immune system of living organisms, high white blood cell (WBC) count can indicate hyperplasia, inflammation, trauma and stress [15]. The toxic substance introduced to experimental tanks containing test fishes in this study result in deleterious

effect in the body system of fishes for instance, ammonia whose value in the test samples exceeded the acceptable and permissible limits is very toxic to fish. [18] reported that ammonia can last for many years in landfill and it can seriously contaminate groundwater. He also said its acute effect in fish includes loss of equilibrium, increase breathing rates, convulsion, coma and death [18]. In fishes, Pb interferes with biosynthesis of haem, and also produced haematological changes such as cellular alteration and aminoaciduria. In this study the elements like Zn, Cd and Cu found in the test sample have acted interactively to elicit toxic response in *Clarias gariepinus* for example, Zn and Cd salts produce additive toxicity in fish while Cu and Zn salts have synergistic action against fresh water fish [19]. Effort made to carefully observe the behavior of fishes during the 96hr study. Behavioral functions are generally quite vulnerable to contaminate exposure and fish often exhibit these responses first and when exposed to pollutants [20]. Behavioral changes such as erratic swimming, uncoordinated movement, darting up and down of fish was observed during this 96hr LC₅₀ study. This may be due to loss of equilibrium at high toxicant concentration which makes the fish to turn upside down and finally die [21]. Although some of the chemicals in the leachate were analyzed, it should be noted that these chemicals might not represent all or even the majority of chemical species in the leachate. Leachate usually contain complex mixture of organic and inorganic chemical and many identified toxicants known as non-conventional pollutants (NCPs) which may pose risk of unknown magnitude to aquatic biota [21]. Chemicals such as benzene, naphthalene, persistent organic pollutants, dioxins, polychlorinated biphenyls, polycyclic aromatic hydrocarbons and alkylating agent were reported to be present in leachate [21,22]. Therefore, the cause of toxicity of leachate assayed in this study could be due to one or component or a combination of known and unknown constituents. Apart from toxic elements microorganism that are potential cause of wide range of infectious was identified in Akomo raw leachate (ARL) for example, the exotoxin produced by the microorganism like *Clostridium botulinum* is lethal to fish and has been the cause of many human death [20]. Entry into ground or surface water bodies, of these microorganisms therefore becomes a potential threat or problem to the environment and public health. High population of coliform and faecal bacteria were observed in this study just as [18] reported evaluated populations of total coliform, faecal coliform bacteria, faecal streptococci, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Websella pneumoniae* in leachate from various landfills. [19,21] also reported the presence of *Staphylococcus spp* and *Streptococcus spp* in landfill leachate, ground water and surface water sample.

5. Conclusion

The result of this study indicated that raw leachate from Akomo landfill contained toxic constituents that can elicit change in haematological profile in *Clarias gariepinus*. The leachate sample also contained microorganisms that are of public health concern. The gradual changes in fish behavior in response to different toxicant concentration

reflected a transient toxicity in the immune system of the fish. More studies are required on the possible bioaccumulation of the toxicant in humans who fed on the polluted fish species.

Competing Interest

The authors have no competing interest.

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