TOXICITY ASSESSMENT OF OLUSOSUN AND IGANDO LEACHATES USING THE AFRICAN CATFISH (*CLARIAS GARIEPINUS*) AS BIOINDICATOR SPECIES PART I

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ABSTRACT

Olusosun and Igando landfill leac ahates were investigated for their toxicity using *Clarias gariepinus* as the bio-indicator species. Physical and chemical analyses of leachates showed that pH was generally basic (11.46; 10.15 respectively). The electrical conductivity, total dissolved solids, total hardness, chloride, nitrate and sulphate for Olusosun and Solous landfills leachates were found to have exceeded the WHO limits for drinking water. Heavy metals analyses showed that the concentration of nickel (75.5 ± 6.9 to 269.7 ± 1.33 μg/L), lead (74.7± 9.8 to 259.83 ± 8.3 μg/L), zinc (20 ± 7.5 to 198.2 ± 5.5 μg/L), cobalt (< detection limit to 12.1 ± 0.7 μg/L), cadmium (4.3 ± 0.6 to 4.7 ±0.2 μg/L) and copper (29.5 ± 1.3 to 220.3 ± 1.9 μg/L) were above the WHO (2007) recommended limits. Toxicity assessments carried out showed that the treatment level of 7.5 % was the least toxic to the fingerlings. The haematological results showed that Packed cell volume, Red Blood Cell count (1.5 $\times 10^{12}$/L, 1.85 x 10^{12}/L), Haemoglobin (6.5 g/dL, 7 g/dL), White Blood Cell (10.95 $\times 10^{9}$/L, 13.5 x 10^{9}/L), Heterophils (28.5 %, 38 %), Lymphocytes (70.5%, 62%), Eosinophils (0.5 %, 00 %), Basophils (0.5 %, 01 %) and Monocytes (00 % 00%) for Olusosun and Solous landfills leachates respectively were higher than in the control fish not exposed to leachate samples. Histopathological analyses of the fishes that survived the leachate exposures showed damage to the gills and liver of the fishes.

Keywords: Leachate, Landfill, Toxicity, Bio-indicator species.

INTRODUCTION

Increase in household waste generation in Nigeria is widely attributed to the rapid urbanization and population growth (Adeolu *et al.*, 2009). Pollutants from solid wastes most often penetrate towards the lower soil horizons as leachates and subsequently polluting the groundwater at varying degrees (Adedosu *et al.,* 2013). Municipal and industrial solid wastes contain a variety of potentially significant chemical constituents and pathogenic organisms that could negatively affect public health, air, soil and groundwater qualities. These constituents include regulated hazardous priority pollutants such as heavy metals, poly aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and other persistent organic pollutants (POPs) (Ikem *et al.*, 2002; Osibanjo, 2003; Anetor *et al.*, 2008).

One of the major environmental issues in Nigeria is the improper management of solid wastes. Clark (2006) described landfill practices as the disposal of solid wastes by infilling depressions on land. Wastes placed in landfills are subject to either groundwater underflow or infiltration from precipitation. Water percolation through the degraded solid wastes cause variety of inorganic and organic compounds to flow out of the wastes and accumulate at the bottom of the landfill. The resulting contaminated water which is termed leachate percolates through the soil into the underground aquifer over time and space (Adeolu *et al.*, 2009). Leachate composition is dependent on the component materials present in landfill, i.e. dissolved organic matters (alcohols, acids, aldehydes, and short chain sugars), inorganic macro-components (common cations and anions including sulphate, chloride, and ammonium), heavy metals (Pb, Ni, Cu, Hg), xenobiotic organics and polychlorinated biphenyls (Ludwig *et al.*, 2003).

Rapid urbanization, industrialization and population growth which are major stressors of the environment lead to problems such as human health problems, eutrophication and fish kill, biodiversity losses, ozone layer depletion and climatic changes (Sadiq, 2002; Bay *et al*., 2003). Effect of leachate from waste dump sites leaching into nearby water bodies could have adverse effect on the aquatic organisms particularly fish, even though, the effect may be reduced with distance from the source of generation (Agatha and Jerimoth, 2011).

Chemical compounds in leachates pose serious risks to ecosystems and human health when the chemicals migrate to surface waters and drinking water wells (Adeolu *et al*., 2009), or are accumulated in the body of fish consumed by man. Such risks are known to be very high when the landfill site lacks an impermeable liner and leachate collection system allowing the direct flow of leachate into groundwater (Adeolu *et al*., 2009). The fish (*Clarias gariepinus*), as a bio-indicator species, plays an increasing important role in the monitoring of water pollution because it responds with great sensitivity to the changes in the aquatic environment (Adeolu *et al*., 2009). The use of fish as a test organism in eco-toxicological studies is essential because of the link to human via food chain. The aim of this study is to assess and compare the toxicity effects of landfill leachates from Olusosun and Igando in Lagos State using *C. gariepinus* as a bio-indicator species.

MATERIALS AND METHODS

The Study Areas

Olusosun and Solous landfill sites are the top two largest dumpsites located in Lagos State, the industrial and commercial centre of Nigeria. Olusosun landfill site Latitude 6^0 35' 7"N and Longitude 3^0 22' 32.92"E is located in Ojota a few kilometres from the Lagos Lagoon and the Bight of Benin (Ogundiran and Afolabi, 2008). The Solous landfill between Longitude $3^0$13'30"E to $3^0$17'15"E and Latitude 6'28^{0}N to 6'42^{0}N is situated in Igando in Alimosho Local Government Area of Lagos State, Nigeria (Akoteyon *et al*., 2011).

Sample Collection Techniques

Raw landfill leachates (100 litre) were collected from each of the leachate ponds at Olusosun and Igando landfill sites using Winchester bottles.

Sample Analyses

Selected physical and chemical parameters of the leachates were analysed either *in situ* or *ex situ*. The parameters analysed *in situ* were: colour, pH, electrical conductivity, temperature, and total dissolved solids. Electronically combined pH/EC/TDS meter (Combo HI 98130, Hanna, USA) was used for *in situ* analyses of the leachate which were first diluted and neutralise with distilled water and then inserting its probe into the leachate to take readings. The chemical parameters determined in the laboratory using APHA (1985) were: chloride, total hardness, alkalinity, sulphate and nitrates. The concentrations of iron, zinc, copper, cadmium, lead, cobalt, and nickel in the leachate samples were also analysed using atomic absorption spectrophotometer (iCE 3300 AA) after initial digestion with nitric acid according to Olujimi *et al.* (2012).

Fish Collection and Acclimatization

Juveniles of the African catfish (280 pieces; Means Total Length average 14 ± 2.0 cm and mean weight 11.8 ± 0.9 g) used for this investigation were purchased from the Federal University of Agriculture Abeokuta Fish Farm. The test fish were transported in a sealed oxygenated polythene bag which contained freshwater from the farm pond. The specimens were kept in four (4) large glass aquaria (20×15×30 cm) containing well aerated borehole water and acclimatized for two weeks to the laboratory conditions. Water in each aquarium was changed at two days interval to prevent the build-up of metabolic wastes. The fish were fed twice daily with fish meal at 3 % body weight (Olaniyi and Salau, 2013). Feeding was discontinued 48 hours prior to the commencement of the experiment.

Toxicity Assessment

Ten acclimated juveniles were introduced into each treatment tank containing five different concentrations of leachate in duplicates (7.5, 15, 25, 50 and 75%). The control experiment was similarly set up in triplicates with aerated borehole water collected from the University. Acute toxicity test conducted followed the biostatistics protocol recommended by UNEP (1989). The fish in the test and control tank were observed for abnormal behaviours and mortality at 12, 24, 48, 72 and 96 hours. Dead fish were removed from test solutions as soon as observed. A fish was considered dead when it was totally immobile with no respiratory/opercula and tail movements. The results obtained were subjected to one way ANOVA at $p<0.05$ level of significance using SPSS version 16.0 for Windows.

Haematological and Histopathology Analyses

For the histopathology analysis, two fishes from each group were subjected to histological procedure, the abdominal cavities of the sacrificed fishes were opened and organs (liver and gills) were removed. The organs were fixed in 10 % buffered formalin before being taken for analysis. For the haematological analyses, two fishes from each group were also subjected to haematological procedure. Blood sample of the test organism were taken from the tail region using caudal puncture and transferred into an EDTA bottle and immediately covered to prevent clotting of blood. The organs and the blood samples were then taken for analysis at the Veterinary Laboratory in the Federal University of Agriculture, Abeokuta (FUNAAB).

RESULTS AND DISCUSSION

Toxicity Assessment

The physico-chemical parameters and heavy metals concentration determined in the leachate samples are presented in Table 1. Comparatively, the raw leachate samples from Olusosun landfill and Solous landfill had higher values of pH, electrical conductivity, total hardness, total dissolved solids, chloride, nitrate, and some heavy metals such as lead, and nickel. This showed that the leachate fails to comply with standard set by WHO (2007). The increased pH in raw leachate sample from these dumpsites could be attributed to basic industrial solvents that were probably dumped with the solid waste. Some of the industrial solvents probably contain ammonium compounds, which likely imparted the basic pH of the leachate at the dumpsite. Also, the very alkaline pH recorded in the leachates could be attributed to the on-going methane fermentation phase at the dumpsites. The high alkaline pH has been reported to support the growth of methanogens which converts much of the organic contaminants in leachate to methane gas (Mcbean *et al.,* 1995).

TABLE 1: Physical and Chemical Parameters of Leachate during the Period of Study

Parameters	WHO Standard	OLUSOSUN	SOLOUS
Colour	NS	Black	Dark brown
Temperature (°C)	NS	34.0±2.37	26.1±0.00
pH	6.5-9.5	11.46	10.15
Electrical Conductivity (µS/cm)	1000	3999	3999
Total hardness (mg/L)	180	100	
Total Dissolved Solids (mg/L)	500	2000±15	2000
Chloride (mg/L)	250	3430 ±17.00	1090± 13
Nitrate (mg/L)	50	76±3.91	73.56±2.69
Sulphate (mg/L)	250	4.6	26.45±4.81
Zinc (µg/L)	500	198.17±5.45	20±7.49
Copper (µg/L)	50	220.3±1.94	29.5±1.34
Cadmium (µg/L)	10	4.7±0.22	4.3±0.62
Lead (µg/L)	50	259.83±8.31	74.73±9.84
Cobalt (µg/L)	NS	12.12±0.69	<DL
Nickel (µg/L)	70	269.7 ± 1.33	75.5±6.86

Exposure of *C. gariepinus* to varying concentration of leachate samples from the two sites caused visible and significant behavioural changes such as the loss of reflexes, skin decolouration, air gulping, erratic swimming, babel deformation and excessive mucus secretion (Table 2). The severity of the behavioural changes increased with increase in leachate concentration, with rapid responses recorded at the highest treatment levels of 50 and 75 %. At the two concentrations, mortality of *C. gariepinus* fingerlings commenced within 10 minutes of introduction into the test leachate samples. However, fingerlings in 15 and 25 % leachate concentrations began to die after some hours of introduction. Fish behaviour on exposure to different Solous leachate concentrations showed loss of reflex at 25, 50 and 75 % leachate concentration which was unnoticeable in the control, and at 7.5 and 15 % leachate concentrations. Skin decolouration in fish fingerlings was observed at 15, 25, 50 and 75 % leachate concentrations (Table 2). The fish retained their normal colour in the control and 7.5 % leachate concentrations. Air gulping which was observed in the fish exposed to 25, 50 and 75 % leachate concentrations was absent at lower concentrations of the leachate. Erratic swimming was recorded at 25, 50 and 75 % test leachate concentrations; this was however absent at lower concentrations (Table 2). Loss of reflex, skin discolouration, air gulping, erratic swimming reported in the fish during in this study were similar to the findings of Fauziah *et al.* (2013) when *Anabas testudineus* was exposed to sanitary landfill leachate. The barbel of the fish was present throughout 96 hours of exposure to leachate from Solous landfill site. Excessive mucus secretion was not noticed in the fish at all the leachate concentrations except for the control and 7.5 % treatment. Normal barbel and moderate mucus production similar to what was observed in *C. gariepinus* during the study was similarly reported in *C. gariepinus* exposed to edible oil mill waste water (Adakole, 2011). However, contrary to what was reported by Adakole (2011), no sign of behavioural changes were noticed at the lowest leachate treatment (7.5 % leachate) and the control. The non-behavioural responses in fish fingerlings in the control and at 7.5 % treatment level probably implied that the condition in the treatment tanks was still tolerable for fish survival.

All fishes in control survived throughout the period of study. However, there was 100 % mortality in treatment levels of 15 %, 25 %, 50 % and 75 % except in 7.5 % treatment level where all the test samples also survived thus showing the level of toxicity of the leachate in treatments above 7.5 % as shown in Figures 1 and 2 while there was no mortality recorded for 7.5 % and 15 % Solous leachate concentrations. There was 100 % mortality in 25 %, 50% and 75% leachate concentrations respectively. This shows that Olusosun dumpsite is relatively more toxic than Solous dumpsite.

TABLE 2: Behavioural Responses of *C. gariepinus* in Different Leachate Treatment

	OLUSOSUN						SOLOUS				
Concentrations	Control	7.5 %	15 %	25 %	50 %	75 %	7.5%	15 %	25 %	50 %	75 %
Loss of reflex	-	-	**	**	**	**	-	-	**	**	**
Skin decolouration	-	-	**	**	**	**	-	**	**	**	**
Air gulping	-	-	**	**	**	**	-	-	**	**	**
Eratic swimming	-	-	**	**	**	**	-	-	**	**	**
Barbel deformation	-	-	**	**	**	**	-	-	-	-	-
Excessive mucus secretion	-	-	**	**	**	**	-	-	**	**	**

NOTE: ** indicates an increase in response, - indicates normal response.

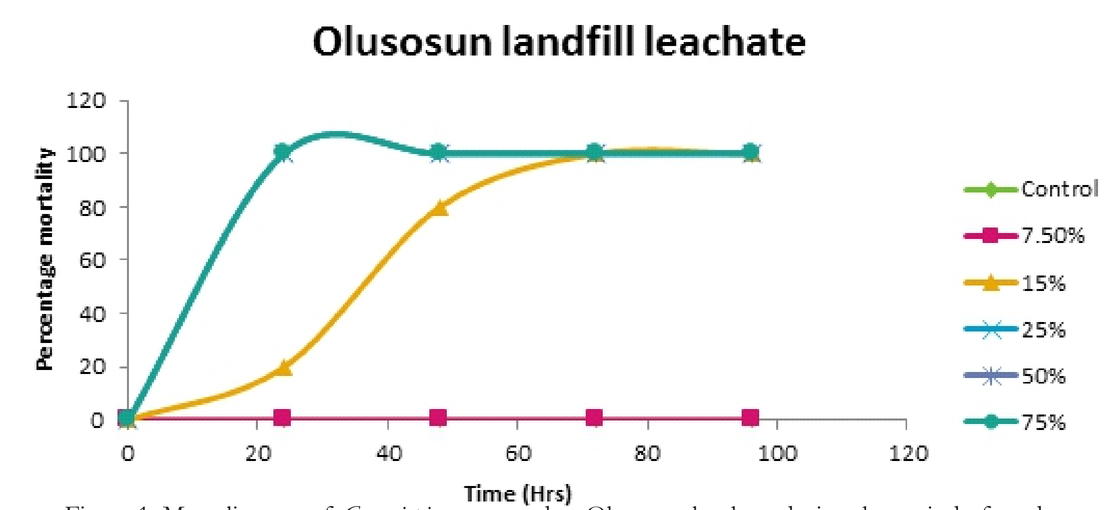

Figure 1: Mortality rate of *C. gariepinus* exposed to Olusosun leachate during the period of study

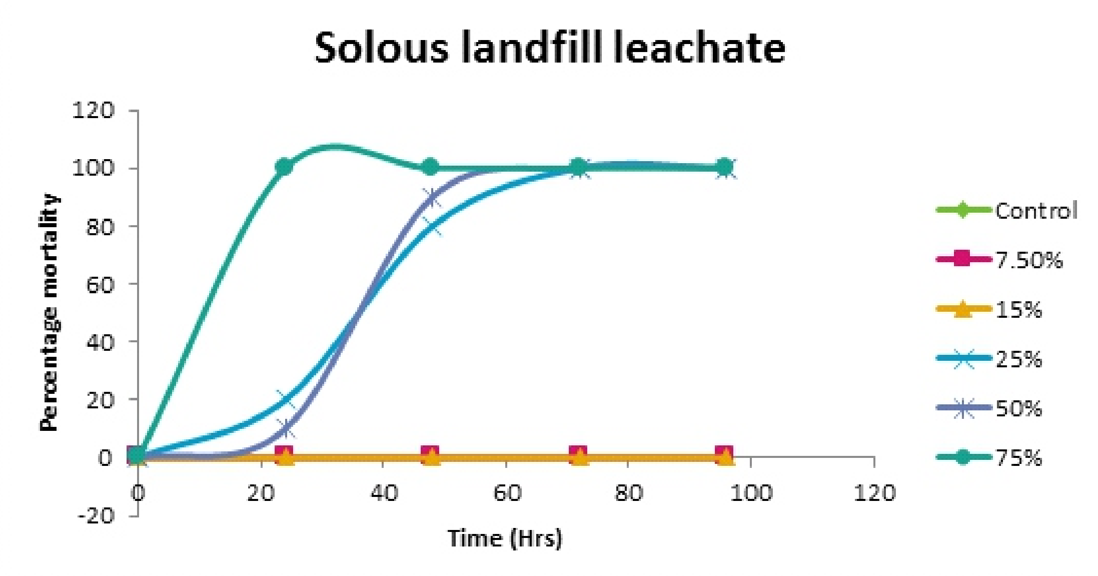

Figure 2: Mortality rate of *C. gariepinus* exposed to Solous leachate during the period of study

Haematological Studies

Haematological parameters were only analysed for the fishes that survived i.e. the control and 7.5 % Solous leachate treatment level. There was an increase in the value of packed cell volume (PCV), hemoglobin (HB), red blood cell (RBC), white blood cell (WBC), heterophils (HET), lymphocytes (LYM), eosinophils (EOS), basophils (BAS) and monocytes (MONO) when compared with the control value (Table 3). Exposure of *C. gariepinus* to 7.5 % treatment of Olusosun leachate caused an increase in their haematological parameters such as PCV, HB, HET, and LYM but a decrease in RBC, BAS and MONO while WBC and EOS show no changes. The increase and decrease observed in the levels of the blood parameters could be attributed to species of fish and the toxicants considered (Ololade and Oginni, 2010). The PCV of *C. gariepinus* exposed to 7.5 % treatment level increased than that of the control by comparing their means (i.e. 16.5 % to 19 %). This may be due to the fact that leachate contains inorganic macro-component that might be useful to the fish. The 7.5 % treatment level was too low to have an adverse effect on the exposed fish; instead it provided nutrients to the water medium which helped in boosting the PCV of the tested sample. The increase in PCV agrees with previous study (Ajani and Awogbade, 2012) where *C. gariepinus* were exposed to different treatments of diurons. Studies have shown that decrease in red blood cells and haemoglobin may indicate anaemia as a result of blood loss, bone marrow failure, and malnutrition such as iron deficiency, over-hydration, or mechanical damage to red blood cells (Marchand *et al.*, 2009). Concerning the decrease in the RBCs and haemoglobin, it is possible that the oxygen uptake by the fishes was affected by the presence of components like benzene, toluene and ethyl benzene which are potential toxicants to fish and might be present in the 7.5 % treatment level or probably an indication of destruction of their red blood cells. Decrease in RBC and haemoglobin recorded in this study is in line with the report obtained on catfish exposed to cassava mill effluent (Olaniyi *et al.*, 2013).

WBC plays an important role in the immune system of a living organism. An unusually high WBC count can indicate hyperplenism, inflammation, trauma and stress. The increased leukocyte count observed during the study may be attributed to immune response of *C. gariepinus* to the toxicants present in the leachate sample and it is similar to the report of Obeamata *et al.* (2012). Increase in eosinophils value obtained is similar to the increase found in *O. mossambicus* during exposure to copper (Nussey *et al.*, 1995). The potential for chemicals to cause damage to the immune system is of considerable public health significance, as alterations in immune function can lead to increased incidence of hypersensitivity disorders, autoimmune and infectious diseases (Oshode *et al.*, 2008). Iron intoxication may have led to an increase in haemoglobin in the blood of the fish. High levels of basophils correspond to an active allergic response. Normal levels vary from 0 to 1% of the existing white blood cells (Nakada *et al.*, 2014). Their elevation does more to establish that a higher WBC is due to a natural, proper physiological response rather than a disease process. The level of RBC may have been boosted by erythropoietin hormone in response to low level of oxygen (hypoxia) in the blood (Haase, 2010). The low level of oxygen may have resulted from the introduction of the fish into the leachate (Aziz, 2015).

TABLE 3: Haematological Parameters of *C. gariepinus* in Control and 7.5 % Treatment

LABEL	**Control (Mean ±SD)**	**Olusosun 7.5 % (Mean ±SD)**	**Solous 7.5 % (Mean ±SD)**
PCV (%)	16.5±1.4	19±1.4	20.5±4.95
HB(g/dl)	5.8±0.3	6.5±0.3	7±1.7
RBC(X 10^{12} /L)	1.6±0.1	1.5±0.1	1.85±1.35
WBC (X 10^{9}/L)	10.95±0.1	10.95±0.1	13.5±0.85
HET (%)	28±0.7	28.5±0.7	38 ±1.41
LYM (%)	68.5±2.1	70.5±2.1	62± 0
EOS (%)	0.5±0.7	0.5±0.7	0 ±0
BAS (%)	1±0.7	0.5±0.7	1± 0
MONO (%)	0.7±0.0	0± 0.0	0± 0

Histopathological Assessment

The histopathological result of the gill and liver (Figures 3 and 4) show no visible lesions in the control while there is a severe and diffuse necrosis and degeneration of the epithelial cells of the primary filament in the gill and liver of the other treatment levels which correlates with the report of Abdel-Moneim (2008) on *C. gariepinus* exposed to dye-stuff and chemical waste water. The gill sample from the 15 % Olusosun treatment level shows the degeneration of the epithelial cells of the primary filament while the 25 %, 50 % and 75 % treatment level showed no single sign of the epithelial cells. The 15 %, 25 %, 50 % and 75 %

treatment levels of the liver showed multiple fossil of periportal congestion and mono-nuclear cells. There was also a severe periportal necrosis of the hepatocells with the presence of bilirubin pigment thereby causing the breakdown of the red blood cells probably leading to the mortality of *C. gariepinus* fingerlings. The changes noticed in the gills and livers tissue of *C. gariepinus* exposed to different concentrations of leachate corroborated the findings of several studies in the literature (Oshode *et al*., 2008; Adeogun *et al*., 2012). The severity in the changes of the gill and liver increased with increase in the treatment levels. The hepatocells of the liver in the 75 % treatment level showed large area of vacuolation and presence of fat covered with bilirubin pigment. The primary filament showed no visible lesions in the control fish. In 25 % Solous leachate treatment there was severe and diffuse necrosis and degeneration of the epithelial cells of the primary filament in the gill of the fish. The liver of the fish showed severe and diffuse vacuolation. In 50 % leachate treatments from the two landfill sites, there were periportal mononuclear cells and fossile necrosis and also bilirubin pigment was noticed in the liver. The presence of bilirubin indicates the breakdown of red blood cells. No single epithelial cell was present in the primary and secondary filament of the gills. In 75 % leachate treatment, the liver of the fish showed severe and diffuse vacuolation and there were periportal mononuclear cells and fossile necrosis. The observed defects in the gills and tissues of *C. gariepinus* exposed to the different treatments were similar to what was discovered in fishes exposed to different toxic wastes (Parvathi *et al*., 2011)

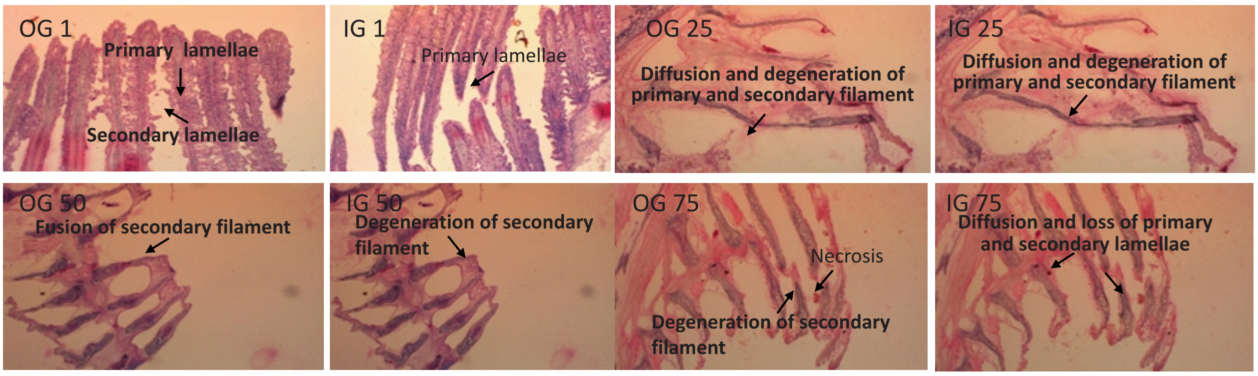

Figure 3. Histopathology *C. gariepinus* in control and leachate: Control Gill (OG1: Olusosun; IG1: Solous control); OG25 (Olusosun 25 %), IG 25 (Solous 25 %); OG50 (Olusosun 50 %), IG 50 (Solous 50 %); OG75 (Olusosun 75 %), IG 75 (Solous 75 %)

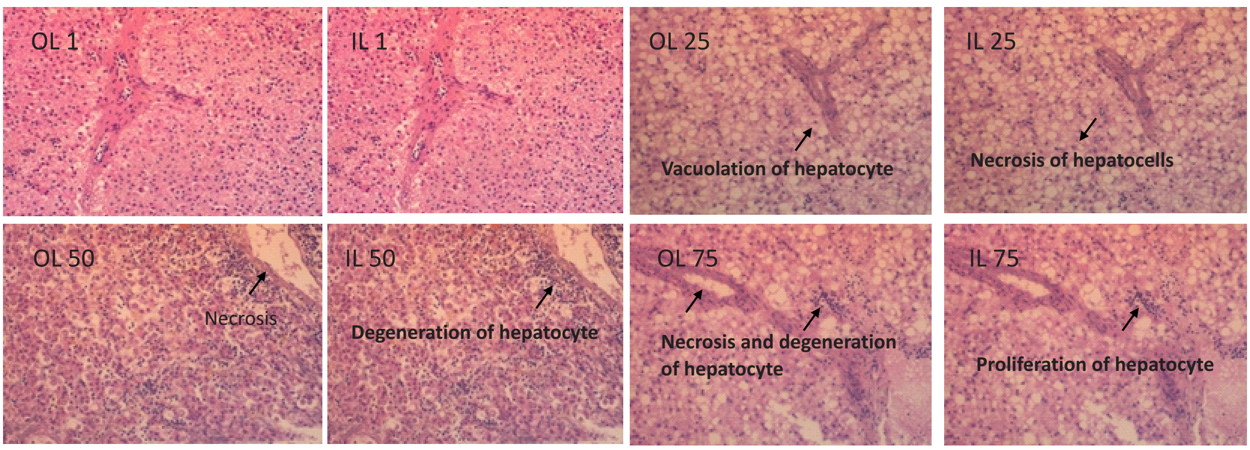

Figure 4. Histopathology *C. gariepinus* in control and leachate: Control Liver (OL1: Olusosun; IG1: Solous control); OL25 (Olusosun 25 %), IL 25 (Solous 25 %); OL50 (Olusosun 50 %), IL 50 (Solous 50 %); OL75 (Olusosun 75 %), IL 75 (Solous 75 %)

CONCLUSION

The analysis of leachate samples from Olusosun and Solous showed that the pH, electrical conductivity, total hardness, total dissolved solids, chloride and nitrates were higher than WHO (2007) recommended standards for drinking water. Also the concentration of copper, lead, and nickel in the two leachate samples were higher than WHO standard. The study also confirmed that exposure of leachate to *C. gariepinus* fingerlings to the leachate sample from the two landfill sites resulted in significant haematological and histopathological changes in the gills and liver of the fish. The results indicated that leachates from the landfill sites at concentrations above 7.5 % were toxic; therefore, further studies are required to evaluate the chronic toxicity and potential environmental risk of leachates.

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