

## Ecotoxicological Assessment Using *Clarias Gariepinus* and Microbial Characterization of Leachate from Municipal Solid Waste Landfill

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**ABSTRACT:** Among the environmental problems in Nigeria is the lack of proper management of municipal solid wastes (MSW), which is capable of releasing hazardous chemicals via leachate to the surrounding environment. In this study, the potential toxic effects of raw leachate obtained from Aba-Eku landfill (AERL) on *Clarias gariepinus*; and the microorganisms that may be present in the leachate were investigated. Physico-chemical analysis showed that the leachate contained toxic constituents. The 96 h LC<sub>50</sub> obtained was 36.6%. Haematocrit %, erythrocyte number, haemoglobin concentration, leukocyte and lymphocyte number increased with increasing leachate concentration. Histopathological lesions were marked in the gills, kidney and liver of exposed fishes and were concentration dependent. A total of 112 bacterial isolates belonging to 17 genera were recorded from samples of AERL. Potential pathogens and toxin producing microorganism were identified. These observations are of prime health concern because there is no known containment or treatment system for the leachate generated from the study site. Our findings would be of assistance in the assessment of hazardous effects of chemicals from waste landfills discharged into the aquatic environment and in making policy on environmental waste management.

**Key words:** Fish, Haematology, Histopathology, Leachate, Microorganism, Physico-chemical parameters

### INTRODUCTION

There are several major environmental issues in Nigeria; among which is lack of proper management of solid wastes. The annual generation of municipal solid wastes (MSW) in Nigeria is  $29.78 \times 10^9$  kg (Ojolo *et al.*, 2004) and this may increase due to rapid urbanization and population growth rate. Wastes are commonly disposed of in uncontrolled and unlined landfills and or improperly sited open dumps located in public places and in wetland or other areas with seasonally high water tables. Uncontrolled burning of dumps as well as burning of refuse from homes and offices is also common. The disposal sites are capable of releasing large amounts of harmful chemicals to nearby water sources and air via leachate and landfill gas respectively (Christensen

*et al.*, 2001; Ikem *et al.*, 2002; Alimba *et al.*, 2006). Researchers worldwide have extensively characterized the chemical and physical composition of solid waste leachates (Loizidou and Kapetanios, 1993; Yasuhara *et al.*, 1997; 1999; Ward 2002; Ikem *et al.*, 2002). Leachate contain a range of chemical compounds, which may leach to the groundwater and pose serious risks to ecosystems and human health if the chemicals migrate to surface waters or to drinking water sources. Apart from the toxic elements, leachate may contain microbes of which some are opportunistic pathogens. These microbes could produce toxins that may cause public health problem (Donnelly *et al.*, 1988). Also, the effect on farm animals drinking leachate-polluted water may portend some health risks. Although the

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effects of leachate are weakened with distance from the source of generation, it can still cause pollution of surface and groundwater, organic carbon affecting odor and taste of groundwater, nitrogen compounds producing eutrophication in surface waters and high nitrates in drinking water, and toxic heavy metals in ground and surface waters (Robinson, 1983).

The realization of the polluting and potential public health effects of solid waste leachate has prompted a number of studies on the toxicity of leachates on microalgae (Cheung *et al.*, 1993, Bernard *et al.*, 1996), bacteria (Day *et al.*, 1993; Pivato and Gaspari, 2005; Koshy *et al.*, 2007), plants (Cureton *et al.*, 1991; McMurphy *et al.*, 1996; Bakare and Wale-Adeyemo, 2004), *Drosophila melanogaster* (Siddique *et al.*, 2005; 2008), *Daphnia* and fishes (Wong, 1989, Ernst *et al.*, 1994; Bernard *et al.*, 1996; Wik and Dave, 2006), mice (Bakare *et al.*, 2003a; Sang and Li, 2005), rats (Alimba *et al.*, 2006) and human cells (Bakare *et al.*, 2007). Amongst these, reports on the potential toxic effects of leachate on fishes are limited, and none so far on the use of tropical fish as test organism. Also, there is paucity of information on microorganism that may be present in solid waste landfill/dumpsites leachate in Nigeria. Little information is currently available about the toxicity of MSW landfill leachates in Nigeria, and such a database would be useful in evaluating treatment options and reuse possibilities. 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 the assessment of water-borne and sediment-deposited toxins where they may provide advanced warning of the potential danger of new chemicals and the possibility of environmental pollution (Powers, 1989). In addition, fish health is essential to the success of aquaculture industry, an industry of growing importance in protein production for humans (Hart, 1996). In Nigeria, fish is regarded as one of the commonest source of proteins, thus contamination of water bodies where fish resides deserves greater attention. In this study, *Clarias gariepinus* was exposed to raw leachate obtained from Aba-Eku municipal solid waste landfill, Ibadan, Nigeria; and the LC<sub>50</sub> and effects on blood parameters, gills, kidney and liver were investigated. We also examined and characterized the microbial

population of leachate from the landfill.

## MATERIALS & METHODS

The study site is Aba-Eku landfill, located in Ona-Ara Local Government Area of Ibadan (longitude 3° 5 East and latitude 7° 23 North), Oyo State, Nigeria. The landfill constructed in 1996 and opened to the public for dumping of refuse in 1999 has since been used for municipal solid waste disposal from public and private waste management operators (Aluko, 2001). This landfill is not equipped with a leachate collection and treatment system; thus, leachate produced is freely discharged into the surrounding aquatic and terrestrial environmental media. Raw leachate samples collected in June 2005 (rainy season) from leachate wells (not less than 20 grabs to form a composite sample for the study site) was transferred to the laboratory in pre-washed plastic containers (10 L capacity), filtered to remove debris and stored at 4 °C until use 48 hr later. This was considered as the stock solution and designated Aba-Eku raw leachate (AERL). Serial dilutions were prepared from the stock in accordance with standard procedures for short-term static bioassay (Reish and Oshida, 1986). The physical and chemical properties of the leachate were determined in accordance with standard methods (USEPA, 1996; APHA, 1998). Standard physical and chemical parameters, including; chemical oxygen demand (COD), biochemical oxygen demand (BOD), dissolved oxygen (DO), conductivity, chloride, sulphate, ammonia and nitrate, were determined. The concentrations of six heavy metals viz. iron (Fe), lead (Pb), cadmium (Cd), manganese (Mn), zinc (Zn) and chromium (Cr) were estimated in the leachate sample using atomic absorption spectrophotometer (Buck AAS model, United Kingdom).

Fingerlings of *Clarias gariepinus* obtained commercially (Oyo state fisheries, Ibadan Nigeria) were utilized for this study. The fish is available, inexpensive and important in aquaculture in Nigeria. The care and use of these fishes was in accordance with international guidelines on the use of fishes for research (American Fisheries Society, 2004). They were acclimatized and maintained in plastic tank (40 L capacity) at 26 ± 2 °C for 14 days, during which they were fed with commercial fish pellet until average weight of 3.05 ± 0.58 g was reached. The natural photoperiod was maintained

during the acclimation and experimental periods and the water was kept oxygen saturated with aeration. Mortality during the period of acclimatization was less than 1% and each experiment was carried out in duplicate. Five concentrations viz: 5, 15, 25, 35 and 45 % of the test leachate and a control group (dechlorinated tap water) were utilized for the acute toxicity test. These concentrations were selected based on a preliminary range finding test (data not shown). Twenty fishes per tank were used to conduct this assay with 2 replicates for each concentration and the control. This set-up was observed for 96 hr, and the test fishes were observed hourly for behavioral/physiological changes and mortality. A fish was considered dead when there was lack of opercular movement when prodded with a glass probe (Adeogun, 2004). The haematological and histopathological examination was based on the  $LC_{50}$  obtained in the acute toxicity study. Three concentrations (3.08%, 6.17% and 12.33%) and a control group in duplicates was set-up. Ten fishes (average weight  $7.53 \pm 4.38$  g) were stocked in each group and were fed at 5% body weight once daily. Each concentration and the control was renewed every 48 hr for 3 weeks to maintain a continuous exposure. Blood samples were collected on the 7<sup>th</sup>, 14<sup>th</sup> and 21<sup>st</sup> day from the start of experiments from the caudal vein of 5 fishes (randomly selected) from each group with 1 mL sterile syringe and needle and mixed in a 5 mL heparinized disposable bottles. Total erythrocyte and leucocyte count, packed cell volume and hemoglobin estimation were carried out in accordance to Blaxhall and Daisley (1973) while derived hematological values (MCV and MCHC) were calculated according to Jain (1986).

Two fishes were sacrificed immediately after the final (21<sup>st</sup> day) collection of blood samples and kidney, liver and gills were surgically removed. These organs were fixed in Bouins fluid for 6 hr, transferred into 10% buffered formalin, and dehydrated in ascending grades of alcohol. They were cleared in xylene, embedded in paraffin wax and sections were cut with a microtome. The sections were stained with haematoxylin and eosin (H and E) and examined for pathological changes at 1000x. Leachate samples (180) for microbiological analysis were collected in sterile universal plastic container from four randomly selected points at the study site during the months of November 2004 to March 2005 (dry season) and June to October 2005

(rainy season). They were analyzed for microorganisms using standard microbiological procedures (Barrow and Fetham, 1993; APHA, 1995). The SPSS 11.0 ® was used for the statistical analysis. Results are expressed as mean  $\pm$  SE. A two-way analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) were used to analyze the data with the level of statistical significance estimated at  $P < 0.05$ .

## RESULTS & DISCUSSION

Table 1 presents the physical and chemical characteristics of AERL. The pH of the sample used for toxicity test is slightly alkaline. The pH of samples used for microbial analysis varied from 6.5 to 8.1 while the temperature (values not shown in the Table) was between 28.6 and 33.5 °C. The samples had foul smell and the concentrations of BOD, ammonia, nitrate, chloride, Pb, Cd, Fe, Mn and Cr were high. When the test leachate was administered into experimental tanks, various behavioral responses like rapid and erratic swimming, uncoordinated movement, darting up and down with occasional jumpy movement, swimming alternately on the lateral and ventral sides and rapid opercula movement were observed. Prior to mortality recorded at some concentrations, there was negative thigmotropism with prolonged gaping

**Table 1. Physical and chemical characteristics of raw leachate from Aba-Eku MSW landfill Nigeria**

parameter <sup>a</sup>	AERL	USEPA <sup>b</sup>	FEPA <sup>c</sup>
pH	8.2	6.5 – 8.5	6 – 9
BOD*	110.7	-	50
COD**	338.19	410	-
Conductivity	915	-	-
Ammonia	83.88	0.02	0.01
Nitrate	38.60	10	-
Sulphate	98.40	250	20
Chloride	4280.0	250	-
Pb	73.30	0.015	0.01
Cd	5.693	0.005	0.05
Fe	50.47	0.30	0.05
Mn	23.81	0.05	0.05
Zn	3.51	5.0	5.0
Cr	2.48	0.10	0.05

<sup>a</sup>All values are in mg/l, except pH with no unit, and conductivity, which is in  $\mu$ s/cm.

<sup>b</sup>([www.epa.gov/safewater/mcl.html](http://www.epa.gov/safewater/mcl.html))

<sup>c</sup>Federal Environmental Protection Agency (2001) Permissible limits for drinking water,

\*BOD Biochemical Oxygen Demand

\*\*COD Chemical Oxygen Demand

of jaws. These observations were more pronounced with increasing concentrations. The colour of exposed fishes became progressively darker with increasing concentration and mortality was directly proportional to the concentration of the test leachate.

In the LC<sub>50</sub> study, no death was recorded after 96 hr in the control, 5% and 15% concentrations of AERL. There was 20%, 40% and 100% mortality at the 25%, 35% and 45% concentrations of AERL, respectively. The 96 hr LC<sub>50</sub> obtained using the probit method is 36.6%. The hematological changes observed are presented in Tables 2 and 3. In general,

haematocrit percentages, erythrocyte number and hemoglobin concentration increased at tested concentrations compared with the control values (Table 2). There were considerable increases in leucocytes and lymphocyte number when compared with the control values (Table 3). When compared with the controls, gills of exposed fishes had stunted and sloughed secondary lamellae at tested concentrations. Mild erosion of the secondary lamellae was observed at 6.17% and 12.33% concentrations of AERL, respectively (Fig. 1). Histopathological investigations of the liver of

**Table 2. Red blood cell indices and thrombocytes of *Clarias gariepinus* exposed to Aba-Eku raw leachate**

Days	Conc. (%)	PCV (%)	RBC count	Hb conc. (mg/dl)	MCV (fc)	MCHC	Thrombocytes (x10 <sup>5</sup> l)
7	Control	21.0±1.0 <sup>a</sup>	1.34±0.02 <sup>a</sup>	6.65±0.15 <sup>c</sup>	157.0±10.0 <sup>a</sup>	32.0±1.0 <sup>a</sup>	20.75±0.62 <sup>c</sup>
	3.08%	18.5±0.5 <sup>a</sup>	1.23±0.01 <sup>a</sup>	6.0±0.0 <sup>a</sup>	150.5±2.5 <sup>a</sup>	32.5±0.5 <sup>a</sup>	24.40±3.4 <sup>a</sup>
	6.17%	21.5±0.5 <sup>a</sup>	1.38±0.38 <sup>a</sup>	7.0±0.0 <sup>a</sup>	156.0±0.0 <sup>a</sup>	32.0±1.0 <sup>a</sup>	21.35±3.95 <sup>a</sup>
	12.33%	22.0±4.0 <sup>a</sup>	1.40±0.14 <sup>a</sup>	7.35±1.15 <sup>a</sup>	155.5±13.5 <sup>a</sup>	33.5±0.5 <sup>a</sup>	22.25±0.55 <sup>a</sup>
14	Control	26.5±3.5 <sup>a</sup>	1.87±0.44 <sup>a</sup>	8.75±0.95 <sup>a</sup>	145.0±16.0 <sup>a</sup>	33.0±1.0 <sup>a</sup>	12.30±0.1 <sup>a</sup>
	3.08%	23.5±1.5 <sup>a</sup>	1.60±0.24 <sup>a</sup>	7.8±0.4 <sup>a</sup>	151.5±31.5 <sup>a</sup>	33.0±0.0 <sup>a</sup>	12.10±0.3 <sup>a</sup>
	6.17%	21.0±1.0 <sup>a</sup>	1.48±0.2 <sup>a</sup>	7.15±0.35 <sup>a</sup>	145.0±26.0 <sup>a</sup>	34.0±0.0 <sup>a</sup>	12.10±0.3 <sup>a</sup>
	12.33%	23.5±3.5 <sup>a</sup>	1.48±0.27 <sup>a</sup>	7.5±1.0 <sup>a</sup>	160.0±5.0 <sup>a</sup>	32.0±1.0 <sup>b</sup>	13.45±0.15 <sup>a</sup>
21	Control	27.0±5.0 <sup>a</sup>	1.84±0.57 <sup>a</sup>	8.4±1.6 <sup>a</sup>	152.5±20.5 <sup>a</sup>	3.50±0.5 <sup>a</sup>	13.75±0.35 <sup>ab</sup>
	3.08%	21.5±1.5 <sup>a</sup>	1.35±0.03 <sup>a</sup>	6.75±0.05 <sup>a</sup>	158.5±7.5 <sup>a</sup>	31.5±2.5 <sup>a</sup>	14.10±0.3 <sup>b</sup>
	6.17%	27.0±2.0 <sup>a</sup>	1.41±0.08 <sup>a</sup>	8.0±0.5 <sup>a</sup>	191.0±4.0 <sup>a</sup>	29.5±0.5 <sup>a</sup>	12.5±0.3 <sup>a</sup>
	12.33%	26.0±4.0 <sup>a</sup>	1.83±0.54 <sup>a</sup>	8.3±1.3 <sup>a</sup>	148.0±22.0 <sup>a</sup>	31.5±0.5 <sup>a</sup>	12.85±0.35 <sup>ab</sup>

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 3. Total and differential white blood cell counts of *Clarias gariepinus* exposed to Aba-Eku raw leachate**

Days	Conc. (%)	Total leucocytes	Lymphocyte (x10 <sup>3</sup> /Nc)	Eosinophilis (x0 <sup>3</sup> /Nc)	Monocytes (x10 <sup>3</sup> /Nc)	Heterophilis (x10 <sup>3</sup> /Nc)
7	Control	15.9±0.2 <sup>a</sup>	10.23±0.11 <sup>a</sup>	0.238±0.077 <sup>a</sup>	0.000±0.00 <sup>a</sup>	5.41±0.386 <sup>a</sup>
	3.08%	17.18±0.38 <sup>a</sup>	11.75±0.52 <sup>a</sup>	0.084±0.084 <sup>a</sup>	0.088±0.088 <sup>a</sup>	5.28±0.887 <sup>c</sup>
	6.17	15.93±3.73 <sup>c</sup>	10.95±2.41 <sup>a</sup>	0.160±0.038 <sup>a</sup>	0.09±0.098 <sup>a</sup>	4.72±1.179 <sup>a</sup>
	12.33%	18.38±1.13 <sup>a</sup>	13.0±1.80 <sup>a</sup>	0.195±0.195 <sup>a</sup>	0.184±0.011 <sup>a</sup>	4.99±0.885 <sup>c</sup>
14	Control	15.73±1.03 <sup>a</sup>	9.90±0.80 <sup>b</sup>	0.231±0.063 <sup>a</sup>	0.157±0.011 <sup>a</sup>	5.43±0.275 <sup>a</sup>
	3.08%	17.08±0.025 <sup>a</sup>	9.95±1.32 <sup>b</sup>	0.083±0.083 <sup>a</sup>	0.083±0.083 <sup>c</sup>	6.07±1.337 <sup>a</sup>
	6.17%	16.40±0.15 <sup>a</sup>	10.15±0.2 <sup>b</sup>	0.171±0.171 <sup>a</sup>	0.086±0.086 <sup>a</sup>	6.66±0.181 <sup>a</sup>
	12.33	15.7±0.4 <sup>a</sup>	6.25±0.63 <sup>a</sup>	0.85±0.371 <sup>a</sup>	0.00±0.00 <sup>a</sup>	8.59±1396 <sup>a</sup>
21	Control	17.65±0.75 <sup>a</sup>	12.0±0.33 <sup>c</sup>	0.281±0.077 <sup>a</sup>	0.184±0.184 <sup>a</sup>	5.21±0.310 <sup>a</sup>
	3.08%	17.18±0.925 <sup>c</sup>	11.55±0.37 <sup>a</sup>	0.272±0.272 <sup>a</sup>	0.253±0.072 <sup>a</sup>	5.07±0.359 <sup>a</sup>
	6.17%	17.83±0.775 <sup>a</sup>	11.6±0.33 <sup>a</sup>	0.284±0.078 <sup>a</sup>	0.171±0.171 <sup>a</sup>	5.82±0.698
	12.33	17.80±0.1 <sup>a</sup>	12.0±0.02 <sup>a</sup>	0.178±0.001 <sup>a</sup>	0.208±0.091 <sup>a</sup>	5.34±0.03 <sup>a</sup>

Values are mean ± standard error.

Means with same superscript along the same column within the sampling day boxes are not significantly different.

P < 0.05 = Level of statistical significance.

the control fish showed hepatocytes arranged in cords around central veins in all the lobules. Glycogenic infiltration of the hepatocytes and congestion of the central veins and portal vessels were observed in exposed fishes and was concentration-dependent. At 21 days exposure period, large focus of hepatic necrosis with mild cellular infiltration was observed in fishes exposed to the highest concentration of test leachate (Fig. 1). Kidney of the control fishes showed well-distended tubules with numerous glomeruli scattered within the renal stoma, while degeneration of renal tubules and tubular calcification was observed in exposed fishes (Fig. 1).

A total of 112 bacterial isolate belonging to 17 genera were recorded from samples of AERL (Table 4). The samples were positive for potential pathogenic species and toxin-producing organisms including *Bacillus cereus*, *E. coli*, *Staphylococcus aureus*, *Clostridium sordelli*, *Clostridium perfringens* and *Salmonella arizonae*. The microbial population ranged between  $0.3 \times 10^6$  and  $9.2 \times 10^9$  cfu/ml. The fungi isolate included *Aspergillus niger*, *A. flavus*, *A. terreus*, *fusarium oxysporum*, *Penicillium spp.* and *Rhizopus sp.*

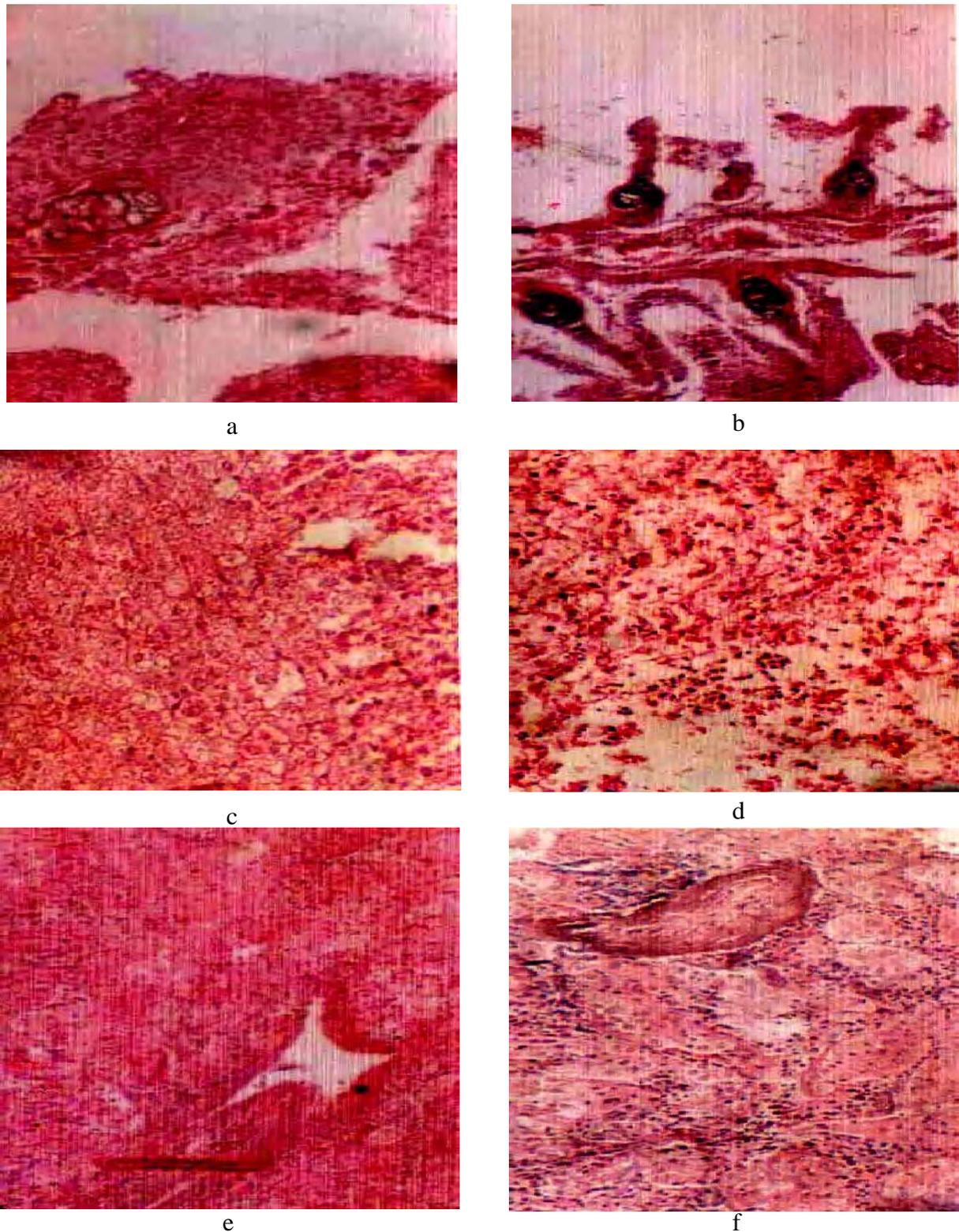
In the present study, the 96 hr LC<sub>50</sub>, haematological and histopathological effects in *C. gariepinus* and microbial characterization of raw leachate obtained from Aba-Eku landfill in Ibadan, Nigeria was investigated. The results indicate that AERL is toxic; producing dose-responsive increases in mortality and abnormalities in the structure of liver, kidney and gills of *C. gariepinus*. The data also shows that the sample contained opportunistic pathogens. The leachate constituents were believed to induce the observed effects. Physico-chemical and heavy metal analysis of the sample showed the presence of the constituents at different concentrations; some of these concentrations were higher than the limits set by international regulatory authorities (Table 1). They were also higher than the values that could support aquatic life. The pH value of 8.2 is in accordance with previous pH value obtained from the study site (Bakare and Wale-Adeyemo, 2004) and from leachate samples from other solid waste dumps sites in Southwest Nigeria (Ikem *et al.*, 2002; Bakare *et al.*, 2003a). In acute toxicity studies, the biological effects can be determined without difficulty. Death is an alternative answer to chemical exposure as it proves the total

**Table 4. Microorganisms analyzed in leachate samples obtained from Aba-Eku landfill, Nigeria**

Isolate	Total isolates (%)
<i>Bacillus spp.</i>	17.9
<i>Citrobacter sp.</i>	1.8
<i>Escherichia coli</i>	18.5
<i>Corynebacterium sp.</i>	1.8
<i>Micrococcus sp.</i>	2.6
<i>Staphylococcus aureus</i>	8.9
<i>Serratia marscescens</i>	5.4
<i>Xanthomonas sp.</i>	0.8
<i>Alcaligenes sp.</i>	1.8
<i>Acinetobacter sp.</i>	1.8
<i>Clostridium spp.</i>	8.5
<i>Pseudomonas aeruginosa</i>	8.5
<i>Pseudomonas fluorescens</i>	1.8
<i>Chromobacterium sp.</i>	1.8
<i>Flavobacterium sp.</i>	1.8
<i>Salmonella arizonae</i>	5.4
<i>Salmonella typhimurium</i>	3.6
<i>Klebsiella spp.</i>	6.2
<i>Enterobacter sp.</i>	1.8

destruction of at least one vital organ system. The LC<sub>50</sub> value of 36.6% shows that the tested sample is toxic, and more toxic than some other leachates obtained from southwest Nigeria assessed for acute toxicity in mice (Bakare *et al.*, 2003a). The value is however, more than (less toxic) those reported for leachate induced toxicity (LC<sub>50</sub>) using *D. magna* (USEPA, 1980; Taylor *et al.*, 1996), rainbow trout (Cameron and Koch, 1980; Day *et al.*, 1993) and *S. mossambicus* (Wong, 1989) as test organisms. The current finding corroborate previous toxicity assessment of AERL wherein the leachate dose-dependently inhibited root growth (EC<sub>50</sub> of 6.10%) and induced various types of chromosomal aberration in *Allium cepa* (Bakare and Wale-Adeyemo, 2004).

Studies have shown that when the water quality is affected by toxicants, physiological changes will be observed in the values of one or more of the haematological parameters (Van Vuren, 1986). Effects of chronic exposure of AERL on hematological parameters in *C. gariepinus* included increased level of erythrocyte number, haemoglobin concentration and haematocrit values. These observations might indicate a compensatory erythropoiesis, which resulted in production of RBC to recompense the older ones that are rapidly destroyed due to decrease in blood's carrying capacity. The high values are in concert with earlier reports on fishes exposed to different toxicants (Alkahem, 1993, 1994; Mazon *et al.*,



**Fig. 1. (x 1000)**a. Section of gill of unexposed *C. gariepinus*. b. Section of gill of *C. gariepinus* exposed to 3.08% concentration of test leachate showing stunted (single arrow) and sloughed(double arrow) secondary lamellae. c. Section of liver of unexposed *C. gariepinus*.d. Section of liver of *C. gariepinus* exposed to 12.33% concentration of test leachate showing necrosis of hepatocytes (single arrow) and cellular infiltration of lymphocytes (double arrow).e. Section of kidney of unexposed *C. gariepinus*.f. Section of kidney of *C. gariepinus* exposed to 12.33% concentration of test leachate showing tubular degeneration (arrowed)

2002). Soivoi and Nikinmaa (1981) reported that increase in haematocrit is an indication of a stress response causing RBC swelling, or haemoconcentration due to plasmatic volume reduction (Wilson and Taylor, 1993). White blood cell (WBC) plays an important role in the immune system of living organisms. An unusually high WBC count can indicate hypersplenism, inflammation, trauma and stress (Nordenson, 2004). The increase in leukocyte count observed during the study may be attributed to immune response of *C. gariepinus* to toxicants present in the leachate sample. Increase in eosinophils value obtained herein correlate with increases found in *O. mossambicus* during exposure to copper (Nussey *et al.*, 1995a, b). 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.

Significant pathological changes like epithelial degeneration of secondary lamellae observed in gill of *C. gariepinus* fingerlings could be attributed to the high content of Cd in the leachate and these changes may lead to an overall reduction in the efficiency of the gill filament to aid diffusion of oxygen across the gill filament. This is in accord with the work of Randi *et al.*, (1996) who reported similar histopathological lesions in gill tissue of freshwater fish *Macropsobrycon uruguayance* following 30 and 60 days of exposure to 1.5 mg<sup>l</sup><sup>-1</sup> of Cd. Necrosis of hepatocytes and periportal oedema observed in the liver of exposed fishes are similar to those observed by Palackova *et al.*, (1994) and Marty *et al.*, (1999). Lesions such as degeneration and necrosis of tubular epithelial cells observed in the kidney could be as a result of high content of heavy metals especially Cd present in the leachate. There were similar observations in the kidney of Tilapia; *O. aureus*, and it was attributed to the presence of Cd in the test chemical (Woo *et al.*, 1993).

The mechanism of the observed effects in *C. gariepinus* is not clearly understood. What is certain is that toxic substances had been introduced into experimental tanks containing the test animals; these substances exert their deleterious effects in living system via different mechanisms and concentrations. Ammonia, whose value in the test sample exceeded the acceptable

and permissible limits, is very toxic to fish. It can last for centuries in landfills and can seriously contaminate groundwater (Cossu *et al.*, 2003; Pivato and Gaspari, 2005). Its' acute effect in fish includes loss of equilibrium, increased breathing rates, convulsions, coma and death. Sublethal concentration of ammonia induces pathological changes in gills, liver and kidney (Odiete, 1999). In fishes, Pb interferes with biosynthesis of haem, and also produces haematological changes such as cellular alterations in erythrocytes resulting to death. Acute exposure to Pb can also result in proximal tubular damage with characteristics histologic features and manifested by glycosuria and aminoaciduria (Loghman-Adham, 1997). These chemicals may also have acted interactively to elicit toxic responses in *C. gariepinus*. For example, Zn and Cd salts produce additive toxicity in fish while Cu (though not analyzed in this study, but is known to be one of the heavy metals usually present in leachates) and Zn salts have synergistic action against freshwater fish (Odiete, 1999).

Although some of the possible chemicals in the leachate were analyzed, it should be recognized that these chemicals might not represent all or even the majority of chemical species in the leachate. Leachate 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 (Lee and Jones-Lee, 1994; Tewari *et al.*, 2005; Cuadra *et al.*, 2006). Alkylating agents, for example, were reported to be electrophilic compounds with affinity for nucleophilic centers in organic macromolecules (Lawley, 1966). 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.

Aside the toxic elements, microorganisms that are potential causes of a wide range of infections were identified in AERL. For example, the exotoxin produced by the microorganism *Clostridium botulinum*, is lethal to mice and has been implicated in the cause of many human deaths (Brown, 1988). Entry into ground or surface water

bodies of these microorganisms therefore becomes a potential threat to the environment and public health. Donnelly and Scarpino (1991) reported elevated population of total coliform, fecal coliform, fecal streptococci, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *E. coli* and *Klebsiella pneumoniae* in leachate from various landfill sites. Obbard (1999) and Bakare *et al.*, (2003b) also reported the presence of *Staphylococcus* and *Streptococcus* species in landfill leachate, ground and surface water samples.

## CONCLUSIONS

The present findings indicate that raw leachate from Aba-Eku landfills contained toxic constituents that can elicit changes in hematological and histopathological profiles in *C. gariepinus*. The leachate also contained microbes that are of public health concern. Leachate generation at the study site is therefore of prime health concern because there is no known containment or treatment system for it. Previous reports from a nearby community to the landfill indicates that there were mortality of domestic animals and destruction of crops resulting from exposure of these organisms to contaminated water by the landfill leachate (Bakare and Wale-Adeyemo, 2004). More studies are required on the possible transfer of contaminants from leachate polluted waters by fish to man. These will assist in assessing the hazardous effects of chemicals from MSW discharged into the aquatic environment and also in policy making on environmental waste management.

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