

## BIOPHYSICO-CHEMICAL AND TOXICOLOGICAL QUALITIES OF LEACHATES FROM SOLID-WASTE DUMPSITES IN BENIN CITY

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### ABSTRACT

The microbial, physico-chemical and toxicological qualities of leachates obtained from dumpsites/landfills in Benin City Edo State were studied. Five dumpsites namely Uzebu,, Iguomo, Oluku, Ugbowo and Ikhueiro were chosen for the study. The study also involved the microbial and physico-chemical examination of water samples from rivers (Ogba and Okhuahe) which are in close proximity to the dumpsites. Collection of raw and simulated leachate samples was carried out within the period of May- August, 2009. Raw leachates samples were collected at depth of 6 and 12 inches respectively. Five bacteria genera were isolated from the leachates and characterized as *Staphylococcus aureus*, *Proteus mirabilis*, *Klebsiella aerogenes*, *Serratia marcescens* and *Alcaligenes spp.* The major fungal genera isolated from leachates were species of *Aspergillus*, *Rhizopus* and yeast. Bacterial counts were higher than fungal counts in all the leachate samples. Equally microbial counts were higher in raw leachate samples (6 inches depth) than simulated leachates samples. However, microbial load was found to decrease with depth. Seven microbial genera were isolated from river waters samples and characterized as species of *Escherichia*, *Pseudomonas*, *Aspergillus*, *Rhizopus*, *Penicillium*, *Mucor* and yeast. Leachates exhibited a toxic effect which appeared bacteriostatic on the test bacteria, however *S. aureus* was more resistant than *E. coli*. In the case of earthworm, all worms died within 48 hours of the experiment.

Keywords: Leachates, Dumpsite, Biophysico-chemical, Toxicological

### INTRODUCTION

In recent times, there has been a tremendous increase in the tendency of residents to generate waste in Nigeria. This increase is due to accelerated industrialization, urbanization, rural-urban migration, population growth and unplanned development. This growth and demographic expansion has not been matched by improvement in the quality the environment and there has been no planned development. Rather, it has resulted in the production of large amounts of wastes (Aluko *et al.*, 2003; Alimba *et al.*, 2006).

According to Aluko *et al.* (2003), solid waste is an asset when properly managed. However, many urban and sub-urban areas in Nigeria do not have adequate waste disposal facilities. Most times, domestic and agricultural wastes are allowed to decompose in the open waste dumps. Thus, Nigeria is currently experiencing the problem of municipal waste disposal in its sprawling cities and state capitals (Aluko and Sridhar, 2005; Iwegbue *et al.*,

2007).

When municipal waste is placed in landfill/dumpsites, facultative and anaerobic conditions develop. These conditions promote decomposition of the wastes. The breakdown of proteins give rise to obnoxious odours due to the formation of H<sub>2</sub>S gas, ammonia, mercaptans and other volatiles substances. Also, the breakdown of carbohydrates by anaerobic bacteria results in the formation of various acids, alcohols and gases (such as CO<sub>2</sub> and methane). It is this biological decomposition of wastes that also gives rise to the formation of leachate (Young and Hahblow, 1995). The leachates formed contains various types of saprophytic and pathogenic microorganisms plus heavy metals and radioactive elements. These leachates migrate vertically and laterally into the environment causing deleterious effects like loss of biodiversity, leukemia, developmental anomalies, low birth weights, release of obnoxious gases into the environment, contamination of domestic water

sources in communities and low farm productivity. (Campbell, 1993; Ademoroti, 1996; Vrijheid, 2000; Aluko *et al.*, 2003).

The exposure of humans to toxic compounds occurs mostly in the form of complex mixtures. Leachate is a mixture of many chemicals and a potential risk to humans (Bakare *et al.*, 2007). Leachates have several harmful and deleterious effects on living organisms (human, animals, plant, microorganisms) and on the environment. This realization has prompted a number of studies. Bloor *et al.* (2005) designed a study to test the short – term acute and longer – term sublethal ex-siu toxicity of leachate reaching the wastes table,. Studies by Li *et al.*, (2006) has shown that landfill leachate induce oxidative damage in hearts, kidney and spleens of mice. Similarly, industrial solid waste and municipal sludge leachates induced DNA damage in human peripheral blood lymphocytes. Leachates from some landfills in southwest Nigeria induced abnormal sperm shape in mouse (Bakare *et al.*, 2005). The range of effects that leachates can manifest upon living organisms and the environment are as varied as the various components of the leachates. This paper examined microbial, physico-chemical and toxicological qualities of leachates from solid-waste dumpsites in Benin City with a view to estimating pollution effects on the surrounding environment.

## MATERIALS AND METHODS

### Study Location

Benin City, the state capital of Edo State in the south-west of Nigeria, lies in the equatorial climate region between latitude  $6^{\circ} 47^{\circ}$  and  $7^{\circ} 15^{\circ}$  and longitude  $5^{\circ} 49^{\circ}$  and  $6^{\circ} 14^{\circ}$  (Ministry of Lands and Survey Edo State, 2008). The city experiences mainly tropical climate with an estimated annual rainfall of about 200mm, an average height of 200m above sea-level with an estimated population of 1.6 million (UNCNS/UNEP, 1997). There are about seven (7) legal (recognised) solid waste dumpsites that serves Benin City and they are all located in the outskirts of the city. These are the Iguomo, Oluku, Ikhueniro I and II, Ugbowo, Temboga and Ekenwan dumpsites. There are also some illegal dumpsites that serve the city. These include Uzebu and

Evbareke dumpsites. In the past these sites were used as burrow pits where sand were mined. They are generally unlined and have no leachate drains.

### Leachate/River sampling and Analyses

Raw leachate were collected with sterile plastic containers from leachates wells (holes in the elevated areas of the dumpsites) at depth of 6 and 12 inches (i.e 0.15 and 0.30m) at the five aforementioned dumpsites namely, Ugbowo, Oluku, Uzebu, Iguomo and Ikhueniro. For leachate simulation, solid wastes were collected from different points on the dumpsites used for raw leachates collection and were shredded to provide representative samples for simulation in the laboratory. Leachate simulation was done using, American Society for Testing and material (ASTM) method. (Perket *et al.*, 1982) with slight modification. Two hundred and fifty grams (250g) for the waste samples collected at the depths of 6 and 12 inches were shredded and packed into 2L flat bottom flasks. A litre of distilled water was added for extraction, the waste mixture was thoroughly mixed and allowed to stand for 48 hours at room temperature, with continuous stirring done manually at regular intervals of 2 hours. After 48 hours, the solid and liquid portions were separated, and then the liquid portion was thoroughly mixed and filtered to remove debris. The pH of the sample was measured and preserved in the refrigerator at  $4^{\circ}\text{C}$  until use. The collection of the samples from dumpsites was done four (4) times within the wet seasons (May – August 2009) using sterile plastic containers. Water samples were collected within same period from Ogba and Okhuahe rivers which are in close proximity to the dumpsite using sterile plastic containers and preserved at  $4^{\circ}\text{C}$  until used.

### Physico-chemical and heavy-metals analyses of leachates and river water

The physico-chemical properties of leachates samples were determined in accordance with standard method (USEPA, 1996; APHA, 1998). Physico-chemical parameters analysed include; chemical oxygen demand (COD), biochemical oxygen demand (BOD), total dissolved solid (TDS), turbidity, conduc-

tivity, pH, temperature, alkalinity, chloride, sulphate, phosphate, ammonia, nitrate, sodium and potassium. The concentrations of the following five heavy metals were measured using atomic absorption spectrophotometer, graphite furnace flame and hydride system – copper, iron, lead, manganese and mercury (APHA, 1998).

### **Media, viable microbial counts and microbial isolation**

All chemical and media were obtained from Aldrich and Merck chemical companies Germany. The total heterotrophic bacterial counts of leachate samples were performed in triplicate by plating-out 0.1ml of leachates samples on nutrient agar plates containing fulcin (anti-fungal agent), using the spread plate techniques. Plates were enumerated after 48 hours of incubation at 35°C. Isolation and enumeration of fungal isolates were equally performed in triplicate by plating-out 0.1ml of the leachate samples in 20ml of molten potato-dextrose agar (PDA) containing two drops of streptomycin (5µg/ml) using the spread plate techniques. Plates were enumerated after 3-7 days of incubation at room temperature (26 ± 2°C) as described by Pelczar *et al.* (1983).

### **Characterization and Identification Leachate Microorganisms**

The bacterial isolates were examined for colonial morphology and cell micro-morphological characteristics. Gram reaction, citrate utilization of glucose, motility test, indole test and urease production test were all carried out according to (Holt *et al.*, 1994). Fungal isolates were identified and characterized according to the accepted morphological Scheme of Barnett and Hunter (1972). Macroscopic features such as texture of the colony, its surface colour and production of pigments seen on the reverse-side of the Petri-dish plates also aided in the identification of fungal isolates. The multiple-tube technique using MacConkey broth was used to detect coliforms and determine the most probable number (MPN) of coliforms.

### **Toxicity of composite leachates samples on bacteria and earthworm (*Aporrectodea***

### ***longa*)**

The composite leachate samples used for physico-chemical/heavy-metal and toxicity testing were obtained by mixing equal volumes of all the samples obtained from a particular dumpsite together. This was done using only raw leachates obtained from all the five (5) dumpsites. Two bacterial test organisms used in this study were found in frequent association with dumpsites and landfills. These were *Escherichia coli* and *Staphylococcus aureus*. Pure cultures of these organisms were obtained from Department of Microbiology, University of Benin Teaching Hospital (UBTH).

The toxicity test was carried out to monitor the effect of the leachates/leachate constituents on microbial growth. The method of Odokuma and Ikpe (2003) was adopted with slight modifications. A loopful of test organisms were collected from slant and dislodged in 10ml of normal saline and allowed to grow over night. This served as the stock culture. Composite leachate samples were sterilized at 121°C for 15 minutes using an autoclave. The sterilized leachate served as diluents from the initial stock (a loopful of bacterial cells in 10ml of normal saline). Various dilutions of 10<sup>-1</sup>, 10<sup>-2</sup> and 10<sup>-3</sup> were prepared using the sterilised leachate as diluent. An aliquot (0.1ml) of these dilutions (10<sup>-1</sup>, 10<sup>-2</sup>, 10<sup>-3</sup>) was plated on molten nutrient agar plates, using the spread plate technique and incubated at 37°C for 48hours, after which viable cells were enumerated. This plating was carried out in duplicates. Control was set – up using normal saline (no organism added) and sterilized leachate.

The effect of raw leachate on earthworm (*Aporrectodea longa*) was evaluated in this study using sample collection methods described by Ezemonye *et al.* (2006). The earthworm was collected from pristine environment at Ugbowo campus, University of Benin, Nigeria. The earthworm was collected by digging and hand sorting from sub-surface litters, identified in the laboratory and then acclimatized to laboratory conditions for seven (7) days. Earthworms were selected based on liveliness (active response when prodded).

Experimental procedure was based on schemes of Heimbach (1984); Delahaut and

Koval (1990). Factors considered in the toxicity experiment were exposure duration and mortality. The toxicity test involved the use of leachate impregnated filter paper. Selected earthworm species were exposed to composite raw-leachate obtained from various dumpsites for a period of 72 hours. The filter paper test is a method for determining contact toxicity of the leachates. Pieces of filter papers were wetted with the composite raw-leachate samples and placed in the bottom half of Petri-dishes. These were allowed to air-dry and then, rewetted with 2ml of sterile deionized water prior to placement of the worms on the wetted filter paper. A single worm was placed on each plate containing leachate-wetted filter paper. The plates were covered with perforated plate covers and incubated at  $24\pm 4^{\circ}\text{C}$  and kept in dark for 48 hours. A control plate was set-up containing only sterile deionized water wetted filter paper with earthworm and incubated in the same condition as those containing test compound raw-leachate. Evaluations were made at 48 and 72 hours after treatment. The set-up was replicated five times for each composite leachate and entire toxicity test was carried out thrice. The chi-square goodness of fit was used to test significant differences between the physico-chemical and heavy metals characteristics of simulated and raw-leachates across the five (5) dumpsites.

## RESULTS AND DISCUSSION

Major bacterial isolates obtained from raw and simulated leachate samples include *Staphylococcus aureus*, *Serratia marcescens*, *Proteus mirabilis*, *Klebsiella aerogenes* and *Alcaligenes spp.* *Staphylococcus aureus* was the most predominant with frequency of isolation of 3.62% (Table 1). Major fungal isolates obtained from combined leachate samples include *Aspergillus niger*, *Aspergillus flavus*, *Rhizopus spp* and yeast. *Aspergillus* species were the most predominant with frequency of isolation of 62.3%. Equally, microbial isolates from nearby rivers of Ogba and Okhuahe are shown on table 1. The result showed that the major bacterial isolates from nearby rivers include *Enterobacter acrogens*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* while fungal isolates include *Aspergillus niger*, *Rhizopus sp.* *Penicillium*

*sp.*, *Mucor sp* and yeast.

Mean total heterotrophic bacterial counts for raw and simulated leachates are shown on table 2a while mean fungal counts are shown on table 2b. The mean total heterotrophic bacterial counts of raw leachates obtained at the 6 inches (0.15m) depth was higher than that of samples obtained at the 12 inches (0.30m) depth. Atuanya and Ejide (2008) obtained similar results in which microbial counts decreased with depth. At 6 inches depth, the highest mean count was obtained from Ugbowo dumpsite ( $10.66\pm 2.8 \times 10^6$  cfu/ml). At 12 inches depth, the highest mean count of  $7.71\pm 0.81 \times 10^6$  cfu/ml was obtained from Iguomo dumpsites, while the lowest mean count was from Uzebu dumpsite ( $4.04\pm 0.98 \times 10^6$  cfu/ml). The mean total heterotrophic bacterial counts of simulated leachates were generally lower than that of raw leachates. The highest mean simulated leachate count of  $8.03\pm 1.4 \times 10^6$  cfu/ml was also recorded at Ugbowo dumpsite. Similarly, the total fungal counts for raw leachates obtained at depth of 6 inches are higher than that of leachates samples obtained at the 12 inches depth. At both 6 and 12 inches depth, Oluku dumpsite recorded the highest mean counts of  $54 \pm 5.4 \times 10^5$  cfu/ml and  $27.11 \pm 13.8 \times 10^5$  cfu/ml respectively. Also, the mean total fungal counts for simulated leachates were lower than that of raw leachates sample with exception of Uzebu and Ikheuniro dumpsites.

Though, this is in contrast to results obtained by Atuanya and Ejide (2008) where raw microbial counts were generally higher than simulated leachate samples, it agrees with the result obtained by Riley *et al.* (1977) in which the viable heterotrophic aerobes and anaerobes for raw leachates were higher than the simulated leachates. From the foregoing, it is evident that the microbial loads and microbial types found in leachates are as varied as the constituents of leachates. Cook *et al.* (1967) isolated *Escherichia*, *Bacillus*, *Lactobacillus*, *Streptomyces*, *Nocardia*, *Clostridium*, *Aspergillus* and *Penicillium spp.* from leachates. Riley *et al.* (1977) identified the following genera from leachate samples – *Pseudomonas*, *Flavobacterium*, *Bacillus*, *Alcaligenes*, *Sarcina*, *Xanthomonas*, *Streptomyces* and *Penicillium*.

Leachates are known to consist of microbes – pathogenic, non-pathogenic and some opportunistic pathogens (Atuanya and Ejide, 2008). According to Riley *et al.* (1977), some factors that affect the microbial loads of dumpsites/Landfills and the resulting leachates are rainfall, surface water runoff, ground cover, wastes and types of soil within waste tip/dumpsite. The high microbial load of leachates can be attributed to the increased nutrient availability resulting from biological decomposition of wastes in the dumpsites/landfills (Charlotte, 1998).

The results of the physico-chemical parameters and heavy metals detected in raw and simulated leachate samples are presented in table 3. The pH values of leachate samples ranged from 5.44 to 7.37 for raw leachates and 6.44 to 8.44 for simulated leachates. These pH values are within FEPA (1991) recommended standards of 6-9. Biochemical oxygen demand (BOD), chemical oxygen demand (COD), phosphate, sulphate, nitrate, ammonia and copper, were high in raw leachates samples compared to their concentrations in simulated samples. The concentrations of phosphate, sulphate, nitrate, ammonia, copper, iron, lead and manganese were found to be above FEPA (1991) recommended standards.

The elevated amount of ammonia in raw leachates compared to values obtained in simulated leachate were caused by the actions of microorganisms that were present in raw leachates. This finding agrees with that of Donnelly *et al.* (1988). The presence of dissolved oxygen (DO), nitrate and ammonia (table 4) in ground and near by surface water at concentrations above acceptable limit as recommended by FEPA (1991) poses concern to public health, as some of these compounds are associated with cancer of the digestive tract, urinary tract and non-Hodgkin lymphoma (Bates *et al.*, 1992). The mean colony counts of water samples obtained from the two nearby rivers are presented on table 5. The table indicated both rivers are highly polluted judging from values obtained in their total coliform and faecal coliform counts. Also Okhuahe river appears a little more polluted than Ogba river.

Higher number of bacterial and fungal loads observed in leachates and river water samples (Tables 1,2 and 5) may be caused by elevated amount of dissolved oxygen and ammonia. Unlined sanitary landfills have been reported to release large amounts of hazardous and deleterious chemicals to nearby ground and surface water and may pose a threat to the environment and public health (Christensen *et al.*, 2001).

**Table 1: Major microbial isolates from combined leachates samples and from two nearby rivers**

Bacterial isolates from leachates	Frequency of Isolates (%)	Fungal isolates From leachates	Frequency of isolation (%)	Bacterial isolates from rivers	Frequency of isolation (%)	Fungal isolates From rivers	Frequency of Isolation (%)
<i>ureus</i>	36.2	<i>Aspergillus spp</i>	62.3	<i>E.aerogenes</i>	26.1	<i>A.niger</i>	32.4
<i>teus. Spp</i>	16.2	<i>Rhizopus</i>	25.2	<i>E. coli</i>	24.7	<i>Rhizopus.spp</i>	12.3
<i>ratia spp</i>	18.2	Yeast	12.5	<i>P. aeruginosa</i>	13.0	<i>Mucor spp</i>	34.1
<i>bsiella sp</i>	16.1			<i>S.aureus</i>	36.0	Yeast	21.2
<i>ahgens sp.</i>	14.2						

**Table 2a: Mean total heterotrophic bacterial counts for raw and simulated Leachates (cfu/ml) x 10<sup>6</sup>**

Dumpsites	Raw leachates counts		
	6 inches depth	12 inches depth	simulated leachates counts
Uzebu	7.12 ± 1.47	4.04±0.98	5.70±0.83
Iguomo	9.26±1.74	7.71±0.81	7.67±0.97
Oluku	8.37±2.09	7.11±1.67	7.40±1.03
Ugbowo	10.66±2.78	5.99±1.89	8.03±1.41
Ikhueniro	10.10±2.56	6.21±1.93	6.43±1.24

**Table 2b: Mean total fungal counts for raw and simulated leachates (cfu/ml) x 10<sup>5</sup>**

Dumpsites	Raw leachates counts		
	6 inches depth	12 inches depth	simulated leachates counts
Uzebu	12.89± 4.96	9.44±5.12	18.33±5.04
Iguomo	21.78±18.1	13.22±7.55	8.33±3.14
Oluku	54.00±5.43	27.11±13.8	7.35±1.81
Ugbowo	15.78±5.41	8.67±4.14	7.67±1.72
Ikhueniro	21.5±13.1	11.4±8.06	15.0±3.13

**Table 3: Physico-chemicals and heavy metals characteristics of composite samples of raw and simulated leachates**

Parameters	IGUOMO		IKHUENIRO		UGBOWO		UZEBU		OLUKU	
	A	B	A	B	A	B	A	B	A	B
pH	7.13	6.44	5.54	6.47	5.44	7.91	6.61	6.90	7.37	8.49
EC (µS/cm)	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Temperature (°C)	23.0	23.0	24.0	23.0	24.0	23.0	23.0	24.0	24.0	32.0
TDS (mg/L)	101.3	95.3	922.0	419.0	103.8	85.8	9.39	196.0	261.0	150.8
COD (mg/L)	69.00	54.00	89.00	50.00	31.00	19.00	99.00	33.00	48.00	42.00
BOD (mg/L)	35.00	30.00	33.10	27.00	14.20	9.00	43.00	29.00	22.00	17.20
Alkalinity (mg/L)	102.0	68.0	50.0	69.0	48.0	122.0	72.0	78.0	112.0	180.0
Chloride (mg/L)	48.6	49.2	56.3	47.5	32.4	37.8	66.7	31.2	30.8	32.1
Phosphate (mg/L)	2.8	2.3	11.4	3.7	3.0	1.8	13.7	1.8	2.1	1.7
Sulphate (mg/L)	0.8	0.6	2.6	1.1	0.4	0.3	3.0	0.6	0.8	0.7
Nitrate (mg/L)	1.4	1.4	4.2	1.9	0.7	0.5	4.2	0.6	0.7	0.7
Ammonia (mg/L)	2.3	2.1	10.4	3.2	1.3	1.0	13.3	1.7	1.9	1.6
Na(mg/L)	26.60	28.50	71.06	94.20	12.64	29.45	38.20	40.75	44.75	39.90
K (mg/L)	36.06	22.45	90.76	88.96	17.94	27.32	86.20	64.30	86.94	57.40
Cu (mg/L)	5.40	4.20	6.70	4.20	2.30	1.80	7.20	5.40	6.70	5.80
Fe (mg/L)	3.20	3.00	2.80	2.00	1.40	1.60	2.30	2.60	2.00	1.90
Pb (mg/L)	2.00	1.80	1.60	1.20	1.00	1.20	2.00	2.00	1.00	1.00
Mn (mg/L)	1.20	1.00	0.90	1.00	0.60	0.50	0.80	0.80	1.00	1.00
Hg (mg/L)	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25

NOTE:

A: Raw leachate B: Simulated leachate

**Table 4: Physicochemical and heavy metals characteristics of water samples obtained from rivers**

	OKHUAHE	OGBA	FEPALIMITS
pH	6.90	6.30	6-9
EC (µS/cm)	.....	.....	.....
Temperature (°C)	24.00	24.00	<40
TDS	24.60	26.90	-
COD	30.01	30.40	50
BOD	11.40	11.80	-
Alkalinity	88.00	62.10	250
Ammonia	3.20	2.30	0.01
DO	2.10	2.00	>6.00
TSS	0.90	1.08	-
Na	0.08	0.10	-
K	0.04	0.05	-
Cu	0.30	0.20	0.3
Fe	0.40	0.40	0.05
Mn	0.10	0.08	0.05
Nitrate	13.30	10.30	10
Zn	0.50	0.60	-
Hg	ND	ND	-

Note: All parameters are in mg/l except pH, Electrical conductivity (EC), Turbidity and Temperature.

**Table 5: Mean colony counts of water samples obtained from rivers**

	OKHUAHE	OGBA
Total heterotrophic count (cfu/ml) x 10 <sup>3</sup>	61.5	55.0
Total fungal count (cfu/ml) x 10 <sup>5</sup>	41.5	37.0
Total coli form count (MPN/100ml)	35	30
Faecal coli form (MPN/100ml)	4	2

**Table 6: Toxicity analysis using bacteria (as test organism)**

Dumpsites		<i>Staphylococcus aureus</i>					<i>Escherichia coli</i>			
		Dilutions					Dilutions			
		10 <sup>-1</sup>	10 <sup>-2</sup>	10 <sup>-3</sup>	Stock	10 <sup>-1</sup>	10 <sup>-2</sup>	10 <sup>-3</sup>	Stock	
Uzebu	A	40.0	21.0	22.5	TNC	21.5	14.5	14.0	TNC	
	B	46.5	34.5	27.5	TNC	19.0	12.5	9.0	TNC	
Iguomo	A	34.0	23.0	10.5	TNC	23.5	19.0	12.0	TNC	
	B	34.0	21.0	11.5	TNC	23.5	19.5	9.0	TNC	
Oluku	A	18.0	14.5	11.0	TNC	19.0	19.0	12.0	TNC	
	B	32.0	23.0	15.0	TNC	22.5	17.0	14.5	TNC	
Ugbowo	A	25.5	17.0	13.0	TNC	25.0	15.0	6.5	TNC	
	B	37.5	33.0	26.0	TNC	34.0	17.0	14.5	TNC	
Ikhueni	A	36.5	28.0	24.0	TNC	21.0	20.0	17.0	TNC	
	B	55.0	42.5	41.5	TNC	38.0	34.0	24.5	TNC	
CONTROL		NG	NG	NG	NG	NG	NG	NG	NG	

NOTE: A = raw leachates: B – simulated leachates NG – No growth, TNC –too numerous to count.

**Table 7: Toxicological analysis using earthworm (as test organism)**

DUMPSITE	Number of worms Tested	Number dead after experiment	Number remaining after 72 hours
Uzebu	5	5	0
Iguomo	5	5	0
Oluku	5	5	0
Ugbowo	5	5	0
Ikhueni	5	5	0
Control	5	0	5

In the present study, considering the levels of contaminants in the river water samples, it is impossible to rule out the contamination of the rivers by the leachates. Previous studies by Riley *et al.* (1977) showed that except in the dry summer months, the stream located about 50 metres from the sources of the leachates contain some simulated leachate diluted with surface runoff water. Equally, according to Aluko and Sridhar (2005), leachates emanating from municipal wastes are a major sources of surface and ground water pollution worldwide. Therefore, there is high tendency to suspect the dumpsites as one of the major sources of surface water contamination in the study area. This is due to the fact that the dumpsites are not properly sited and do not have geoliner or concrete walls that could prevent leachate from percolating into ground water or diffusing into the nearby rivers.

Toxicological analysis of the leachates carried out using bacteria (*Staphylococcus aureus* and *Escherichia coli*) and earthworms (*Aporreectodea Longa*) as the test organisms are shown on tables 6 and 7 respectively. From the results of the toxicological analysis using bacteria as test organism, it was observed that the leachates exhibited a toxic effect against the organisms. However, the effect appears to be bacteriostatic. It was also observed in all cases that the bacterial growth was lower in raw leachates (A) than in simulated leachates (B). This implies that raw leachates is more toxic to test bacterial organisms than simulated leachates. This toxicity can be accrued to the presence of inhibitory substances in the leachates. These inhibitory substances could be heavy metals and other pollutants contained in the leachate and / or the acids produced from the decomposition of

the waste (Riley *et al.*, 1977).

Equally, result of this study showed *Staphylococcus aureus* to be more resistant to the leachate toxicity than *E.coli* because the population of *S. aureus* that grew was higher than that of *E.coli* (table 6). A plausible explanation for this is the presence of a thick peptidoglycan layer in the cell wall of Gram-positive bacteria, which confers it resistance to environmental stress (Odokuma and Ikpe, 2003).

It was observed (Table 7) in all cases of the leachates tested that all worms were dead within 48 hours, with many dying within the first several hours of exposure to the leachates. The worms exhibited a writhing behaviour and violent seizures when they were placed on the leachate impregnated filter paper. This study showed the vulnerability of earthworms to leachates. Mortality of earthworm was very high, this may be attributed to the toxic constituents of the leachate. The implication of this is that if the release of leachates into the soil is not monitored or curtailed, it will result in great detrimental effect on soil organisms especially earthworm. Earthworm play a vital role in the enhancement of soil structure and fertility (Muthakarrupan and Paramasamy, 2010).

This study also agrees with previous reports on the toxicity of leachates to living organisms. Report abound on leachates induced genotoxicity in *Allium cepa* (Bakare and Wale-Adeyemo, 2004; Chandra *et al.*, 2005), leachate induced chromosome aberration in rat (Alimba *et al.*, 2006). Globally, leachates have been implicated in low yield of farm produce, developmental anomalies, low birth weights, leukaemia incidence and other cancers in communities around the dumpsites (Aluko and Sridhar, 2005). The problems of ectotoxicity posed by leachates must be properly handled in order to reduce it to the barest minimum.

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