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## The Genotoxicity Screening of Simulated Leachate from Semi-urban Waste Dumps in the Niger Delta, Nigeria Analysed by *Allium* Test

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**Abstract:** This communication present the potential genotoxicity effects of simulated leachate from semi-urban waste dumps in the Niger Delta, Nigeria, on *Allium cepa*. The root of *Allium* at about 2-5 cm long was treated with 1, 2.5, 5, 10, 25 and 50% concentration of the leachate for 24 h. These were then used to prepare for observation of chromosomal aberrations and frequency of mitotic division. Different types of chromosomal aberrations was induced (sticky chromosome, disturbed spindle, anaphase bridge etc.). There was significant ( $p \geq 0.05$ ) reduction in mitotic index at all levels of treatment compared to the control. The observed genotoxic effect was due to presence of certain chemical compounds inherent in the semi-urban waste leachate.

**Key words:** Genotoxicity, chromosome aberration, *Allium cepa*, leachate, semi-urban waste dumps, Niger Delta

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### INTRODUCTION

Management of solid wastes is often a problem in urban and sub-urban environment in Nigeria. Most urban and sub-urban areas in Nigeria do not have waste disposal facilities; most at times domestic and agricultural wastes are allow to decompose in the open waste dumps. These open dumps do not have geoliner or concrete walls that could prevent the leachate from percolating into the soils. Leachate and/or runoff from such dumps could contaminate the adjoining surface water bodies and groundwater which form the major sources of drinking water. The various water drinking water sources are abstracted without any form of further treatment. Cytotoxicity assessment of the leachate is therefore necessary to safeguard these communities from the effects of hazardous chemicals found in the waste dumps. Leachates are produced as a result of the action of water on decomposing organic materials. Leachate can be characterized as extremely complex mixture containing numerous inorganic as well as organic compounds; the complexity makes it almost impossible to carry out a hazardous assessment base on chemical analysis.

The *Allium* test employing rooted bulbs of *Allium cepa* has been used as standard short-term test in environmental monitoring and as well as tool for evaluating and ranking chemical with reference to their toxicity by various agencies (Mishra, 1993). This test has been frequently used by many researchers (Fiskesjo, 1981, 1985; Grant, 1982; Mishra, 1993; Rank and Nielsen, 1994; Bakare *et al.*, 1999a, 2001).

*Allium cepa* test is easy to handle, low cost and good correlation with mammalian test system (Fiskesjo, 1985). The test system comprises of rapid and sensitive method at microscopic level by measuring growth inhibition of roots as well as at microscopic level comprising harmful qualitative and quantitative effects on cells and tissues (Mishra, 1993). The microscopic level also include the *Allium*

micronucleus assay which has been used in studying genotoxicity and bioavailability of solid deposits (containing mercury) from chloralkali plant (Dash *et al.*, 1988; Panda *et al.*, 1989, waste water (Amin, 2002) and urban and rural dumps leachates (Bakare *et al.*, 1999a, 1999b, 2001).

The present study was undertaken to assess cytotoxic effects of simulated leachate from sub-urban communities in the Niger Delta. This will provide information for assessment of the hazardous effects of the chemicals from solid waste dumpsites.

## MATERIALS AND METHODS

For leachate simulation, solid wastes were collected from Abraka, Eku and Obiaruku (all in Delta State of Nigeria) in June 2005. Simulation was done using the extraction procedure of the American Society of Testing and Material (ASTM) method (Perkel *et al.*, 1982) with slight modification. From the initial sample of 1 kg, 700 g of the wastes were shredded and extracted with a volume of distilled water four times the waste sample. The waste mixture was mixed thoroughly and allowed to stand for 48 h at room temperature and stirring was done manually at regular interval of 2 h. At 48 h the solid and liquid portions were separated and the pH is recorded and stored at 4°C (Bakare *et al.*, 1999a). The physicochemical properties of the leachate samples were examined following the method described in APHA (1992). The heavy metals was determined by atomic absorption spectrophotometer after extraction with Ammonium pyrrolidine dithiocarbamate-Methyl isobutyl ketone (APDC-MIBK) (APHA, 1992).

### *Allium cepa* Assay

Equal-size bulbs were chosen from a commercial variety of *Allium cepa* (2n = 16). The outer scale of the bulb and brownish plate were removed. The root of *Allium cepa* were generated by suspending the bulb over 50 mL beaker containing distilled water for 48 h. When the roots were about 2-3cm long, they were placed directly in 1, 2.5, 5, 10, 25 and 50% concentrations of the simulated leachate of 2.4 h. Onion root generated in distilled water served as negative control (Bakare *et al.*, 1999a,b). The experiment was performed at about 25±2°C and was protected against sunlight. Root from treated bulbs and those from the control were harvested and fixed in ethanol: acetic acid (3:1) V/V for 24 h. After fixing the root were hydrolyzed with 1 M HCl at 65°C for 3 min.

Two root tips were then squashed in each slide, stained with acetocarmine for 10 min and cover slips were carefully lowered on them to exclude air bubbles. The cover slips were sealed on the slides with clear fingernail polish as suggested by Grant (1982). Five slides were prepared for each treatment and 100 cells were scored for each treatment and control. The data were expressed in terms of mitotic index, number and percentages of chromosome aberrations.

## RESULTS AND DISCUSSION

The results for the physicochemical analysis of the simulated leachate from sub-urban waste dumps is presented in Table 1. Table 2, 3 and 4 present the results of genotoxicity of simulated leachate on *Allium cepa*.

Leachate contains a variety of chemical agents and their interaction and reactions under changing environmental condition further complicate the issue. The synergistic and antagonistic effects among the chemicals are inevitable. As shown in Table 1, the concentration of heavy metals we found in this study exceeds the WHO limits for drinking water. Some of the metals (Cd, Pb and Ni) are known to be carcinogens (Fowler *et al.*, 1994; Haugen *et al.*, 1994; Elinder and Jarub, 1996). Leachate containing significant amount of these metals as part of its content can induce cancer. For example lead

Table 1: Physicochemical and heavy metal characteristic of simulated leachates

Properties	Abraka (ABK)	Eku (EK)	Obiaruku (OBI)
pH	6.34	8.24	6.76
Total dissolved solid (TDS) (mg L <sup>-1</sup> )	218.00	101.30	84.40
Conductivity (µS cm <sup>-1</sup> )	477.00	746.00	176.80
Salinity (mg L <sup>-1</sup> )	0.20	0.20	0.10
Dissolved oxygen (mg L <sup>-1</sup> )	0.40	0.60	1.00
Biochemical oxygen demand (BOD) (mg L <sup>-1</sup> )	160.00	128.00	84.00
Total Alkalinity (mg L <sup>-1</sup> )	130.00	940.00	140.00
Total Hardness (mg L <sup>-1</sup> )	616.00	1382.40	220.00
Total solids (mg L <sup>-1</sup> )	3018.00	6611.30	3054.00
Ammonia (mg L <sup>-1</sup> )	12.80	11.40	46.20
Sulphate (mg L <sup>-1</sup> )	26.00	24.00	170.00
Nitrate (mg L <sup>-1</sup> )	11.20	13.50	77.50
Chloride (mg L <sup>-1</sup> )	29.22	304.28	260.29
Copper (mg L <sup>-1</sup> )	<0.002	<0.002	<0.002
Iron (mg L <sup>-1</sup> )	2.64	0.63	12.56
Lead (mg L <sup>-1</sup> )	1.26	2.08	0.90
Cadmium (mg L <sup>-1</sup> )	0.01	0.09	0.04
Silver (mg L <sup>-1</sup> )	<0.002	<0.002	<0.002
Nickel (mg L <sup>-1</sup> )	0.31	0.56	0.09
Manganese (mg L <sup>-1</sup> )	<0.002	0.03	0.01
Magnesium (mg L <sup>-1</sup> )	168.56	317.93	67.13

\*Mean of triplicate analysis: CV = <5%

was reported to be a strong clastogen, which break chromosome in Chinese hamster ovary cells (Bauchinger and Schmid, 1972), in bone marrow erythrocytes of rat and in cells of *Allium cepa* (Lerda, 1992; Bakare, 2001). Nickel produces highly selective damage to heterochromatic in Chinese hamster genomes (Costa *et al.*, 1994). While cadmium (Elinder and Jarub, 1996), lead (Fowler *et al.*, 1994) and nickel (Haugen *et al.*, 1994) have been reported to induce of variety of tumors in animal studies.

The Biochemical Oxygen Demand (BOD) of leachate reported herein is greater than 40 mg L<sup>-1</sup> in all samples (Table 1). Samples with such high BOD values have been demonstrated to have corresponding high mutagenic activities (Omura *et al.*, 1992). Thus high activities as seen with the induction of various types of aberration are associated with high BOD. This is inline with observation of Bakare *et al.* (2001). Table 2-4 present the results of microscopic studies. The suppression of mitotic activities was often used for tracing cytotoxicity (Smaka-Mkinel *et al.*, 1996). This is usually accompanied by an increase in the fraction of cells with c-mitosis, multi groups, sticky and abnormal chromosome orientation (Amin and Mighid, 2000; Amin, 2002). At each treatment level for all leachate samples, there was a significant decrease in mitotic index compared to the control. Mitotic index decreases with increasing concentrations of leachate, which indicates that, the mixture was mitodepressant. For leachate samples the lowest mitotic index observed was 2.80 (for Obia), 4.50 (for ABK) and 5.58 (for Eku). The lowest mitotic index was observed at 50% concentration in all samples. In the present study, a decrease in mitotic index was found to be significant with increase in the concentrations of the leachate. The suppression of mitotic index was probably due to either the blocking of G<sub>1</sub>, suppressing cells from DNA synthesis (Amin, 2002) or blocking of G<sub>2</sub>, preventing cells from entering mitosis (Amin, 2002). This occurred probably through the uncoupling of respiration processes and carbohydrate metabolism (Andrew *et al.*, 1983) leading to low ATP content, which is essential for progress of mitosis.

Sticky chromosomes indicate highly toxic effect, usually not reversible and probably leading to cell death (Fiskesjo, 1985; Bakare *et al.*, 1999a). The highest amount of sticky chromosome was observed at 50% concentration of leachate from Abraka (ABK) and Obiaruku while 25% concentration gave the highest amount of sticky chromosome for leachate from Eku. Chromosome stickness is caused probably through intermediate reaction of DNA during its inhibition period causing DNA-DNA or

Table 2: Frequency and spectrum of cytological effects of treatment with different concentrations of (EKUSL) simulated leachate

Concentration of samples (%)	Control	1	2.5	5	10	25	50
<b>No. of classified cells</b>							
Cells in division	510.00	278.00	290.00	293.00	302.00	260.00	223.00
Mitotic index	12.75	6.95	7.25	7.33	7.55	6.50	5.58
Cells at anaphase	92.00	60.00	57.00	54.00	49.00	51.00	44.00
Cells at metaphase	73.00	25.00	14.00	29.00	18.00	29.00	36.00
<b>Binucleus cell</b>							
Disturbed spindle		3.00	11.00	9.00	7.00	8.00	1.00
Anaphase bridge		1.00	1.00			2.00	3.00
<b>Chromosome fragment</b>							
Stricky chromosomes		8.00	17.00	19.00	12.00	28.00	24.00
Micronucleus		0.00					
Very big nucleus			1.00	1.00	3.00	-	-
<b>Frequency of aberrant cells</b>							
Total aberrant cells		12.00	29.00	29.00	22.90	38.00	28.00
Total cells scored (%)		0.30	0.73	0.73	0.55	0.95	0.70
No. of dividing cells (%)		6.30	15.22	15.26	11.57	20.00	14.77

Table 3: Frequency and spectrum of cytological effect of treatment with different concentrations of Abraka Simulated Leachate (ABK SL)

Concentration of samples (%)	Control	1	2.5	5	10	25	50
<b>No. of classified cells</b>							
Cells in division	510.00	220.00	231.00	221.00	242.00	231.00	180.00
Mitotic index	12.75	5.50	5.78	5.53	6.05	5.78	4.50
Cells at anaphase	92.00	30.00	44.00	53.00	35.00	53.00	44.00
Cells at metaphase	73.00	31.00	52.00	43.00	32.00	31.00	32.00
<b>Binucleus cell</b>							
Disturbed spindle		7.00	3.00	46.00	38.00	22.00	54.00
Anaphase bridge				1.00	1.00	1.00	-
<b>Chromosome fragment</b>							
Stricky chromosomes			9.00	4.00	19.00	5.00	20.00
Micronucleus							
Very big nucleus					2.00	-	4.00
<b>Frequency of aberrant cells</b>							
Total aberrant cells		7.00	12.00	59.00	62.00	34.00	96.00
Total cells scored (%)		0.18	0.30	1.48	1.55	0.85	2.40
No. of dividing cells		3.64	6.32	31.05	32.63	17.89	50.52

DNA-protein cross linking (Ashour, 1998; Amin, 1991; 2002) or through reactions with the lysosomal system altering the physicochemical properties of nucleic acid and/or nucleiprotein, liquidification of the chromatin materials (Adam and Farah, 1989), which consequently lead to hindrance of normal chromosome separation at anaphase giving rise to sticky chromosome bridges. Sticky bridges might also result from incomplete replication of chromosomes by defective or less active replication enzymes (Sinha, 1979) or late replicating DNA sequences of the felomeric heterochromatin (Amin, 2002). If heterochromatin blocks did not finish DNA replication when the nucleus is ready to divide, bridge formation would occur (Kaltisikes *et al.*, 1984).

Chromosomal aberrations occur due to lesion in both DNA and chromosomal and spindle protein causing genetic damage. The different types of aberrations observed in our study were probably due to the effects of genotoxic compound found in waste dumps (e.g., household chemicals such as chlorophenol, heavy metals etc.). This caused drastic changes in chromatin, spindle apparatus and centromeres, leading to impairment of chromosome alignment onto metaphase plate, abnormal spindle orientation, abnormal chromosome movement and c-mitosis. The abnormal chromosome orientation was due to the altered quality and quantity of kinetochore heterochromatin (Jannifer *et al.*, 1988). In the present study spindle disturbances occur high frequency in Abraka, Eku and Obiaruku leachate

Table 4: Frequency and spectrum of cytological effect of treatment with different concentrations of Obiaruku simulated leachate (OBIA SL)

Concentration of samples (%)	Control	1	2.5	5	10	25	50
<b>No. of classified cells</b>							
Cells in division	510.00	228.00	250.00	140.00	230.00	160.00	112.00
Mitotic index	12.75	5.70	6.25	3.50	5.75	4.00	2.80
Cells at anaphase	92.00	40.00	48.00	28.00	50.00	29.00	14.00
Cells at metaphase	73.00	29.00	37.00	22.00	39.00	23.00	21.00
<b>Binucleus cell</b>							
Disturbed spindle		01.00	6.00	14.00	4.00	3.00	01.00
Anaphase bridge		01.00				01.00	
Chromosome fragment							2.00
Stricky Chromosomes		2.00	6.00	4.00	3.00	9.00	12.00
Micronucleus							
Very big nucleus							4.00
<b>Frequency of aberrant cells (%)</b>							
Total aberrant cells		4.00	12.00	16.00	7.00	11.00	19.00
Total cells scored		0.10	0.30	0.40	0.18	0.27	0.48
Number of dividing cells		2.10	6.32	8.42	3.68	5.79	10.00

samples. There was no observable trend in the frequency of spindle disturbances with increasing concentration of the leachates. The mechanistic background to spindle disturbances with compounds might be partially due to partitioning of some household chemicals into hydrophobic components of the cell (Onfelt, 1987a, b; Amin, 2002).

The results of this study agrees previous reports from animal studies (Bakare, 1999,b) where the raw leachate from the dumpsite induced abnormally shaped sperm head in albino mice. Further more, Bakare *et al.* (1999a, b, 2000, 2001) reported the clastogenic, mutagenic and cytotoxic effects of raw and simulated leachate from institutional, domestic, municipal and industrial waste dumpsites in south west Nigeria. Similarly, Cabrera *et al.* (1999) and Cabrera and Todriguez (1999) reported the genotoxic effect of landfill leachate and extracts from the organic and total municipal garbage in *Tradescantia* and *A. cepa*.

The genomic disturbance recorded in this study presents damage to the DNA ranging from point of aberrations to chromosomal mutations. The effect of this to the present and future generations of the communities in the vicinity of the dumpsites could be grievous. This is because the dumpsites were not properly sited and do not have geoliner or concrete walls that could prevent the leachate from perculating into the groundwater or diffusing into the nearby river. Communities in vicinity of these dump depend solely on water from boreholes and river water for domestic and commercial purposes. This is confirmed by the reports of several researches who presented different forms of genetic abnormalities e.g., Hen *et al.*, (1988) reported that water from a well near a waste dumpsite induced chromosomal alterations in human lymphocytes and Gonsebatt *et al.* (1995) reported significantly high frequencies of chromatid and chromosomal deletions in individual at landfill for harzadous wastes. Increased incidences of bladder and gastrointestinal cancers (Griffith *et al.*, 1989) reproductive and congenital malformations (Goldman *et al.*, 1985) has reported for people living close to hazardous waste dump sites.

Bakare (2001) suggested that considering the high correlation between mutagenicity and carcinogenicity, it may be pertinent to add that organisms that are predisposed to cancer and when exposed to landfill leachate may be at a very high risk of developing the disease. This is because of the possibility that the leachate contains chemicals possessing initiation, promotion and progression properties working in concert to bring about neoplastic transformation.

This study has shown that sub-urban refuse leachate induced chromosomal aberration in *Allium cepa* through interaction with DNA and protein leading to chromosome stickness, mitotic disturbances and/or cell damage. In addition, semi-urban waste dumps leachate contained pollutant

compounds causing high rate of chromosomal abnormalities. Therefore, it is highly recommended that adequate leachate treatment process and concrete liners or geoliner be provided for these waste dumps to prevent the leachate from percolating into the subsurface or diffusing to adjoining water body.

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