

Full Length Research Paper

Municipal landfill leachates induced chromosome aberrations in rat bone marrow cells

C. G. Alimba, A. A. Bakare* and C. A. Latunji

Cell Biology and Genetics Unit, Department of Zoology, University of Ibadan, Ibadan. Nigeria.

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This study examined the potential mutagenic effects of raw and simulated leachates from Olushosun municipal solid waste (MSW) landfill using rat bone marrow chromosome aberration assay. Raw leachate obtained directly from the landfill and simulated leachate obtained via the American Society for Testing and Materials (ASTM) category-A extraction procedure were examined for their physical and chemical properties. Rats were intraperitoneally exposed to 1 - 25% concentrations of the leachates for 48 h. The erythroblasts of the bone marrow cells examined post treatment show structural chromosomal abnormalities such as breaks, gaps, rings and acentrics. The induction was dose dependent ($r = 0.80$ and 0.85 for ORL and OSL, respectively). Physico-chemical and heavy metal analysis of the test samples showed that they contained high concentrations of toxic anions and cations that are capable of inducing mutation in living cells. The interaction of these constituents with the genetic material in the bone marrow cells of rat caused the observed chromosome aberrations. Our data indicate that MSW leachates can induce genotoxicity in rat and suggest potential health risk to human populations.

Key words: Municipal landfill leachate, chromosome aberrations, genotoxicity, toxic chemicals, rat.

INTRODUCTION

In Nigeria, the propensity of residents to generate waste seems to have heightened in recent times. This increase is due largely to accelerated industrialization, urbanization, and population growth, which have elicited strong international and national concerns about the possible environmental, health and safety effects of living in the vicinity of these wastes. Landfilling and or open dumping of wastes are very common methods of managing these wastes. These landfills/dumpsites are unlined and are located in public places surrounded by residential quarters and in wetland or other areas with seasonally high water tables. Unlined sanitary landfills have been reported to release large amounts of hazardous and deleterious chemicals to nearby ground water and to the air, via leachate and landfill gas respectively (Lee and Jones-Lee, 1994; Christensen et al., 2001; Ikem et al., 2002). This indicates that leachates from landfills/dumpsites can potentially pollute ground

water in these cities and may pose a threat to the environment and public health.

Exposure to multiple chemical combinations in populations living near waste dump sites has led to series of human health disorders (Vrijheid, 2000; Palmer et al., 2005). This formed the basis of our studies on leachate induced cytotoxicity and mutagenicity in eukaryotic systems. Results obtained in these studies have shown that leachates from some landfills in southwest Nigeria induced chromosome aberrations in *Allium cepa* (Bakare, 2001; Bakare and Wale-Adeyemo, 2004) and abnormal sperm shape in mouse (Bakare et al., 2005). In these studies and others available in the broad review of literature, information is particularly lacking on the potential clastogenic effect of landfill leachate on rat chromosome. Among available experimental mammals, rat has certain advantages, which include their well-defined karyotype and chromosome number ($2n = 42$) that approximates that of human. They have been successfully used in several cytogenetic studies (Celik et al., 2003; Kanabay and Oguz, 2005). Also, information on chromosome aberration (CA) is important, as it has been implicated in several human genetic disorders.

In this report, we present an extension of our study on the use of rat bone marrow metaphase chromosome aber-

Corresponding author E-mail:

adekunle.bakare@mail.ui.edu.ng, adebakar19@yahoo.com.

Tel: +234-(0) 802-325-5904, +234-(0)-27514185

ration assay to evaluate the potential mutagenic effects of raw and simulated leachates from Olushosun landfill, Lagos state, Nigeria. The landfill, which receives varieties of domestic, industrial and commercial wastes, is not equipped with a leachate collection and treatment system. As a result, leachates produced are discharged into the surrounding environment. Some physical and chemical properties of the leachate samples were also analyzed.

MATERIALS AND METHODS

Sampling site, leachate sampling and simulation from solid waste

The sampling site is Olushosun landfill at Ojota, located on the Northeastern part of Lagos State (Latitude 6°34' N and Longitude 3° 24' E), Nigeria. The site was swampy prior to the landfilling operations, which commenced in 1977 (LAWMA, 1998). It covers about 42-hectares of land and with an excavation of about 18 m deep into the landfill area.

Raw leachate was collected from leachate wells (holes in the ground) and was thoroughly mixed. For leachate simulation, solid wastes were collected from different points on the landfill site, 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 A extraction procedure (Perket et al., 1982), with slight modification. Briefly, an initial solid waste sample of 2 kg was collected at different points of the same depth from the landfill, and representative samples of 0.7 kg were packed into 4 different 5 L flat bottom flasks. A volume of distilled water, four times the sample weight, was added. The waste mixture was mixed thoroughly and allowed to stand for 48 h at room temperature. Continuous stirring was done manually at regular intervals of 2 h. At 48 h the solid and liquid portions were separated, and then the liquid portions were thoroughly mixed. The raw and simulated samples were filtered to remove debris, the pH was measured, and the sample stored at 4°C until use. They were designated Olushosun raw leachate (ORL) and Olushosun simulated leachate (OSL), respectively.

Physico-chemical parameters and heavy metal analysis

The physical and chemical properties of the leachate samples 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), total dissolved solids (TDS), alkalinity, chlorides, sulphates, ammonia and nitrates, were analyzed. The concentrations of seven heavy metals namely copper (Cu), iron (Fe), lead (Pb), cadmium (Cd), manganese (Mn), mercury (Hg), and arsenic (As) were estimated in each of the two leachate samples using atomic absorption spectrophotometer.

Animal exposure and bone marrow metaphase chromosome slide preparation

Pathogen free male rats, weighing 155 – 197 g, obtained from animal breeding units of Physiology Department, University of Ibadan, Nigeria, were used for this study. Five concentrations of 1.0, 2.5, 5.0, 10.0 and 25.0% of each leachate sample were tested using 4 rats in each group. Each rat in each group was given a single intraperitoneal injection of 1.0 ml of the test sample for 2 consecutive days (Brusick, 1980). The negative and positive control rats received same volume of distilled water and cyclopho-

sphamide (20 mg/kg), respectively. Each rat was injected with Vinblastin Sulphate (0.1 mg/kg) 2-h prior to sacrifice. At 48 h post treatment, the animals were sacrificed by cervical dislocation and bone marrow cells were prepared from the femoral bone marrow by the conventional method (Brusick, 1980). Briefly, bone marrow cells were flushed from both femurs in 2.2% sodium citrate and cells were centrifuged at 1500 rpm for 10 min. Pelleted bone marrow cells were then resuspended in 5 ml of a hypotonic solution of 0.075 M KCl for 20 min at 37°C. The cells were centrifuged again and fixed with three changes of 5 ml each of ice – cold Carnoy's fixative (methanol - acetic acid, 3:1, v/v) for 30 min at 25°C. The cells were then dropped onto clean, grease free microscope slides which were air-dried and stained with 5% Giemsa for 15 min. All slides were evaluated blindly at X1000 magnification for structural chromosome aberrations. 50 well spread complete metaphases were scored per slide and 4 slides were prepared per rat at each concentration.

Statistical analysis

The SPSS 10.0 statistical package was applied to evaluate the distribution of abnormal chromosome. Results are presented as Mean \pm SE for each leachate sample (experimental unit (n) for this analysis was the individual rat). The levels of statistical significance were estimated at $P < 0.05$ and $P < 0.01$ using the student's t-test. Correlation coefficients (r) were also calculated to analyze concentration-response relationships.

RESULTS

The result of the physico-chemical parameters and heavy metals detected in ORL and OSL are presented in Table 1. Both samples were dark brown and their pH was about neutral. BOD, COD, chlorides, nitrates and sulphates were high in ORL compared to their concentrations in OSL, while TDS, alkalinity and ammonia were high in OSL compared to the ORL. The concentrations of the heavy metals were higher in ORL than in OSL. The concentrations of Pb and Fe in ORL were comparatively higher than those of other heavy metals in the two samples.

Table 2 shows the result on genotoxicity. Compared to the negative control, both samples induced dose-responsive increases in abnormal chromosome in rat bone marrow. This was statistically significant at most of the tested concentrations of the two leachates. The correlation coefficient (r) for the least squares linear dose-responses was 0.80 and 0.85 for ORL and OSL, respectively. The chromosome aberrations observed were breaks, rings, gaps and acentrics. The frequency of abnormal chromosomes (25.83%) in the positive control group was higher than those at the tested concentrations of the leachates. There were low mitotic indices in marrow of animals exposed to all the concentration levels of ORL and OSL as indicated by few numbers of metaphase spread.

DISCUSSION

Chromosome analysis of bone marrow cells *in vivo* has become a standard method for testing for the potential mutagenic effects of viruses, radiations, drugs and chemical pollutants (Celik et al., 2003; Kanabay and Oguz, 2005). In the present study, rats were intraperitoneally exp-

Table 1. Physico-chemical characteristics and heavy metals detected in raw and simulated leachates obtained from Olushosun landfill.

Parameters *	ORL	OSL	FEPA ^a	USEPA ^b
pH	7.3	6.8	6 - 9	6.5 – 8.5
BOD ⁺	598	590	50	-
COD ⁺⁺	480	370	-	410
Total Dissolved Solid	0.320	1.320	2000	500
Hardness	540	300	-	0 - 75
Total Alkalinity	480	540	250	20
Chlorides	770	240	-	250
Sulphates	68.58	48.20	20	250
Nitrates	3.86	2.46	-	10
Ammonia	0.86	78.68	0.01	0.02
Copper	0.77	0.44	0.3	1.0
Iron	1.90	0.83	0.05	0.3
Lead	1.40	0.69	0.01	0.015
Cadmium	0.58	0.46	0.05	0.05
Manganese	0.79	0.46	0.05	0.05
Mercury	0.41	0.23	0.1	-
Arsenate	0.36	0.27	-	-

*All values are in mg/L except pH.

+: Biochemical oxygen demand.

++: Chemical oxygen demand.

^aFederal Environmental Protection Agency (1991) Permissible limits for drinking water.

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

Table 2. Chromosome aberrations induced in bone marrow cells of rats exposed to raw and simulated leachates obtained from Olushosun landfill.

Test samples	Concentration (%)	Chromosome breaks	Acentrics	Rings	Gaps	Mean ± SE
Negative control	0	1	-	-	2	0.75 0.41
ORL	1.0	2	1	-	2	1.25 0.32
	2.5	8	4	-	5	4.25 1.65*
	5.0	7	6	-	5	4.50 1.55**
	10.0	9	7	-	6	5.50 1.93**
	25.0	10	9	1	7	6.50 1.93**
OSL	1.0	1	1	1	1	1.0 0.11
	2.5	5	6	-	4	3.75 1.32*
	5.0	6	5	1	5	4.25 1.11*
	10.0	8	8	1	7	6.00 1.68**
	25.0	10	9	2	7	7.00 1.78**
Cyclophosphamide	20 mg/kg bw	10	12	2	7	7.75 2.17**

*P<0.05, ** P<0.01 = levels of significance of chromosome damage in leachate-treated bone marrow cells compared with the negative control.

used to different concentrations of Olushosun raw and simulated leachates containing complex mixtures of toxic substances. Chromosome aberrations were detected in the bone marrow cells in a concentration dependent manner and showing statistical significance at P<0.05 and P<0.01. These observations may be due to

the various organic and inorganic constituents present in the leachates. These chemicals could cause clastogenic effects individually or in combinations. Heavy metals have been implicated in leachate-induced genotoxicity (Siddique et al., 2005; Tewari et al., 2005; Bakare et al., 2005). In this study, Cd, Cu, Fe, Pb, Mn, As and Hg were found at high

concentrations compared to acceptable limits by regulatory authorities [USEPA, 1989 (www.epa.gov/safewater/mcl.html); FEPA, 2001]. Many studies have reported the mutagenic, clastogenic and carcinogenic effects of these metals in mammals (Buchet et al., 1980; Bates et al., 1992; Fowler et al., 1994; Elinder and Jarup, 1996; Godet et al., 1996; Galaris and Evangelou, 2002).

The mechanisms for CA induction in mammals by MSW leachates are not clearly understood. It could be via inhibition of DNA repair processes in the bone marrow cells, and metals have been reported to exert unique mechanism(s) of repair inhibition (Hartwig, 1998). It may also involve the generation of free radicals in the exposed animals. These radicals could bind to the purine and pyrimidines of nucleic acids to cause base substitution and breakage of DNA and eventually induce mutation (Sang and Li, 2004). They could also interact with proteins in a way that will affect their structures and functions (Reist et al., 1998). In this regards, it would be noteworthy to state that Cd, Cu and Fe have been reported to induce the production of reactive oxygen species in eukaryotic systems (Ghio et al., 2002; Radetski et al., 2004). Further studies on the generation of free radicals by the components in leachates and the effects of the radicals on DNA is required to understand the mechanism of leachate-induced genotoxicity in mammals.

The elevated amount of ammonia in OSL than in ORL may be caused by the action of microorganisms that may be present in the leachates. Landfill leachate has been known to consist of microbes, some of which are opportunistic pathogens. Some of these microbes could produce toxins that may cause a public health problem (Donnelly et al., 1988). The presence of nitrate and ammonia in ground water at concentration above acceptable limit, poses concern to public health, as these compounds are associated with cancer of the digestive tract, urinary tract, and non-Hodgkin lymphoma (Gullis et al., 2002).

Although this study did not analyze all chemical substances that could be present in the leachates, it has been reported that many toxic chemicals such as benzene, naphthalene, persistent organic pollutants, dioxins, polychlorinated biphenyls and alkylating agents are 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 like DNA (Lawley, 1966); they alkylate with the ring nitrogen and oxygen of DNA to form phosphotriester bond, which weakens the DNA backbone and can modify the N-glycosylic bond to cause depurination or depyrimidination of the DNA (Loeb and Preston, 1986).

To the best of our knowledge, this is the first report on leachate induced chromosome aberration in rat. Other

reports on leachate induced genotoxicity in *Allium cepa* (Cabrera and Rodriguez, 1999; Bakare and Wale-Adeyemo, 2004; Chandra et al., 2005.), *Vicia faba* (Radetski et al., 2004; Sang and Li, 2004), *Drosophila melanogaster* (Siddique et al., 2005) and mouse (Tewari et al., 2005; Sang and Li, 2005; Bakare et al., 2005) are in concert with our study. Chromosome aberration is a major heritable genetic damage and is associated with cancer predisposition. It has been established that there exists a frequent occurrence of chromosomal aberrations in cancer cells. Thus, it may be plausible to suggest that landfill leachate may induce chromosome breakages that may consequently lead to cancer. Evidences from epidemiological studies of populations living near landfill sites are in support of this assertion (Fielder et al., 2000; Vrijheid, 2000; Elliot et al., 2001; Palmer et al., 2005).

The results of this study indicate that MSW leachates contain constituents, which can produce chromosome aberrations in rat bone marrow cells. This suggests a potential risk to humans that may come in contact with this mixture of contaminants, since chromosome is universal to all living organisms.

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