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Genotoxicity of leachates from a rural waste dump using two bioassays

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ABSTRACT: The potential mutagenic effect of raw and simulated leachate from "Odo-Oba" refuse dump site in South-West Nigeria was evaluated. Two genetic bioassays: The *Allium cepa* assay and the murine sperm-head morphology test were utilized. In the *A. cepa* assay, roots of *Allium* at about 2 – 3 cm long were treated with 1%, 2.5%, 5%, 10% and 25% concentrations of the raw and simulated leachate samples for 24 hours. Different types of chromosomal aberrations were induced in the root meristems and this was significant at $P < 0.05$ level. There was also reduction in the number of cells dividing at the tested concentrations. In the murine sperm-head morphology test, five different concentrations of 1%, 2.5%, 5%, 10% and 25% of the raw leachates were administered to groups of 5 male mice. The sperm of the mice from the cauda epididymis were examined 5 weeks after treatment. A significant dose-responsive mutagenic effect was observed, and this was concetration-dependent with the highest concetration inducing the largest number of sperm-head deformity. The physico-chemical analysis of the leachate samples show substances with known mutagenic and carcinogenic effects. Results obtained may be useful in the assessment of the hazardous effects of the chemicals in the leachate from waste dump sites.

Key Words: Waste dumps; Leachates; Mutagenicity; Genotoxicity.

Introduction

The production of solid wastes in the world varies from 0.5 to 4.5 kg person^{-1} day^{-1}, which constitute an important management problem. There are three major ways of managing these wastes: landfill, incineration and production of compost (Cabrera *et al*., 1999). In Nigeria, land filling and or open dumping of wastes is very common (Bakare *et al*. 1999a).

One of the impacts of greatest concern of an existing landfill or dumpsite is the pollution of surface and or ground waters by its leachate. It is known that many potential mutagens are present in the garbage and others are formed during their degradation. All these chemical and biological agents may go into the leachate to pollute the environment. Leachate could be natural or stimulated. Natural raw leachate is one originating from full-scale existing landfill operation. Stimulated ones are those existing from test lysimeters containing a composite of typical solid waste materials and operated under a carefully controlled set of temperature and precipitation rates to stimulate actual landfill condition (Cameron and Koch, 1980).

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Small amount of landfill leachate can pollute large volume of groundwater, rendering them unusable for domestic and many other purposes (Lee and Jones-Lee, 1996). The long range effects of these chemicals carried in the water table and accumulated in the aquifers are the worries of the generations to come.

Human exposure to industrial wastes have led to health effects ranging from headaches, lung and skin irritations and nausea to serious impairments of liver and neurological function (Buffler *et al*., 1985). Evidence of genotoxic health effects, such as cancer, birth defects and reproductive anomalies, have also been cited (Houk, 1992). Increased incidences of bladder and gastrointestinal cancers (Griffith *et al*., 1989), reproductive abnormalities (Vianna and Polan, 1984; Goldman *et al*., 1985; Paigen and Goldman, 1987) and congenital malformations (Goldman *et al*., 1985) have been found in populations living near hazardous waste dump sites.

Although reports on the mutagenicity of leachate from domestic, municipal, industrial and co-disposed solid waste dumps have been reported (USEPA, 1980; Kamiya *et al*., 1989; Omura *et al.*, 1991, 1992; Bakare *et al*., 199a, 199b, 2000), genotoxic tests on leachates from rural refuse dumps are so far very rare. Due to this and the high pollution potential of refuse leachate, the potential genotoxic effect of raw and stimulated leachates from a rural refuse dump in South-Western Nigeria was evaluated using two short-term bioassays.

The dump site, located at "Odo-Oba", a village near Ogbomoso, Oyo State, Nigeria (Fig. 1) is a major refuse dump site with domestic and market wastes as well as waste from the local "garri" (Cassava flakes) processing centre located near the dump site. This dump site also shares boundary with a portion of the "Oba" river which serves the community for domestic and commercial purposes. The dump site neither has a membrane liner at the bottom, a layer of compacted soil with the desired hydraulic conductivity nor a run-off control system, thus its leachate contaminates the nearby river directly.

The bioassays used are the *Allium cepa* test (Fiskesjo, 1985; Bakare, *et al*., 199b) and the murine sperm-head morphology test (Wyrobek *et al*., 1983). Both assays are simple, inexpensive and the methods are relatively rapid for screening chemicals for their ability to induce genotoxicity.

Materials and Methods

Leachates Sampling

Raw Leachate

Raw leachate samples were collected from ten different spots where leachate seeps out of the dump site. Samples were collected on 21 June, and 11 and 19 July, 1998. These samples were mixed together, filtered to remove debris, P^H taken and stored at 4°C.

Simulated Leachate

For leachate simulation, solid wastes were collected from this dump site thrice in December, 1998. Simulation was done using the American Society for Testing and Material (ASTM) method An extraction procedure (Perket *et al*., 1982), with slight modification. 0.7 kg of the waste from an initial sample of 2 kg were shredded and packed in a 2L glass flasks. A volume of distilled water dour times the sample weight was added. The waste mixture was mixed thoroughly and allowed to stand for 48 hr. at room temperature. Continuous stirring was done manually at regular intervals of 2 hr. At 48 hr the solid and liquid portions were separated and P^H of the liquid portions recorded and stored at 4°C.

The physico-chemical properties of the leachate samples were determined in accordance with the standard method (APHA), 1985). The metals were analysed with atomic absorption spectrophotometer.

A. cepa assay

The details were described by Fiskesjo (1985) and Bakare *et al*., (1999b). The roots of *A .cepa* were generated by suspending the bulbs over 50 ml beakers containing distilled water for 48 hr. When the roots were about 2-3 cm long they were treated with 1%, 2.5%, 5%, 10% and 25% concentrations of the raw and simulated leachates for 24 hr. Onion roots generated in distilled water served as the negative control.

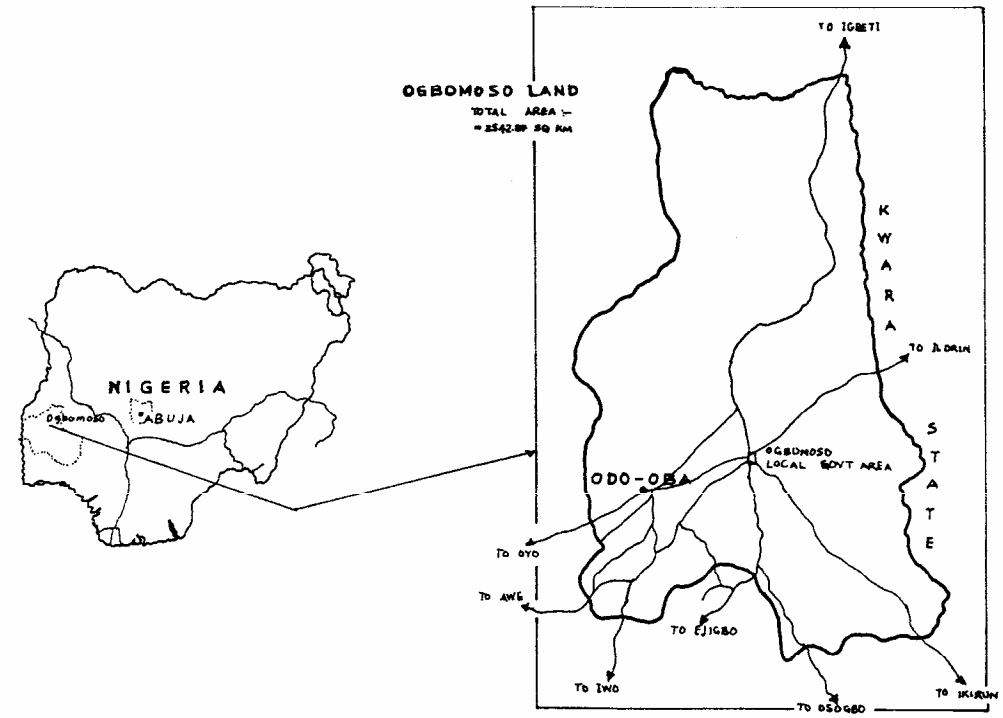

Fig. 1: Geographic location of the village containing the sampling site.

Roots from treated bulbs and those from the control were harvested and fixed in ethanol:acetic acid (3:1, v/v) for 24 hr. After fixing, the roots were hydrolyzed with 1N Hcl at 65°C for 3 min. Slides were prepared with the hydrolyzed roots using the acetocarmine stain squash method. The slides were then observed under the microscope at X1000 magnification and the cells scored for chromosomal aberrations. Seven slides were prepared out of which five were scored (at 1000 cells/slide) for each concentration and control. The data were expressed in terms of mitotic index, number and percentages of cytological aberrations.

Murine Sperm-head Morphological test

Only the raw leachate was evaluated with this assay. The test was carried out with UCH F1 mice obtained from the postgraduate Institute of Medical Research and Training (PIMRAT), University College Hospital, Ibadan, Nigeria. The animals were housed in plastic cages in a pathogen free environment (25-27°C), and were allowed food (Ladokun pelleted mouse cubes) and water *ad libitum*.

Freshly prepared diluted raw leachate at 1%, 2.5%, 5%, 105 and 25% concentrations were used in the experiment. Groups of 5 male mice (24-31g), 12-14 weeks of age, received intraperitoneally (IP) 0.5 ml of the leachate dilutions for 5 consecutive days. The control mice received (IP) o.5ml of normal saline for the same number of days. The animals were exposed for 5 weeks from the first injection after which they were sacrificed by cervical dislocation and sperm obtained from the cauda epididymides. The sperm suspension were fixed and stained with 1% eosin Y.45 minutes later smears were prepared on slide, air-dried and cooled for subsequent microscopic examination at 1000x magnification. 600 sperm/mouse were scored for abnormal morphologies using the categories of Wyrobek and Bruce (1975) and Mosuro (1991).

Statistical Analysis

The statistical significance of differences from control values was determined by means of the student's t-test at the 0.05 and 0.10 level.

Result and Discussion

Tables 1 and 2 show the result of the genotoxicity of the leachate samples in *A. cepa*. The mitotic indices were lower in all the treatments when compared with the control. The lowest value of 3.58 (for the raw sample) and 4.52 (for the simulated sample) was obtained at the 25% concentration; while the highest value of 7.94 (for the raw sample) and 9.02 (for the simulated sample) was obtained at the 1% and 2.5% concentrations respectively. These findings show that the leachates depressed cell division at the tested concentrations, with the lowest number of dividing cells at the highest concentration of bot samples.

Chromosomal aberration study was carried out at all concentrations. At the 1% concentration of the simulated sample there was no aberrant cell. At other concentrations for both samples, aberrant cells were observed. This was significant at $P < 0.05$ for the raw leachate, and at $P < 0.10$ for the simulated leachate. The observed aberrations include sticky chromosomes at metaphase and anaphase, vagrant chromosomes, chromosome bridge at anaphase, mitotic spindle disturbance at metaphase and anaphase, and chromosome condensation. However, there were more aberrant cells with the raw leachate sample.

Figure 2 presents the effect of the raw leachate on mouse sperm-head morphology after 5 weeks exposure. There was a concentration-dependent increase in the incidence of sperm-head abnormalities, and this was statistically significant at $P < 0.05$ level. Figure 3 shows the types and frequency of induced abnormal sperm.

Results of the *A. cepa* test provide cytological evidence that rural refuse leachate can be mutagenic at the chromosome level. The two samples contained chemicals (Table 3) that probably interacted with **DNA** and thus induced mutation in *A. cepa.*

An increase of sperm abnormalities possibly consequent on a mutagenic effect was detected at 5 weeks following treatment with the raw leachate. Spermatozoa observed at these times were presumably exposed to the leachate while they were spermatids and primary spermatocytes. This is suggestive of the fact that the leachate constituents had been able to interfere with the integrity of **DNA** in the sex cells.

Table 1: Observed chromosomal aberrations during mitosis in *A.* cepa treated with the raw leachate.

Concentration (%)	Cells in division	Mitotic Index	Cytological aberrations				Total aberrant cells	Percentage aberration
			Anaphase bridge	Sticky chromosome	Disturbed spindle	Chromosome condensation		
1	397	7.94	–	2	5	–	7*	1.80
2.5	365	7.30	2	1	10	3	16*	4.40
5	241	4.82	7	4	15	5	31*	12.90
10	238	4.76	5	6	42	12	65*	27.30
25	179	3.58	9	16	23	7	55*	30.70
Control	653	13.06						

*Significantly different from the control at $P < 0.05$

Table 2: Observed chromosomal aberrations during mitosis in *A.* cepa treated with the simulated leachate.

Concentration (%)	Cells in division	Mitotic Index	Cytological aberrations					Total aberrant cells	Percentage aberration
			Anaphase bridge	Sticky chromosome	Disturbed spindle	Vagrant chromosome	Chromosome condensation		
1	423	8.46	–	–	–	–	–	–	–
2.5	451	9.02	1	1	2	–	–	4*	0.90
5	372	7.44	3	1	12	5	5	26*	7.00
10	339	6.78	2	5	9	–	2	18*	5.30
25	226	4.52	6	3	28	2	14	53*	23.50
Control	653	13.06							

*Significantly different from the control at $P < 0.10$.

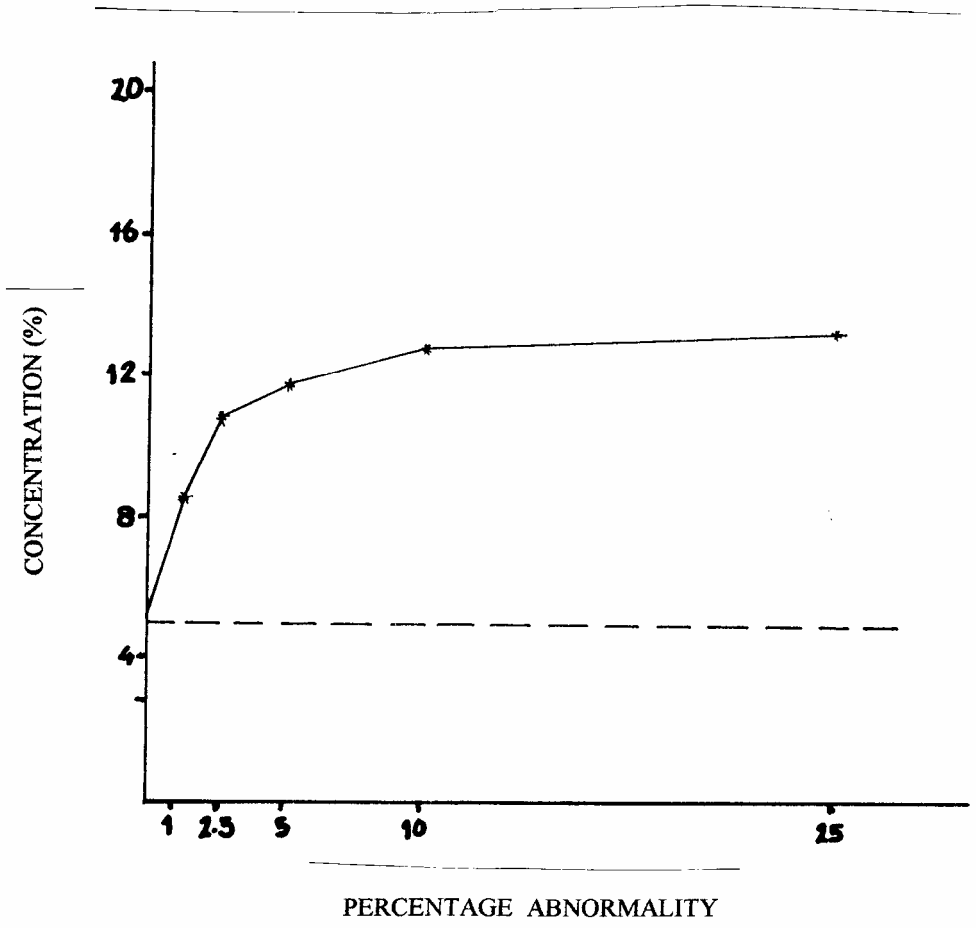

Fig. 2: Concentration-response relationship for the raw leachate induced morphologically abnormal sperm in mice. (The horizontal line represents the average frequency observed in the control animals.

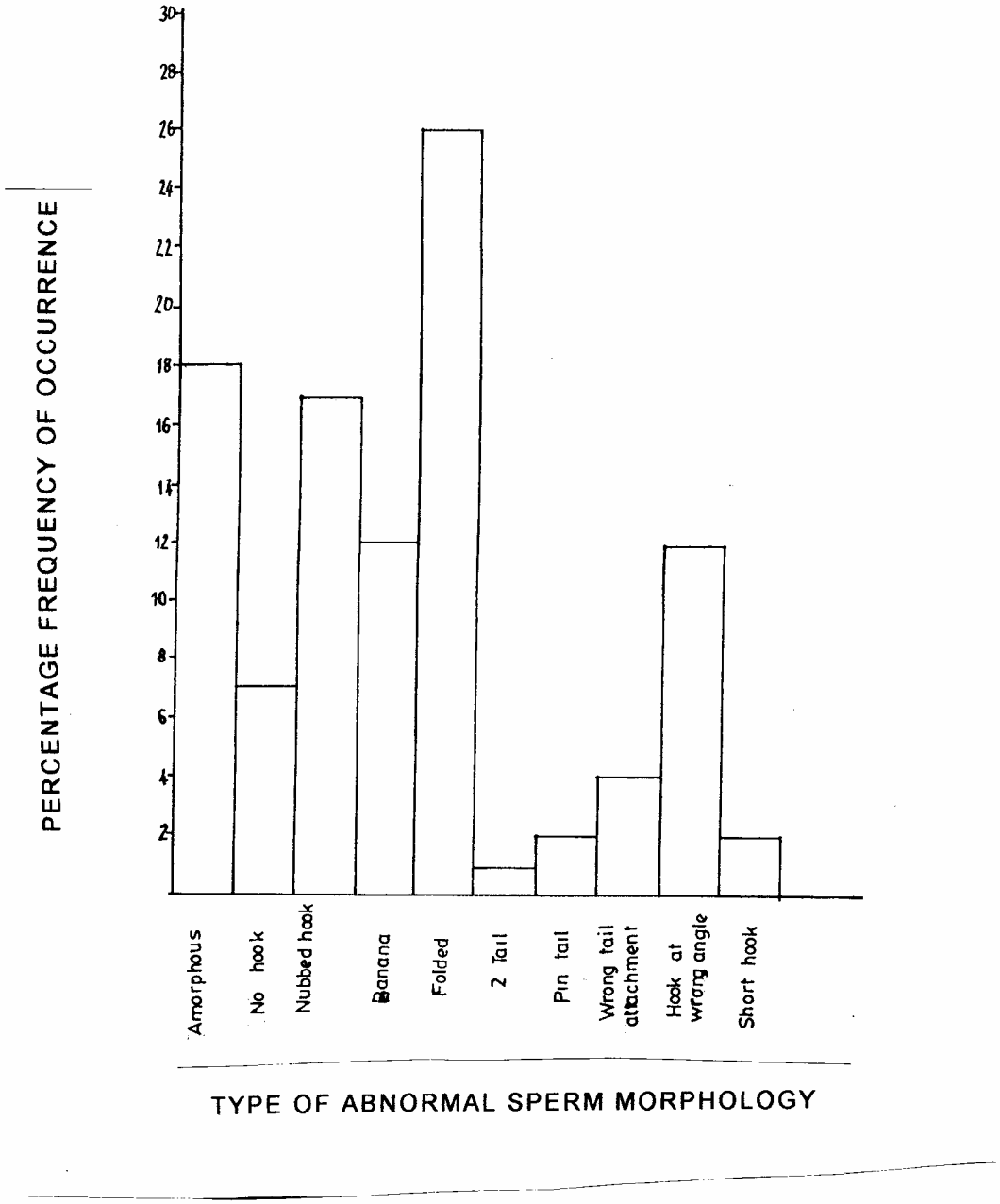

Fig. 3: Types and frequency of abnormal sperm morphology induced by the raw leachate in mice after 5-week exposure

Table 3: Physico-chemical parameters of the leachates from the dump site.

Parameters	Raw leachate	Simulated leachate
pH	6.25	6.14
Colour	Dark brown	Pale Black
Total Solid	5072.17	167.34
Total dissolved solid	3400	100
Total hardness	259.36	48.13
Chloride	30	6
Copper	0.0935	0.0000311
Lead	0.0588	0.000176
Iron	8.321	0.741
Cadmium	0.0385	0.00769
Silver	0.0163	0.0000325
Manganese	0.253	0.0421
Nickel	0.249	0.000497.

*All values are in mg/l except pH

The type of chemical interaction that produced the observed genotoxic effects could be individual, synergistic or antagonistic. However, the synergistic and antagonistic effects are inevitable. Some of the chemicals present in the tested leachates (Table 3) are known mutagens and carcinogens.

Pb was reported to be a strong clasogen which breaks chromosomes in chinese hamster ovary cells (Bauchinger and Schmid, 1972), in bone marrow erythrocytes of rats (Tachi *et al*., 1985) and in cells of *A. cepa* (Lerda, 1992). Ni produced highly selective damage to heterochromatin in chinese hamster genome (Costa *et al*., 1994), while Cd (Elinder and Jarup, 1996), Pb (Fowler *et al*., 1994) and Ni (Haugen *et al*., 1994) have been reported to induce a variety of tumors in animal studies. In rodents, Pb (Kharchenko and Andreera., 1987) and Cd (Dalton *et al*., 1996) are known to inhibit spermatogenesis. It may be pertinent to add that organisms that are predisposed to cancer and genetic birth defects, and that are exposed to leachate from waste dumps may be at a very high risk of developing the disease.

This study further confirms the mutagenic capacity of landfill leachates. Previously, Bakare *et al*., (1999a, 199b, 2000) reported that clastogenic, mutagenic and cytotoxic effects of raw and simulated leachates from institutional, domestic and industrial waste dump sites in South-West Nigeria. Similarly, Cabrera *et al*., (1999) and Cabrera and Rodriguez (1999) reported the geotoxic effect of landfill leachates and extracts from the compost of the organic and the total municipal garbage in *Tradescantia* and *A. cepa*.

The genomic disruptions detected represents damages to **DNA** ranging from point mutations to chromosomal mutations. The consequence of this to the present and future generations of the communities in the vicinity of the dump site could be grievous. Also, other communities that may be exposed to leachate-contaminated waters are not spared of this genetic risk. The relevance of this kind of study cannot be overlooked as results from genetic bioassay are relevant to human health because the toxicological target is DNA, which exists in all cellular forms (Houk, 1992).

This study has shown that refuse leachate contain constituents that are toxic and capable of inducing mutation in *A. cepa* and albino mice. Results of this kind may be informative in environmental waste management and for the assessment of the hazardous effects of the chemicals from solid waste dump sites.

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