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Short communication

THE POTENTIAL MUTAGENIC EFFECT OF THE LEACHATES OF RURAL SOLID WASTE LANDFILL ON *ALLIUM CEPA* (L.)

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ABSTRACT: The potential mutagenic effect of raw and simulated leachate from a rural refuse dump site at "*Odo Oba*", in South-West Nigeria, on *Allium cepa* was evaluated. Roots of *Allium* at about 2–3 cm long were treated with 1%, 2.5%, 5%, 10% and 25% concentrations of the leachate samples for 24 hr. These were then used to prepare slides for the observation of chromosomal aberrations and frequency of mitotic division. Different types of chromosomal aberrations were induced and this was significant at $P < 0.05$ level at all doses tested except at 1% concentration of the simulated leachate. There was also reduction in the number of cells dividing at the tested concentrations when compared with the control. The observed effects may be provoked by genotoxic chemicals found in the leachate samples. This finding may be useful in the practical aspects of waste management and for the assessment of the hazardous effects of the chemicals in the leachate from solid waste dumpsites.

Key words/phrases: ***Allium cepa*, chromosomal aberration, mitotic index, mutagenicity, rural refuse leachate**

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, landfilling and or open dumping of wastes is very common (Bakare *et al.*, 1999a).

One serious concern of an existing landfill is the pollution of surface and or ground waters by landfill leachate. It is known that many potential mutagens are present in the garbage and others are formed during their degradation. Such chemicals and biological agents may go into the leachate to pollute the environment. Small amounts 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 worries for generations to come.

Although studies 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.*, 1999a, 1999b, 2000) genotoxicity tests on leachate from rural refuse dumps are few. Due to this and the high pollution potential of refuse leachate, the potential genotoxic effect of raw and simulated leachates from a rural refuse dump in South-West 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 "*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.

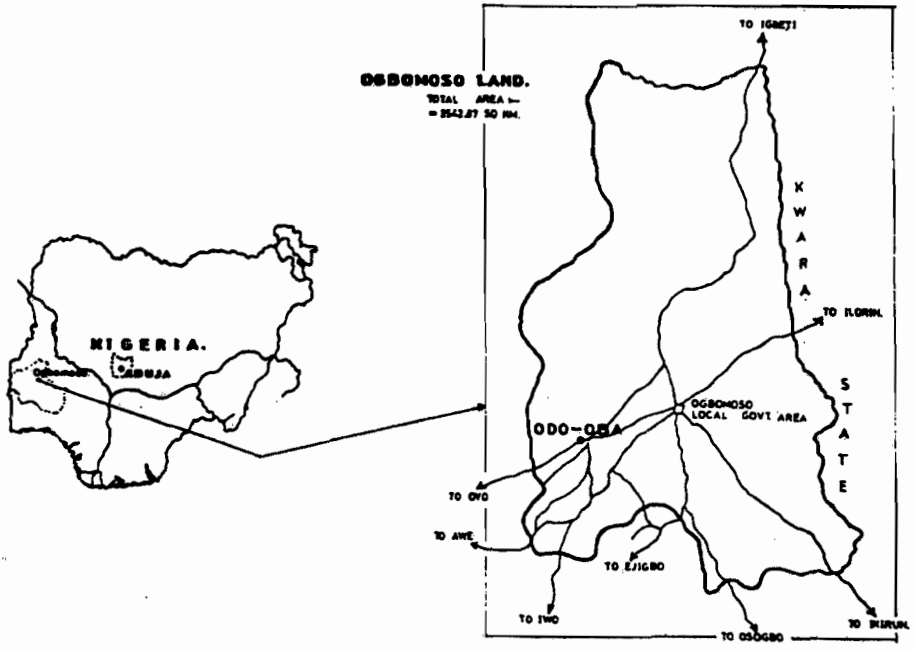

Fig. 1. Geographic location of the village containing the sampling site.

The results from the first assay, mouse sperm-head shape abnormality test, has been reported (Bakare, 1999). In the current study, the *Allium cepa* chromosome aberration assay, a well suited, short-term test system, has been used to determine the potential mutagenic and clastogenic effects of the leachates. Plant bioassays have been validated and particularly, the use of *A. cepa* for the determination of the mutagenicity of landfill leachates (Fiskesjo, 1997; Cabrera and Rodriguez, 1999; Bakare *et al.*, 1999b).

MATERIALS AND METHODS

Leachate sampling

Raw leachate samples were collected from ten different spots where leachate seeps out of the dump site. Samples were collected on 21 June, 11 and 19 July, and 26 August, 1998. These samples were mixed together, filtered to remove debris, pH taken and stored at 4° C.

For leachate simulation, solid wastes were collected from this dump site thrice and in December, 1998 during dry season. Simulation was done using the extraction procedure of the American Society for Testing and Material (ASTM) method (Perket *et al.*, 1982), with slight modification. From an initial sample of 2 kg 0.7 kg of the waste were shredded and packed in a 2 L glass 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 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 pH of the liquid portions recorded and stored at 4° C.

The physico-chemical properties of the leachate samples were determined following APHA (1985). The metals were analysed with atomic absorption spectrophotometer.

A. cepa *assay*

Common onion (*A. cepa*, 2n = 16) is a monocotyledonous plant that belongs to the family *Amaryllidaceae*. Also, it is a genetic population. Variations in the materials was compensated for by the use of root materials from a number of onions. The details of the assay were described by Fiskesjo (1997) 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 raw and simulated leachates for 24 hr. Onion roots generated in distilled water served as the negative control. 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 NCI at 65° C for 3 min.

Two root tips were then squashed on each slide, stained with acetocarmine for 10 minutes and coverslips were carefully lowered on them to exclude air bubbles. The coverslips were sealed on the slides with clear fingernail polish as suggested by Grant (1982). The slides were then observed under microscope at X1000 magnification and the cells were scored for chromosomal aberrations. Five slides were prepared for each concentration and 1000 cells were scored from each treatment and control. The data were expressed in terms of mitotic index, number and percentages of chromosomal aberrations. The students' t-test at the 0.05 level of significance was used to test the level of significance of the data.

RESULTS AND DISCUSSION

Tables 1 and 2 show the result of the genotoxicity of the leachate samples in *A. cepa*. The mitotic indices were found to be lower in all 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 concentrations for both samples.

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

Concentration (%)	Cells in division	Mitotic index	Chromosomal aberrations				Total aberrant cells	% aberration based on cells in division
			AB	SC	DS	CS		
1.0	397	7.94	-	2	5	-	7*	1.76
2.5	365	7.30	2	1	10	3	16*	4.38
5.0	241	4.28	7	4	15	5	31*	12.86
10.0	238	4.76	5	6	42	12	65*	27.31
25.0	179	3.58	9	16	23	7	55*	30.72
Control	453	9.06						

* Statistically different from the control at the 0.05 level (Students' t-test). AB, Anaphase bridge; SC, Sticky chromosomes; DS , Disturbed spindle; CS, Chromosome condensation.

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

Concentration (%)	Cells in division	Mitotic index	Chromosomal aberrations					Total aberrant cells	% aberration based on cells in division
			AB	SC	DS	VC	CS		
1	423	8.46	-	-	-	-	-		
2.5	451	9.02	1	1	2	-	-	4*	0.89
5	372	7.44	3	1	12	5	5	26*	6.99
10	339	6.78	2	5	9	-	2	18*	5.31
25	226	4.52	6	3	28	2	14	53*	23.45
Control	453	9.06							

* Statistically different from the control at the 0.05 level (Students' t-test). AB, Anaphase bridge; SC, Sticky chromosomes; DS, Disturbed spindle; VC, Vagrant chromosome; CS, Chromosomes condensation.

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 $P<0.05$. The observed aberrations included sticky chromosomes at metaphase and anaphase, vagrant chromosomes, chromosome bridge at anaphase, mitotic spindle disturbance at metaphase and anaphase and chromosome condensation.

These findings 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*. Though only few of the possible genotoxic compounds putatively present in the leachate samples were analyzed, landfill leachate is known to contain different types of organic and inorganic contaminants, many of which are known to be toxic and capable of inducing mutation in *A. cepa* (Cabrera and Rodriguez, 1999; Bakare *et al.*, 2000). Their potential to cause cancer in man and hazards to public health and ground water quality are also known (Jones - Lee and Lee, 1993). Pb was reported to be a strong clastogen which breaks chromosomes in Chinese hamster ovary cells (Bauchinger and Schmid, 1972), in bone marrow erythrocytes of rat (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 tumours in animal studies.

Table 3. Physico-Chemical parameters of leachates.

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 mgl^{-1} except pH.

The type of chemical interaction that produced the observed effects could be individual, synergistic or antagonistic. However, the synergistic and antagonistic effects are inevitable. The mutagenic effect of the simulated leachate was less than that of the raw sample. This may probably be due to the fact that the sample was obtained from an extraction procedure; a short-term test in which the time may not be enough for complete degradation and dissolution of the solid waste components. Unlike the raw leachate that was obtained from the open landfill with waste at different stages and degrees of decomposition. Thus, the quantity and concentration of genotoxic ingredients in the raw leachate was more than in the simulated leachate.

The results from this study corroborates previous reports from animal studies (Bakare, 1999) where the raw leachate from the dumpsite induced abnormally shaped sperm-head in albino mice. This study further confirms the mutagenic capacity of landfill leachates. Previously, Bakare *et al.* (1999a, 1999b, 2000) reported the clastogenic, mutagenic and cytotoxic effects of raw and simulated leachates from institutional, domestic, municipal and industrial waste dumpsites in Southwest Nigeria. Similarly, Cabrera *et al.* (1999) and Cabrera and Rodriguez (1999) reported the genotoxic effect of landfill leachates and extracts from the compost of the organic and total municipal garbage in *Tradescantia* and *A. cepa.*

The genomic disruptions detected herein and in previous studies represent damages to the 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 dumpsite could be grievous. This is because the dumpsite is not properly sited and is poorly constructed such

that leachates diffuse into the nearby river, which serves the community for domestic and commercial purposes. Also, other communities that may be exposed to leachate-contaminated waters are not spared of this genetic risk. This is attested to by the report of Hens *et al.* (1988) wherein water fraction from a well near a waste deposit site induced chromosomal aberrations in human lymphocytes. Gonsebatt *et al.* (1995) also reported significantly high frequencies of chromatid and chromosomal deletions in individuals working at a landfill for hazardous waste disposal. Increased incidences of bladder and gastrointestinal cancers (Griffith *et al.*, 1989), reproductive abnormalities and congenital malformations (Goldman *et al., 1985)* have been found in populations living near hazardous waste dump sites. Considering the high correlation between mutagenicity and carcinogenicity, it may be pertinent to add that organisms that are predisposed to cancer and that are exposed to landfill leachate may be at a very high risk of developing the disease. This is because of the possibility that leachate contains chemicals possessing initiation, promotion, and progression properties working in concert to bring about neoplastic transformations.

This study has shown that rural refuse leachate is mutagenic in *A. cepa*. This finding, though with plant system, 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). Results obtained may be informative in environmental waste management and for the assessment of the hazardous effects of the chemicals from solid waste dumpsites.

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