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Research Article

Genotoxicity Evaluation of Landfill Solid Leachates from Waste Dumpsite in Lagos State, Nigeria Using the *Allium cepa* Assay

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ABSTRACT

The management of municipal landfill's leachate is considered one of the most significant environmental issues. Potential mutagenic and genotoxic effects of solid waste leachates (SWL) were investigated using the *Allium cepa* root meristematic cells assay technique. In *Allium* root growth inhibition test, experimental onion bulbs were cultivated in various concentrations of the SWL and distilled water was used as a negative control. The root tips from the treated bulb were processedby orcein squash technique after 72 h. The mean lengths of root bundles were obtained andeffective concentration (EC) values calculated. Some of the physicochemical properties of the WL were also determined. The *A. cepa* assay was carried out at concentrations of 0.05, 0.1, 2.0, 5, 10, 20, 50 and 100% of the WL. There was a statistically different ($p < 0.05$), concentration-dependent inhibition of onion root growth and mitotic index, and induction of chromosomal aberrations in the onion test. The results of the physicochemical analysis revealed that the concentrations of some parameters were above the maximum permissible limit set by the world health organization (WHO) and could partly be correlated with the toxicity of wastewater. The landfill leachates pose a risk to human health and the environment in general. Hence, ecotoxicity/genotoxicity assays would be useful in leachate risk assessment when coupled with physicochemical analysis.

Keywords:Genotoxicity, chromosomal aberration, solid waste leachates, mitotic index, Allium cepa.

Introduction

The expansion of industrial activities population growth and lifestyle changes have led to the exponential generation of municipal solid wastes (MSW) over the years [1]. Sanitary landfill is a process in the solid waste management system and it can be considered as a method of refuse disposal on land without constituting nuisances or hazards to public health and safety [2]. In Nigeria, 25 million tonnes of municipal solid waste are generated annually and the waste generation rates ranged from 0.66kg/cap/d in urban areas to 0.44kg/cap/d in rural areas as opposed to 0.7-1.8kg/cap/day in developed countries [3]. However, municipal solid wastes disposal in the landfill is the most common option in most countries [4]. Landfill leachate is produced by excess rainwater and seepage water, infiltrating through the waste layers in the landfill [5]. There is a danger of groundwater contamination by landfill leachate containing inorganic ions or organic components derived from the original refuse as organics produced by microbial decomposition [6]. Furthermore, it has direct health effects, the elusive menace of pollutants lies in the fact that they may be mutagenic or toxic and lead to several human diseases like cancer, atherosclerosis, cardiovascular diseases and premature

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aging [7]. Several studies have established the existence of genotoxic activity in wastewater extracts of both industrial and urban origin [7, 8, 9, 10]. Merely little reports [11, 12, 13] concentrated on the genotoxicity evaluation of leachates from solid industrial/domestic wastes and landfills. According to Bakare et al. [14], cases of water pollution by solid wastes in Nigeria are very common and this has made many surfaces and groundwater sources unsuitable for drinking and for other purposes. The *Allium cepa* test appears to have some benefits which Levan [15] advocated for genotoxicity evaluation using the onion bulbs (*Allium cepa*) and it has been used on wastewater from various other studies [16, 17, 18 19]. Fiskesjo [20] recommended the *Allium cepa* test as a reference method in environmental monitoring and toxicity screening of wastewater. This study aims to evaluate the genotoxic and mutagenic potential of solid waste leachates obtained from Olusosun landfill, Ojota, Lagos-Nigeria, using *Allium cepa*.

Material and Methods

Sampling site, collection of dilution of solid waste leachates

Olusosun dumpsite is a major landfill in Lagos State Nigeria. Samples of solid waste leachates were collected from discharge points in sterile 5-litre bottles and used for physicochemical and genotoxicity assays. Figure 1 shows the location of the dumpsite, in Ojota area of Lagos State. Dilutions of leachate effluents were made using distilled water as diluent to obtain graded concentrations of the leachate for the genotoxic study.

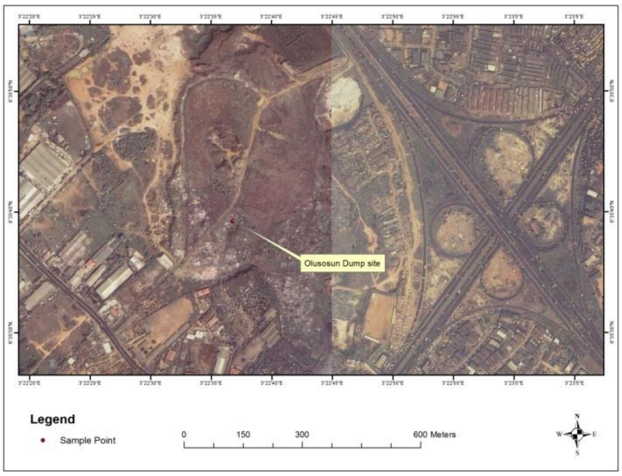


Figure 1. Satellite image showing Olusosun dumpsite at Ojota, Lagos, Nigeria

Data analysis Determination of physico-chemical properties and heavy metal analysis

Chemical analyses included total dissolved solids (TDS), total alkalinity (TA), chemical oxygen demand (COD), biochemical oxygen demand (BOD), nitrate (NO_3), phosphates (PO_4), sulphate (SO_4) and cyanide (CN) were determined according to standard analytical methods (21) while the electrical conductivity (EC), total solids (TS), hardness and chloride (Cl) were determined by method described by Ademoroti (21). Fourteen metals (including ten heavy metals) namely aluminum (Al), silver (Ag), copper (Cu), chromium (Cr), cadmium (Cd), lead (Pb), manganese (Mn), iron (Fe), zinc (Zn) and nickel (Ni) were determined in the wastewater samples according to standard analytical methods (21). Wastewater pH was measured electrometrically with Orion 3 Star bench top pH meter (Thermo-scientific, USA) [22].

Sampling Source and treatment of Allium cepa bulbs

Onion bulbs (*A. cepa*, 2n = 16) onion bulbs, of an average size of 15-20 mm in diameter, were purchased locally in Lagos State, Nigeria. They were dried for about six weeks and the dried roots present at the base of the onion bulbs were carefully shaved off with a new razor blade in order to expose the fresh meristematic tissues. The root length (cm) was measured with a graduated meter rule for three consecutive days and the mean root length of the four bulbs for each test sample concentration was determined and recorded.

Allium cepa assay

The *Allium* test for macroscopic as well as microscopic evaluations adopted in this study has previously described by Fiskesjo [23] and Bakare and Wale-Adeyemo [24]. The outer scales of the onion bulbs and brownish bottom plate were carefully removed thereby leaving the ring of fresh root primordia intact. The peeled bulbs were transferred into distilled water during the cleaning procedure to prevent the primordia from drying. This followed with the bulbs being exposed directly in 0, 0.05, 0.1, 0.5, 1.0, 5, 10, 25, 50 and 100% concentrations (v/v, effluent/distilled water), of each of the test sample (solid waste leachate). Eight onion bulbs were set up in each series for each sample, out of which the best four with good root growth were selected for analysis of root growth inhibition. Distilled water was used as negative control.

The experiment was set up in the dark at 28 °C for 72 h. Test liquids were changed daily. Photographs of test materials were taken with *Nikon* Digital Camera D80 (Nikon Corp., Japan) and special note was taken of the change in colour of root tip and morphological changes. After 72 h, one root tip was removed from each bulb, fixed in ethanol: glacial acetic acid (3:1, v/v) and hydrolyzed with a solution of 1 N HCl at 65°C for 3 min. After staining the tissue, the specimen on the slide was gently covered with a coverslip, allowing the orcein stain to spread evenly over the square parts of the coverslip to eliminate air bubble. The slide with the specimen was then placed in between two folds of the filter paper and using the blunt end of a pen, gentle tapping and pressure were applied around the square area of the cover slip for even squashing of the specimen. Finally, the square edges of the coverslip of the squashed slide were sealed with white transparent nail hardener as suggested by Grant (25) to prevent drying out of the preparation by the heat of the microscope (26). Three slides were prepared for each concentration and control.

After 72 h, mean lengths of root bundles were obtained as described by Fiskesjo (18) and the EC_{50} values at 95 % confidence interval were determined from a plot of root length, % of control against the sample concentrations using the Graphpad prism 5.0 version. The slides were viewed under the microscope to observe mitotic stages and chromosomal aberrations for preparation of photomicrographs. The mitotic index (MI) was calculated as the ratio of the number of dividing cells to the number of observed cells (23). The frequency of aberrant cells (%) was calculated based on the number of aberrant cells per total cells scored at each concentration of each solid waste leachate sample. These are calculated as follows [27]:

$$\text{Mitotic index}=\frac{Number\ of\ dividing\ cells}{Number\ of\ cells\ scored}\times 100$$

$$\text{Frequency (\%) of Aberation}=\frac{Number\ of\ aberant\ cells}{Number\ of\ cells\ scored}\times 100$$

Statistical Analysis

SPSS 20.0 statistical package was used for all data analysis. The results of the root inhibition at each concentration of the effluents were expressed as mean ± standard deviation. The differences between the control and different concentrations of the CWW effluents were compared using one-way analysis of variance (ANOVA) [28]. To assess whether the means of groups are statistically different from each other, the least significant difference (LSD) test was adopted. In all cases, a value of $p<0.05$ was considered significant.

Results and Discussion

Statistical Physicochemical characteristics of solid waste leachate samples

The concentrations of heavy metals were relatively high (Table 1). Of all the elements and ions measured, the average concentration of these metals at the site are 16.04±0.32 for potassium, 39.68±7.41 for sodium, 42.87±3.19 for calcium, 12.16±2.11 for magnesium, 9.32±2.18 for aluminum, 0.814±0.02 for chromium, 0.609±0.03 for cadmium, 0.091±0.008 for manganese, 0.588±0.042 for iron and 0.127±0.0092 for zinc while average concentrations of 19.5±5.29 for nitrate, 94.8±6.35 for sulphate and 395±13.0 for chloride, were obtained while 2.21±0.47 was recorded for phosphate. Interestingly, the cyanide anion, of considerable interest was detectable at a concentration of 3.12±0.61 mgL^{-1}. Reports of Ivanosa [29] and Staykova [30] on the effects of heavy metals and cyanide corroborates with the findings in this study which found the concentrations of metals and cyanide far above the standard limit (as recommended by World health organization (WHO). The results from this study suggest that the chromosome aberration induction in the *Allium cepa* root meristem could be as a result of heavy metals-cyanide interaction in the wastewaters. Yilmaz et al. [31] while working on metals (major, essential to non-essential) composition of the different tissues of three demersal fish species reported that leachates apparently contain vast amounts of organic material in the forms of heavy metals and biodegradable and biorefractory carbon nitrogen. The chromosomal aberrations as a result of genotoxic effects of various physical and chemical agents have been reported [32].

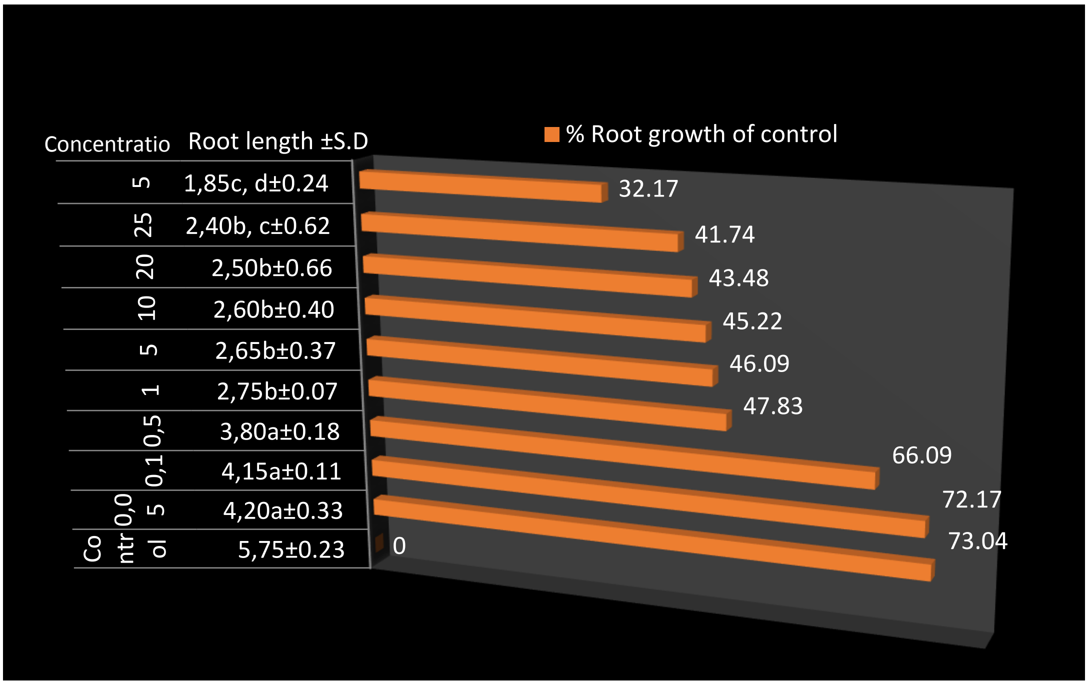
Table 1. Physicochemical properties of solid waste leachate samples collected from Olusosun dumpsite

Parameters	Value/Observation	WHO Standard limit
Colour	Dark	Unobjectionable
pH	5.11±0.071	6.5-9.5
Appearance	Cloudy	Unobjectionable
Odour	Objectionable	Unobjectionable
Conductivity	1336±59.3	1200
Total solid	1260±46.0	1500
Total dissolved solids	630±5.0	2000
Alkalinity	240±15.0	100
Hardness	40±3.0	500
Biochemical oxygen demand	132±14.0	50
Chemical oxygen demand	185±13.0	1000
Potassium(K^+)	16±0.32	-
Sodium (Na^+)	39.68±7.41	-
Calcium (Ca^{2+})	42.87±3.19	-
Magnesium (Mg^{2+})	12.16±2.11	20
Nitrate (NO_3^-)	19.5±5.29	50
Phosphate ($PO_4^2)^-$	2.21±0.47	-
Sulphate (SO_4^{2-})	94.8±6.35	500
Cyanide (CN^{-1})	3.12±0.61	0.07
Chloride (Cl^{-1})	395±13.0	250
Aluminium (Al^{3+})	9.32±2.18	0.2
Silver (Ag^+)	BDL	-
Copper (Cu^{2+})	BDL	2.0
Chromium (Cr^{2+})	0.814±0.02	0.05
Cadmium (Cd^+)	0.609±0.03	0.003
Lead (Pb^{2+})	BDL	0.01
Manganese (Mn^{2+})	0.091±0.008	0.4
Iron (Fe^{2+})	0.588±0.042	3.0
Zinc (Zn^{2+})	0.127±0.0092	3.0
Nickel (Ni^{2+})	0.437±0.049	0.2

All values are means of three replicates and are expressed in mgL^{-1} except colour, odour, conductivity (μScm^{-1}) and pH (no unit). BDL-Below detectable level, ±-Standard deviation

Statistical Macroscopic effects

Morphological properties employed as pointers for genotoxicity are shown in Figure 2. There was no root growth at all in onion bulbs treated with the neat (undiluted) sample, while at 50 % concentration, there was about 32.17 % growth retardation in relation to root lengths in the control. The root growth retardation or inhibition is concentration dependent with an EC_{50} value of 1.2 (Figure 3). The changes in mitotic index (MI) of *Allium cepa* cells are gauges of cytotoxic and genotoxic potential activity of the solid waste leachates. Some investigators have found that the *Allium* test was very useful for evaluating and ranking aquatic toxicities for a number of metals including mercury [32,33]. The cytotoxicity level is a function of the decreased rate of the mitotic index. The macroscopic effect appears to be the most sensitive parameter since any genotoxic effect that shows in a test sample either directly or indirectly is likely to result in inhibition of growth [22].



Means with similar letters are not significantly different from each other at P value = 0.05.
*Values are significantly different from the control at $p < 0.05$ (One-way ANOVA test)

Figure 2. Results of *Allium* root growth inhibition test in solid waste leachate (SWL) samples

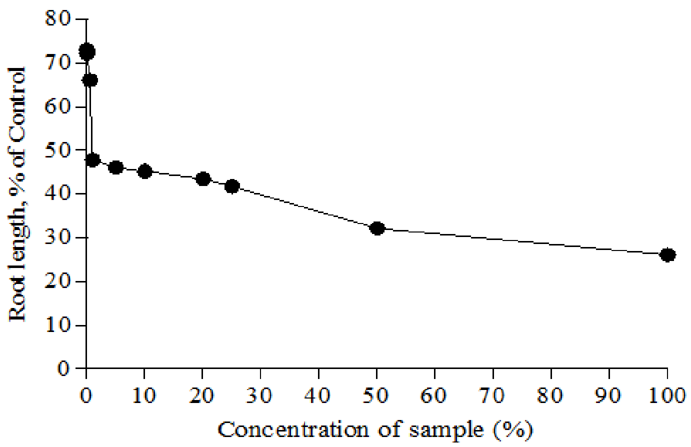


Figure 3. Growth curve of *Allium* roots (in relation to control) after treatment with solid leachate wastes (the curve plotted by Graph pad prism 5.0 version)

Microscopic effects

The cytological effect of the solid waste leachates on cell division and chromosome behaviour of *Allium cepa* are shown in Table 3._No chromosomal aberration in the control which had a mitotic index (MI) value of 10.2. All concentrations induced chromosomal aberrations (statistically significant (p<0.05)). Figure 3 represents the micrograph of the observed chromosome aberrations. As the concentration of the effluents increases, there was a decrease in the mitotic index. Thus the mitotic index could be another endpoint for general toxicity assessment.

Table 2. Cytological effects of treatments with different concentrations of solid waste leachates

Concentration (%)	Mitotic index[1*]	No. of cells[2]	No. of dividing cells	Stickiness	C-Mitosis
Control	10.2±0.20	500	51	0	0
0.05	9.49±030	453	43	8	0
0.1	8.69±0.31	449	39	7	0
0.5	7.87±0.22	445	35	6	0
1.0	7.03±0.16	441	31	4	0
5.0	6.18±0.20	437	27	5	0
10	5.31[a]±0.25	433	23	5	2
20	4.90±0.27	429	21	4	2
25	3.98±0.21	427	17	3	1
50	3.12[b]±0.16	417	13	2	3
100	2.69[b]±0.53	409	11	2	1

Concentration (%)	Vagrants	Fragments	Attached	Laggards	Total Aberant cells	%Aberrant cells
Control	0	0	0	0	0±0.00	0
0.05	5	7	5	0	6.25±1.41*	58.14
0.1	5	6	3	0	5.25±1.63*	53.85
0.5	3	5	4	0	4.50±2.16*	51.43
1.0	5	5	4	0	4.50±2.94*	58.06
5.0	3	3	2	2	3.00±0.82*	55.56
10	2	3	2	1	2.167±2.45*	65.22
20	3	3	1	0	2.60±2.83*	61.91
25	2	2	2	1	1.83±3.56*	64.71
50	1	3	0	0	2.25±3.27	69.23
100	1	1	1	1	1.17±0.82	63.64

[1]Mitotic index was calculated as: (number of dividing cells / number of cell) × 100
[2]Chromosome aberrations were scored on 500 cells/slide
Means with similar letters are not significantly different from each other at P value = 0.05.
*Values are significant different from control at P<0.05 (One-way ANOVA test), ±-Standard deviation

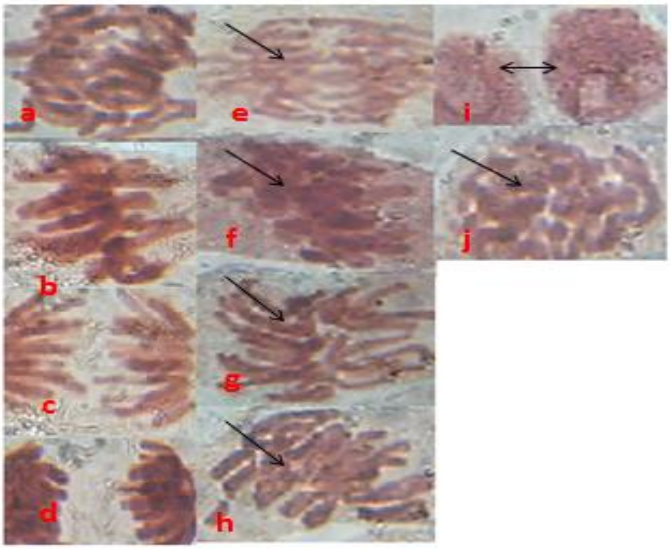


Figure 4. Chromosomal aberrations (arrowed) induced in *Allium cepa* exposed to solid waste leachates. Chromosomal aberrations induced in *Allium cepa* exposed to solid waste leachates (a) prophase control (b) metaphase control (c) anaphase control; (d) telophase control (e) bridged anaphase (f) attached chromosome (g) vagrant chromosome (h) laggard chromosome (i) sticky telophase (j) c-mitosis magnification (x1000).

The solid waste leachates showed a low mitotic index (2.69: control 10.2). These values represent 26.37 % of the control meaning that the leachate samples from the site had sublethal effects. A decrease in mitotic index in onion root meristem was provento be a reliable means for rapid determination of the presence of cytotoxic substances. El-Shahaby et al. [35] reported that the parameter is sensitive enough also to be used for monitoring the pollution levels of slightly polluted water. This validated with the findings of Bakare et al. [12], Bakare et al.[13], Amin [34] and Bakare et al. [14] who recorded lower mitotic index values in treated onion root cells compared with the controls. Microscopic examination allowed for assessing chromosome damage and cell-division disturbances thus providing additional information regarding the severity or mechanism of the toxic effect or of potential mutagenicity. Olorunfemi and Ehwre [37] stressed fact that whenever there were chromosomes aberrations there would be certain growth restrictions [24].

Conclusion

The genotoxicity bioassays should be a useful tool in the evaluation of wastewater toxicity preceding to its discharge into the environment. It should also be used for keeping under surveillance the surface water quality, as it would provide data useful in risk assessment. This study showed the usefulness of combining physicochemical analysis with cytogenetic methods which contribute to environmental risk assessment means is of immense significance.

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