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Full Length Research Paper

Cytogenotoxicity evaluation of two industrial effluents using *Allium cepa* assay

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The cytogenotoxic effects of the industrial effluents from paint (0, 7.2, 18, 36 and 72%) and textile (0, 1.6, 4, 8 and 16%) manufacturing were evaluated using root tip cells of *Allium cepa*. In this study, root length and chromosomal aberration assays were used to determine the 96 h effective concentration (96 h EC_{50}), root growth inhibition, mitotic index and chromosome aberration rate. Based on the 96 h EC_{50}, textile effluent was 4.5 times more toxic than the paint effluent. Analysis of Variance (ANOVA) showed that there was significant difference ($P < 0.05$) in the mean root length of *A. cepa* exposed to different concentrations of the industrial effluents. This indicated that the root growth inhibition was concentration dependent. The mitotic index (MI) decreased with increasing concentrations of paint and textile industrial effluents. The two industrial effluents induced chromosomal aberrations in root tip cells of *A. cepa* with vagrant chromosome, bridges and fragments and sticky chromosomes being most frequently observed. At lower concentrations bridges and fragments were the most common aberration. The suitability of *A. cepa* chromosomal assay as a tool for monitoring the genotoxic effects of industrial effluents and wastewater is discussed.

Key words: Genotoxicity, paint, textile, industrial effluents, *Allium cepa*, mutation, pollution, chromosomal aberration.

INTRODUCTION

Population, poverty and pollution are the three major problems of developing countries (Grover and Kaur, 1999), including Nigeria. With rapid strides in industrialization of some developing countries, the problem is being further accentuated. In Nigeria, the pressure for the improvement of various aspects of living is tremendous and economic development is always on the highest priority of any government. Over the last 20 years and beyond, it has become fashionable for state and local governments in Nigeria to designate certain areas in their major town as industrial estate. Therefore, tax and other concessions are given to lure industrialists to establish industries in their domain. The peculiar characteristic of these Industrial estates is that most of them lack central waste treatment plants and therefore, discharge their wastewater directly or indirectly into water bodies. There is no enforcement of industrial pollution and hazardous wastes laws because industrialization is wholly considered a key indicator of development.

Industrial effluents are a main source of direct and often continuous input of pollutants/toxicants into aquatic ecosystems with long-term implications on ecosystem functioning (Odeigah and Osanyipeju, 1995; Chan et al., 2003; Lah et al., 2004; Smolders et al., 2004). It is well established that pollution lowers the quality of life in various aspects and affects health and life span (Grover and Kaur, 1999) . Besides the direct health effects, the subtle danger of pollutants lies in the fact that they may be mutagenic or toxic and lead to several human afflictions like cancer, atherosclerosis, cardiovascular diseases and premature ageing (Grover and Kaur, 1999).

Many studies (Grover and Kaur, 1999; Lah et al., 2004; Abdel-Migid et al., 2007; Junior et al., 2007) have demonstrated the existence of genotoxic activity in wastewater extracts of both industrial and urban origin. A

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growing interest in genotoxicity caused by environmental pollutants has led to the development of several biological tests for detecting and identifying genotoxicants in the air, water and soil (Grisolia and Cordeiro, 2000). Genotoxicity assays are used specifically to evaluate the genotoxic potential of environmental and industrial effluent samples (Cotelle et al., 1999; Grover and Kaur, 1999; Abdel-Migid et al., 2007).

In Lagos, Nigeria, there are many industries that discharge their effluents into the different water bodies around the metropolis. Of particular interest are the textiles and paint industries which are major industries in Lagos and discharge large amount of effluent among all the industries in the metropolis. There are very few reports on genotoxicity of industrial effluents in Nigeria. Most reports (Odeigah et al., 1997b; Bakare et al., 1999, 2000) concentrated on the genotoxicity evaluation of leachates from solid industrial/domestic wastes and landfills.

Since the usual assays carried out on experimental animals normally require much time and money, alternatives for the determination of adverse effects have been sought. These include bioassays with plants roots. The plant root is extremely useful in biological testing. The root tips are often the first to be exposed to chemical spread in nature, in soil and water. Observation of the root tip system therefore constitutes a rapid and sensitive method for environmental monitoring (Majer et al., 2005). Cytotoxicity and environmental pollution (El- Shahaby et al., 2003) have been assessed by the *in vivo* onion (*Allium cepa*) roots tip cell test, which is known to give similar results to *in vitro* animal cytotoxicity tests (Chauhan et al., 1999; Vicentini et al., 2001; Teixeira et al., 2003). Studies made by many authors pointed out that the *Allium* test is useful for the detection of potentially genotoxic substance in water screening programmes (Rank and Nielson, 1998; Cotelle et al., 1999; Moraes and Jordao, 2001) . *A. cepa* test has been used (Rank and Nielson, 1998; Grover and Kaur, 1999; Junior et al., 2007; Abdel-Migid et al., 2007) to evaluate toxicity and genotoxicity of industrial effluents.

In this study, *A. cepa* root-tip assay was used to evaluate the cytogenotoxicity effects of "treated" industrial effluents discharge from textile and paint industries in Lagos metropolis. The results of this study will be useful to environmental regulatory agencies in developing *A. cepa* assay as a useful tool in detecting the presence and action of mutagenic/clastogenic agents in industrial effluents discharges. Therefore this will set pace for toxicity identification evaluation (TIE) studies of industrial effluents found to be mutagenic.

MATERIALS AND METHODS

Test organism

The common purple onion, *A. cepa* (Stuttgarter Reisen) bulbs (2.5 – 2.8 cm diameter) used for this study was procured from Mile 12 International Market, Lagos, Nigeria.

Test agents

Paint and textile industrial effluents samples were used for this study. The paint industrial effluent was collected from Eagle paint factory in Agidingbi, Ikeja, Lagos, while the textile effluents was collected from Nichemtex factory, Ikorodu, Lagos. Effluents were collected directly from the industrial wastewater discharge pipe of the factories. The effluents were collected in plastic container and stored in the refrigerator at 4°C pending use (Odeigah et al., 1997a). Before each test was carried out, the effluent was equilibrated to room temperature (26 ± 2°C) and diluted with dechlorinated tap water to produce the series of dilutions investigated.

Assay procedure

The assay was carried out using a plastic tube (diameter, 2.3 cm; length, 7.8 cm) in a rack. Dechlorinated tap water was used as control and for dilution of industrial effluents. The yellow shallows and dry bottom plate inside the root primordial of *A. cepa* were carefully removed prior to the test.

Root growth inhibition test

The growth inhibition assay was performed as a 96 h semi- static exposure test (Rank, 2003). *A. cepa* was exposed for 96 h to different dilutions of the industrial effluents as follows:

Paint effluent: 0, 5, 10, 20, 40, 60, 80 and 100%
Textile effluent: 0, 2, 5, 10, 15, 20, 25 and 30%

Each concentration was set-up in 5 replicates. The test solutions were replaced every 24 h with fresh solutions. At the termination of exposure, one onion (out of five) with the poorest growth was discarded and the length of the root bundle was measured for the rest four onions. Two longest roots in each bulb were measured. Growth inhibition was estimated as EC_{50} (the effective concentration of a chemical producing 50% of the total effect).

Genotoxicity assay

The genotoxicity assay was carried out with four effluent concentrations. The concentrations were composed of the EC_{50} as the highest concentration, followed by 50, 25 and 10% of the EC_{50} (Rank, 2003). Five onions were exposed to each concentration for 48 h (Rank, 2003) as follows:

Paint effluent: 0, 7.2, 18, 36 and 72%.
Textile effluent: 0, 1.6, 4, 8 and 16%

As for the growth inhibition test, the test solutions were changed after 24h and at 48h, one root tip (10 mm) from each bulb was harvested for cytological study. The root tips were fixed and macerated in 45% acetic acid-IN HCl (9:1) solution and heated for 5 min at 50°C (Odeigah et al., 1997b).

Subsequently, the roots were placed on a slide and the terminal root tips (1 - 2 mm) were cut off and used for further preparation. The rest of the material was removed from the slide and the excess liquid was sucked up with a piece of blotting paper. Two drops of fresh filtrated 2% orcein solution was added and mixed with the root tips by stirring and knocking with a blunt stick of stainless steel. A cover slip was placed on the root cells and allowed to absorb stain for 5 - 10 min afterwards, the cells were squashed by placing layers of blotting paper on the cover slip and pressing slightly down with the thumb. The cover slip was fixed carefully to the slide with nail cortex.

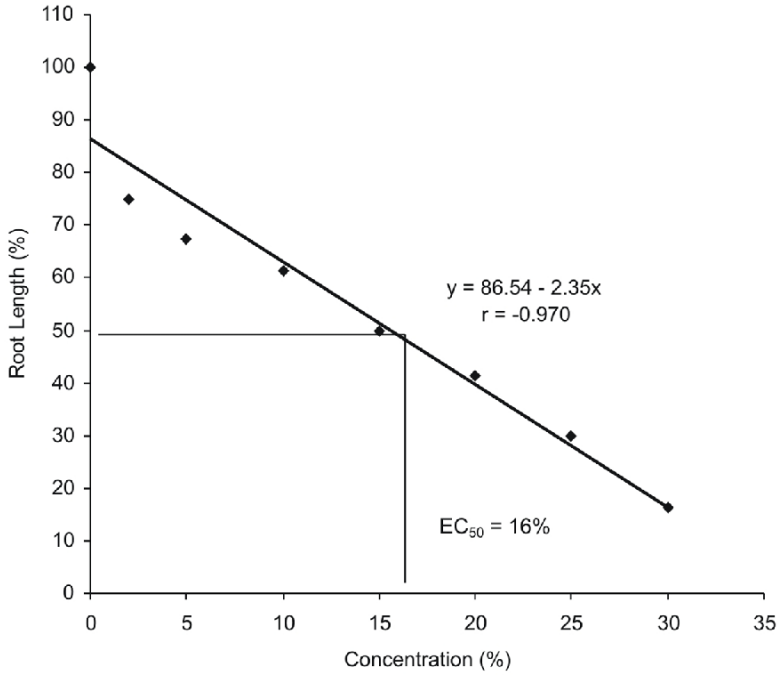

Figure 1. Growth inhibition of *Allium cepa* roots exposed to textile effluent.

Microscopic examination

All slides were coded and examined blind. The mitotic index (MI) was determined by the examination of 500 cells per concentration (100 cells per slide). Characterization of mitosis and chromosomal aberrations were scored in 100 cells per slide.

Statistical analysis

The EC_{50} and regression equation was determined from a plot of root length as a percentage of control against the sample concentrations, by using a Microsoft Excel computer program. Pearson correlation analysis was carried out to test for significant relationship (Positive or negative) between the root length and effluent concentrations. Analysis of Variance (ANOVA) and Student Newman Keul's (SNK) tests were used to test for significant differences in the mean root lengths of *A. cepa* exposed to different concentrations of paint and textile effluents. The tests were carried out at 5% significant level. The analysis was performed using SPSS 10.0 computer program.

RESULTS

Root growth inhibition

The results of the macroscopic parameters (root length) used in testing for general toxicity (root growth inhibition) of *A. cepa* exposed to paint and textile industrial effluents are presented in Figures 1, 2 and Table 1. The estimated EC_{50} (concentration of a chemical producing 50% of the total effect) of *A. cepa* exposed to paint and textile effluent was 72 and 16%, respectively (Figures 1 and 2). No growth was observed in the *A. cepa* exposed to textile effluent concentration greater than 30% (Table 1). Generally, growth retardation was observed in onion exposed to high concentration of paint and textile effluents (Table 1). Further analysis using Pearson correlation revealed that the root growth retardation or inhibition was signifi-cantly concentration dependent ($P < 0.01$, $r = -0.970$ for textile effluent and $r = -0.974$ for paint effluent) (Figures 1 and 2). High growth rate was observed in onions exposed to low concentrations of either paint or textile effluent (Table 1).

Statistical analysis using analysis of variance (ANOVA) showed that there was significant difference ($P < 0.05$) in the mean root lengths of *A. cepa* exposed to different concentrations of paint and textile effluents. Further analysis using Student Newman Keul's (SNK) test revealed that the root length of the control group *A. cepa* (0% of paint or textile effluent) was significantly different ($P < 0.05$) from the root length of *A. cepa* exposed to all other concentrations (Table 1). No significant difference ($P > 0.05$) was observed in root growth of *A. cepa* exposed to the pair of 5 and 10% of paint effluent on one hand and that of 40 and 60% on the other (Table 1). However, SNK at $P = 0.05$ showed that there was significant difference ($P < 0.05$) in the root length of *A. cepa* exposed to textile effluent at all concentrations pairings (Table 1).

Microscopic effects

The microscopic results of *A. cepa* roots exposed to paint and textile effluents are summarized in Tables 2 and 3

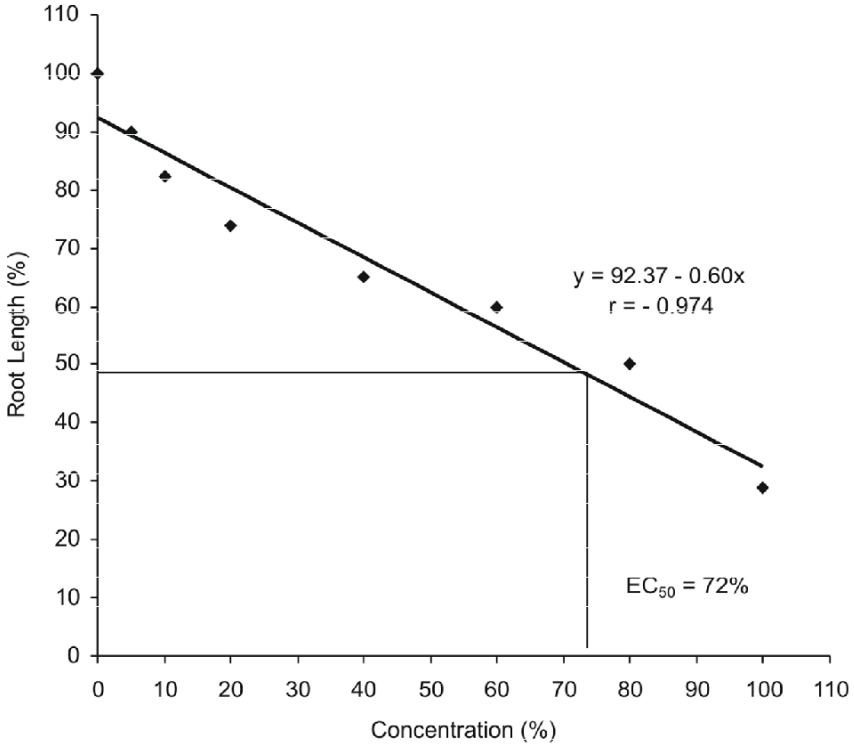

Figure 2. Growth inhibition of *Allium cepa* roots exposed to paint effluent.

Table 1. Mean (± SD) root length of *A. cepa* exposed to different concentrations of industrial effluents

Paint effluent		Textile effluent	
Concentration (%)	**Mean root length (cm)**	**Concentration (%)**	**Mean root length (cm)**
Control (0)	8.0 ± 0.44^{F}	Control (0)	8.0 ± 0.44^{H}
5	7.2 ± 0.65^{E}	2	6.0 ± 0.34^{G}
10	6.6 ± 1.00^{E}	5	5.4 ± 0.37^{F}
20	5.9 ± 0.69^{D}	10	4.9 ± 0.52^{E}
40	5.2 ± 0.64^{C}	15	4.0 ± 0.33^{D}
60	4.8 ± 0.75^{C}	20	3.3 ± 0.58^{C}
80	4.0 ± 0.44^{B}	25	2.4 ± 0.31^{B}
100	2.3 ± 0.48^{A}	30	1.3 ± 0.41^{A}

Means with the same superscript letter along the column are not significantly different (P > 0.05) in the SNK test. SD = standard deviation

respectively. Except in the *A. cepa* exposed to 72% of paint effluent, there was a decrease in the mitotic index (MI) with increasing concentration of the wastewaters. In all the experiments with the two industrial effluents, it was impossible to obtain 500 mitoses for chromosome screening as was obtained in the control (Tables 2 and 3).

Analysis of the chromosomes showed that the two industrial effluents induced chromosomal aberrations significantly when compared to the control. No aberration was recorded in the chromosome of *A. cepa* exposed to the control. C. mitosis was observed in *A. cepa* exposed to 7.2 and 16% of paint and textile effluent, respectively.

Table 2. Effects of treatments with different concentrations of paint industrial effluent

Concentration (%)	Mitotic index	Number of cell	Number of dividing cell	Chromosome aberrations							
				Stickiness	C-mitosis	Vagrant	Bridges fragment	Binuclei	Multipolar anaphase	Attached	% Aberration (± SD)
Control (0)	7.00	500	35[$P_{13}M_{10}$ A_3 T_9]	0	0	0	0	0	0	0	0.00 ± 0.00
7.2	8.82	272	24[P_7 M_7 A_5 T_5]	5	1	5	5	0	1	0	6.25 ± 1.14
18.0	7.25	262	19[P_7M_5 A_4 T_3]	3	0	3	4	0	0	1	4.20 ± 0.55
36.0	5.56	216	12[P_2M_2 A_4 T_4]	2	0	0	4	0	0	0	2.78 ± 0.84
72.0	9.90	202	20[P_6M_5 A_3 T_6]	6	0	4	3	0	0	1	6.93 ± 1.30

Mitotic index was calculated as: (number of dividing cells / number of cell) × 100

Table 3. Effects of treatments with different concentrations of textile industrial effluent

Concentration (%)	Mitotic index	Number of cell	Number of dividing cell	Chromosome aberrations							
				Stickiness	C-mitosis	Vagrant	Bridges fragment	Binuclei	Multipolar anaphase	Attached	% Aberration (± SD)
Control (0)	7.00	500	35[$P_{13}M_{10}$ A_3 T_9]	0	0	0	0	0	0	0	0.00 ± 0.00
1.6	9.71	412	40[P_{12} M_8 A_9 T_{11}]	3	0	4	5	2	1	2	4.13 ± 0.89
4.0	9.59	271	26[P_6 M_6 A_3 T_{11}]	6	0	2	2	0	1	0	4.06 ± 0.84
8.0	8.47	189	16[P_3M_6 A_4 T_3]	1	0	5	2	0	1	0	4.77 ± 0.45
16.0	7.69	156	12[P_6M_2 A_2 T_2]	2	1	1	2	0	0	0	3.84 ± 0.45

Mitotic index was calculated as: (number of dividing cells / number of cell) × 100

Vagrant chromosomes were observed in all concentrations except 36% paint effluent. Bridged and fragments were also observed in the chromosome of onions exposed to different concentrations of paint and textile effluents. Abnormal and multipolar anaphase was observed in *A. cepa* exposed to 1.6, 4, 8% of textile effluent and 7.2% of paint effluent. *A. cepa* exposed to 16% textile effluent, 36 and 72% paint effluent showed attached metaphase. Stickiness was observed in all the concentrations of paint and textile effluent to which *A. cepa* was exposed (Tables 2 and 3).

DISCUSSION

The results obtained in this study indicated that the textile effluent (96 h EC_{50} value of 16%) was 4.5 times more toxic than the paint effluent (96 h EC_{50} = 72%) when tested with *A*. *cepa* using root growth inhibition (Figure 1 and 2). There was no root growth at all in onion bulbs treated with textile effluent concentration above 30%, while at 100% concentration of paint effluent there was 28.8% root growth relative to control. ANOVA showed that there was significant difference in the mean root length of *A. cepa* exposed to different concentrations of higher growth rate observed in onion bulbs exposed to low concentration of either paint (r = -0.974) or textile (r = -0.970) effluent than those exposed to high concentrations (Figures 1 and 2). This relationship was also supported by Figures 1 and 2 used in estimating the 96 h EC_{50}, thus indicating a fundamental similarity in dose-response effect. This was in agreement with the findings of Odeigah et al. (1997a, 1997b). This study has shown that onion root growth test can be fully integrated into the

Whole Effluent Toxicity (WET) program by giving a particular EC_{50} that an industrial effluent must met before being allowed to be discharged into the aquatic environment.

The cytogenetic effects of different industrial effluents treatments on mitotic division in the root tip cells of *A. cepa* are given in Tables 2 and 3. The mitotic index (MI), except in the control experiment decreased with increasing concentrations of textile industrial effluents. Similar results were obtained after treating *A. cepa* root cells with leachates from solid industrial wastes (Odeigah et al., 1997b), sodium metabisulphite (Rencuzogullari et al., 2001) and oil field wastewater (Odeigah et al., 1997a). Inhibition of mitotic activities is often used for tracing cytotoxic substances. However, in *A. cepa* exposed to 72% of paint effluent, the MI was higher than those exposed to each of the 7.2, 18.0 and 36% of the same effluent.

The two industrial effluents studied induced chromosomal aberrations in root tip cells of *A. cepa* with vagrant chromosomes, bridges and fragments and sticky chromosomes being most frequently observed. This suggests the presence of certain cytotoxic/genotoxic substances in the industrial effluents. Vagrant chromosomes have been described as a weak C mitotic effects indicating risk of aneuploidy while sticky chromosomes indicate a highly toxic, irreversible effect probably leading to cell death (Fiskesjo, 1985, 1988). According to Kong and Ma (1999), there is a hypothesis that stickiness of chromosomes may cause incomplete separation of daughter chromosomes as a result of cross-linkage chromoproteins.

The number of aberrant mitotic cells caused by all concentrations of the paint and textile effluent was apparently different from that of the control. No aberration was observed in the *A. cepa* exposed to the control (0%). The variation in the number of chromosomal aberration observed in this study was not dose dependent, except in bridges and fragments where the number of observations reduced with higher concentrations of effluents. This is in disagreement with Qian (2004) which reported that aberrant rate goes up with the concentrations but in agreement with Odeigah et al. (1997a). According to Odeigah et al. (1997a), a possible explanation for this is that, with increasing concentration and consequently, increasing toxicity, there was an inhibitory effect on cell division. This might occur in pre-prophase, where cells are prevented from entering prophase or there may be prophase arrest where cells enter into mitosis but are arrested during prophase resulting in a high frequency of prophase cells. It is suggested that prophase - arrest is the most likely explanation, as it could also explain the decline in chromosome aberrations, without any parallel decline in the mitotic index values (Odeigah et al., 1997a).

The proportion of aberrant cells in *A. cepa* exposed to paint effluent was higher than those exposed to textile effluent (Tables 2 and 3) even though textile effluent was found to be more toxic than paint effluent based on their EC_{50}. In general, the induction of chromosomal aberration in the root tip of *A. cepa* by the industrial effluents indicates their genotoxic effects. The *Allium* test has been used in the screening of other types of industrial waste and wastewater. Odeigah et al. (1997a) reported the genotoxicity of oil field wastewater, Odeigah et al. (1997b) reported the genotoxicity screening of leachates from solid industrial wastes and Rank and Nielsen (1994) evaluated the suitability of the *Allium* test in the screening of wastewater for genotoxicity. Grover and Kaur (1999) have evaluated the genotoxicity of wastewater samples from sewage and industrial effluent using *Allium* root anaphase aberration, while Abdel-Migid et al. (2007) evaluated the efficiency of algal biofilters in bioremediation of toxic industrial effluent using *Allium* genotoxicity bioassay.

According to Odeigah et al. (1997a), the impact of genotoxic wastewater on the environment and the significance to human health are difficult to predict, because wastewater are complex mixtures of chemical substances. Complete interpretation of their effect often requires, in addition chemical analysis of the constituents that may indicate the components of the wastewater that can persist and accumulate in exposed biota and thus potentially pose a hazard to human health.

This study has shown that the genotoxic potential of paint and textile industrial effluents can easily be detected using the *A. cepa* chromosomal aberration assay. It would be beneficial to apply *A. cepa* chromosomal assay as a tool for monitoring the genotoxic effects of industrial effluent and wastewaters thereby providing information on the need for environmental managers to further subject treated industrial effluent to Toxicity Identification Evaluation (TIE) and Toxicity Reduction Evaluation (TRE) before they are finally discharged. This will enable proper chemical analysis of industrial effluent in order to identify the constituent that is really genotoxic and its prompt removal from the effluent before discharge.

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