Genotoxic Endpoints in *Allium cepa* and *Clarias gariepinus* Exposed to Textile Effluent


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**Abstract** The genotoxic effects of textile effluent were investigated in *Clarias gariepinus* and *Allium cepa* using the micronucleus assay and induction of chromosomal aberration in respectively. The aim of the study is to provide genotoxic endpoints that can serve as biomarkers of exposure to textile effluents in the environment. The physico-chemical characterization of the effluent revealed high levels of chemical oxygen demand (COD), biological oxygen demand (BOD) and total suspended solids (TSS) above the standards set by regulatory body. Exposure of *Clarias gariepinus* to sub-lethal concentrations of effluent resulted in a dose-dependent and significant (P<0.05) increase in the formation of micronuclei and nuclear anomalies in blood erythrocytes. The textile effluent also caused a significant (P<0.5) reduction in mean root length and increased chromosomal aberrations such as vagrant, sticky chromosomes, binucleus and c-tumors in exposed *Allium cepa* compared to control. The results obtained from this study indicate the textile effluent being discharged into the Lagos lagoon have genotoxic potential and is capable of causing significant ecological disruption in the receiving environment.

**Keywords** textile effluent; genotoxic effects; *Clarias gariepinus; Allium cepa*

**I. INTRODUCTION**

The textile industry is one of the prominent industries in Nigeria, with attendant indiscriminate discharge of effluents into the surrounding water bodies. The textile industry poses severe environmental problems mainly due to the large volumes of wastewater it generates, and the complex composition of the effluents produced [5]. The chemical reagents used are very diverse in chemical composition, ranging from inorganic compounds to polymers and organic products such as dyes, plasticizers, detergents [22]. These effluents contain selected important environmental pollutants that tend to accumulate in organisms, and persist in the environment due to their chemical stability or poor biodegradability. In developing countries, due to the high cost of treatment, these effluents are often not treated prior to discharge into the environment. The introduction of such effluents into the aquatic system has caused several disruptions to aquatic productivity and eventually leads to some physiological dysfunction in the resident organisms [14]. A variety of biomarkers and bio-assays can be used in the laboratory and field studies to determine the effects of genotoxic pollutants on biotic systems, these include the presence of DNA adducts, chromosomal aberrations and DNA strand breaks. The micronucleus test is widely applied, due to its simplicity, reliability, sensitivity and proven suitability for fish species [11]. It detects both clastogenic and aneugenic effects and therefore can detect the genotoxicity of a wide range of compounds [10]. The *Allium cepa* test is a simple, sensitive and rapid bioassay that has been widely used as a standard for biomonitoring of environmental contaminants using various genotoxicity parameters [9]. Based on the significance of the micronucleus test and Allium test this study is aimed at assessing the genotoxic effects of textile effluents on a plant, *A. cepa* and an animal, *C. gariepinus*.

**II. MATERIALS AND METHODS**

**A. Collection of Effluent**

Effluent was collected at different outlets discharge from a creek receiving direct effluent discharge from a textile mill at Ikorodu, Lagos, Nigeria. The effluent kept in plastic containers, were mixed and stored at 4°C prior to analysis.

**B. Physical and Chemical Characterization**

The physico-chemical properties of the effluent sample were determined in accordance with standard analytical methods.

**C. Test Organisms**

Fish: *Clarias gariepinus* (diploid chromosome 2n = 56) was chosen for this study because of its common availability in most fish farms in Nigeria. *Clarias gariepinus* juveniles of similar sizes (12-14cm) were purchased from a commercial fish farm. The juveniles were kept in glass tanks in the laboratory and allowed to acclimatize for at least 7 days to laboratory conditions.

Onion Bulb: Purple strain of *Allium cepa* (2n = 16) of size 2.0 to 2.5 cm and weigh 2 to 4g was used for the Allium cepa test. The onion was purchased from the local market. The onion roots were intact and dry, free from decay and other forms of mechanical damage.

**D. Micronuclei Assay and Nuclear Abnormalities in C. gariepinus**

Preliminary screening was carried out to determine the appropriate concentration range for effluent, prior to experiment. At the end of 96h, the concentration in which all fishes stay alive or no mortality was observed - LC<sub>50</sub> was determined at 1.6ml L⁻¹. The concentrations 0.16 and 0.016 ml L⁻¹(1/10th and 1/100th respectively of the LC<sub>50</sub>) were selected for the experiment. Fishes were placed in tank containing dechlorinated tap water (control) and two different concentrations of textile effluents (0.16 and 0.016 ml L⁻¹). For the micronucleus test, blood samples were obtained from...
the caudal sinus on the 5th, 10th, and 15th day of exposure. Twenty-five (25) fishes were used for the assay which was done in duplicates (5 fishes per replicate for each concentration and 5 for the control).

Peripheral blood samples were obtained from the caudal sinus using a syringe. Blood was smeared immediately on clean grease free microscope slides, air dried for 12 hrs and then fixed in absolute methanol for 20min. Each slide was stained with 5% Giemsa solution for 30mins. Three slides were prepared for each fish from the experimental and control experiments were viewed microscopically. Small, non-refractile, circular or ovoid chromatin bodies showing the same staining pattern as the main nucleus were considered as micronuclei (MN) [13]. Studies from [4] classify nuclear abnormalities into blebbed nuclei (BL), lobed nuclei (LB), notched nuclei (NT) and binuclei (BN). The frequencies of micronuclei and other nuclear lesions were expressed per 1000 cells (0/00).

E. Root Growth Inhibition and Chromosomal Aberration in A. cepa

The assay was carried out using a plastic tube (diameter, 2.3 cm; length, 7.8 cm) in a rack. Dechlorinated 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.

The toxicity assay is performed as a 96 h semi – static exposure test, and six concentrations (10, 20, 30, 40, 50 and 100%) of the textile effluents are used. Every 24 h the test solutions are replaced with fresh solutions and the length of the root bundle is measured. At the termination of the exposure, one onion (out of six) with the poorest growth is discarded and the length of the root bundle is measured for the remaining five onions. Growth inhibition was estimated as EC50 (the effective concentration of a chemical producing 50% of the total effect).

For each of the concentration there were 4 replicates. The root tip of each replicate was selected and fixed in 4 different fixative bottles using acetic acid. From each bottle containing fixed roots, 5 microscopic slides were made using the aceto orcein squash method. From each concentration, 8 microscopic slides were prepared. A total of 48 slides were made. The slides were viewed microscopically for mitotic or cytological aberrations and micrographs were taken.

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.

F. Statistical Analysis

Fish (Clarias gariepinus):

Probit analysis was used to determine the 96 h LC50 value of the effluent. All data were expressed as means±SE. A two-way analysis of variance (ANOVA) was used to determine the significance of micronuclear tests. Bonferroni post tests were used to compare frequency of nuclear abnormalities and micronucleus between control and treatment groups. Statistical tests were performed using the GraphPad Prism for PC computers.

1) Onion (Allium cepa):

The EC50 and regression equation were 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 textile effluents. The tests were carried out at 5% significant level. The analysis was performed using SPSS 10.0 computer program [20].

III. RESULTS

A. Physico-Chemical Characterisation of Textile Effluent

The results of the physico-chemical characterization of textile effluent revealed higher values of most parameters than the standards set by environmental protection agency (Table 1).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Concentration (mg/L)</th>
<th>Effluent Standards(mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BOD</td>
<td>152</td>
<td>20</td>
</tr>
<tr>
<td>COD</td>
<td>319</td>
<td>8</td>
</tr>
<tr>
<td>TSS</td>
<td>1160</td>
<td>30</td>
</tr>
<tr>
<td>Oil &amp; grease</td>
<td>612</td>
<td>10</td>
</tr>
<tr>
<td>pH</td>
<td>6.9</td>
<td>6-9</td>
</tr>
<tr>
<td>Cr</td>
<td>1.20</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Cu</td>
<td>0.02</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Phenol</td>
<td>Not detected</td>
<td>0.01</td>
</tr>
<tr>
<td>NH3</td>
<td>0.50</td>
<td>-</td>
</tr>
<tr>
<td>Free chlorine</td>
<td>Not detected</td>
<td>1.0</td>
</tr>
</tbody>
</table>

TABLE II MEAN±SE OF MICRONUCLEUS FREQUENCIES (0/00) IN ERYTHROCYTES OF C. GARIEPINUS EXPOSED TO DIFFERENT CONCENTRATIONS OF TEXTILE EFFLUENT

<table>
<thead>
<tr>
<th>Concentration ml L⁻¹</th>
<th>Time of exposure</th>
<th>Day 5</th>
<th>Day 10</th>
<th>Day 15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>0.50±0.28</td>
<td>0.25±0.25</td>
<td>0.25±0.25</td>
</tr>
<tr>
<td>0.16</td>
<td></td>
<td>4.75±0.62</td>
<td>2.75±0.47</td>
<td>1.50±0.28</td>
</tr>
<tr>
<td>0.016</td>
<td></td>
<td>2.50±0.28</td>
<td>1.25±0.47</td>
<td>0.75±0.47</td>
</tr>
</tbody>
</table>

B. Effect of Textile Effluents on Micronucleus Formation and Nuclear Abnormalities.

An LC50 value of 1.639ml L⁻¹ was obtained at the end of the 96th acute toxicity assay. It was observed that the micronucleus frequencies increased dose-dependent as they decreased time-dependently (Table 2). Formation of micronuclei were found to be significant (P<0.01) and (P<0.001) at day 5 and day 10 respectively.

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Similarly, frequencies of nuclear abnormality in erythrocytes were also positively dose-dependent and negatively time-dependent (Fig. 1). 0.16ml L-1 of effluent was significant (P<0.01) and (P<0.05) at days 5 and 10 respectively. Anomalies of the erythrocytes are presented in Fig. 2.

![Fig. 1 Changes in the frequencies of nuclear abnormalities in erythrocytes](image)

Fig. 1 Changes in the frequencies of nuclear abnormalities in erythrocytes

![Fig. 2 Various nuclear abnormalities. a) micronucleus; b) notched nucleus](image)

Fig. 2 Various nuclear abnormalities. a) micronucleus; b) notched nucleus

![Fig. 3 Growth inhibition of Allium cepa roots exposed to textile effluent](image)

Fig. 3 Growth inhibition of Allium cepa roots exposed to textile effluent

**TABLE III**

<table>
<thead>
<tr>
<th>Textile effluent concentration (%)</th>
<th>Mean root length (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (0)</td>
<td>4.60&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>10</td>
<td>2.70&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>20</td>
<td>1.98&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>40</td>
<td>1.65&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>50</td>
<td>1.30&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>100</td>
<td>0.83&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means with the same superscript letter (A, B, C, D and E) along the column are not significantly different in the SNK test at P<0.05

**C. Effect of Textile Effluent on Root Growth and Chromosomal Aberration in A. cepa**

The estimated EC50 of A. cepa exposed to textile effluent was 25% (Fig. 3). Reduction in root lengths was observed in onion root tips exposed to all concentrations of textile effluent. The root growth inhibition was concentration dependent as shown in Fig. 3. Statistical analysis revealed that the root length of A. cepa exposed to control was significant (P<0.05) from the root length of A. cepa exposed to all other concentrations (Table 3). Results of microscopic effects and chromosomal aberrations of A. cepa roots exposed to textile effluent are summarized in Table 4 and Fig. 4. There was rapid decrease in the mitotic index (MI) with increasing concentration of the effluent. Five hundred mitoses could not be attained for chromosome screening compared to control due to the effects of effluent on cells. Analysis of the chromosomes showed that the effluent induced chromosomal
aberrations significantly when compared to the control. In the control no aberration was recorded in the chromosome of *A. cepa.*

**IV. DISCUSSION**

The physicochemical properties of the effluent obtained were mostly higher than the Federal Environmental Protection Agency (FEPA) safe limit for effluents discharged into water bodies and this resonates the belief that industry operators within the country disregard safe limits set by the regulatory agency. All these point to a lag in the mechanism for the part of the regulators. Oil and grease concentration was over 60 times the set limit while total suspended solid was also about 39 times above the limit. These raise concerns about the health of the Lagos Lagoon ecosystem where these effluents eventually deposits. Further concern is raised by the depletion of available oxygen for aquatic residents, while high amount of COD in effluent is toxic to biological life. Heavy metals like cadmium and mercury were not detected and this is in consonance with earlier findings in the Lagos Lagoon and water bodies elsewhere in the country where the concentrations recorded range from very little to complete absence [18].

The low LC₅₀ acute toxicity tests results reflects the toxicity of effluents discharged into water bodies which raises serious concern about the continued introduction of the effluent into the Lagoon ecosystem. The result derived from this study is consistent with the low toxicity values of effluents from paint, textile and chemical as obtained by several author [20].

The induction of micronuclei in erythrocytes of exposed fish in this study agrees with the observation of [17] who reported a significant increase in the frequency of micronucleated erythrocytes of *Clarias lazera* exposed to textile mill effluent. Similarly, induction of micronuclei in peripheral erythrocytes of *Oncorhynchus mykiss* exposed to textile industry effluent was shown by [15]. Molecular and cellular level effects have also been linked with exposure to effluents from industries including textile industries [21] observed that after 15 days exposure to sub-lethal concentrations of the effluent, assessment revealed cellular aberrations (genotoxic effects) which impacts at the cellular and possibly sub cellular levels. This generates serious concerns about the far reaching effects of effluents which are continuously introduced into surrounding water bodies on fishes, shell fishes and other aquatic animals especially edible ones. Micronuclei assay with fish show potential as monitoring techniques for detecting genotoxic substances in water.

The decrease in the mitotic index (MI) with increasing concentrations of industrial textile effluents as well as the formation of chromosomal aberrations such as vagrant, sticky chromosomes, binucleus and c-tumors, severe reduction in root length can be attributed to the presence of some cytotoxic and genotoxic compounds in the effluent. Further studies need to be carried out to identify the specific agents responsible for the genotoxic effects.

**V. CONCLUSION**

The results obtained from this study indicate the textile effluent being discharged into the Lagos lagoon have
genotoxic potential and is capable of causing significant ecological disruption in the receiving environment. The increase in the formation of micronuclei and nuclear anomalies in blood erythrocytes of *Clarias gariepinus*, as well as, chromosomal aberrations such as vagrant, sticky, binucleus and c-tumors in *Allium cepa* were identified as good genotoxic biomarkers for monitoring impact of industrial effluents in the environment.

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REFERENCES


