Research Article

Cytotoxic effect of sewage effluent on root tip cells of Allium cepa L.

Teena M.T1, Soumya K.R1; Sudha K.S 1,*

1Post Graduate Department of Botany and Research Centre, Sree Krishna College, Ariyannoor, Guruvayoor 680102, Kerala, India
* Corresponding author: Sudha KS; E-mail: sudha.soman@gmail.com

Received 16 July 2015; Revised 12 September 2015; Accepted 22 September 2015; Published 2 January 2016

Abstract

Screening for mutagens in complex environmental mixtures, such as surface water or industrial wastewater, is gradually being accepted as a routine method in environmental monitoring programs. In the present work, the simplified Allium cepa root assay was utilized to evaluate the possible cytotoxic effect of sewage effluent on root tip cells of A. cepa L. Sewage effluents were collected from Kunnamkulam market. Treatments were done in five different time durations such as 3, 6, 12, 24, and 48 hours. Allium cepa exposed to sewage effluents resulted to cells with chromosomal aberration. The Mitotic index was decreased when the time durations were increased. The mitotic index of the control was 49.65±3.35. The mitotic index of the sewage effluent in 3 hr was 42.28±3.37. From this a gradual decrease in the mitotic index when the duration of time increased. The mitotic index 42.15±3.17, 32.27±3.04, 31.65±3.19 and 28.19±2.87 are 6 h, 12 hr, 24 h and 48 h respectively chromosomal abnormalities such as bridges, fragments, vagrant chromosomes, sticky chromosomes, C-mitosis, spindle abnormalities and disoriented chromosomes were observed. The impacts of using sewage effluent as fertilizer on the environment are difficult to predict. So they may be treated to remove its toxic particles prior to its release into the environment. Toxicity or genotoxicity bioassays should be an integral tool in the evaluation of waste water toxicity.

Keywords: Allium cepa, Cytotoxicity, Sewage effluent, Chromosomal Aberrations, Mitotic Index

1. Introduction

Environmental pollution is the greatest problem of the present world. Among that, pollution of water resources is a serious and growing problem. The pollution of the aquatic environment by toxic chemical pollutants continuous to occur, with domestic and industrial effluents, being the main sources responsible for the contamination of aquatic environments (Claxton et al., 1998). Water pollution is the contamination of water bodies for e.g. lakes, rivers, oceans and ground water. This form of
environmental degradation occurs when pollutants directly or indirectly discharged into water bodies without adequate treatment to remove the harmful compounds.

The polluted water contains toxic chemical compounds that are directly discharged to the agricultural fields, where untreated waste water is used for irrigation. The polluted water containing the urban waste, including sewage, heavy metal contents etc. This cause significant health hazards by using water loaded with pathogens, especially when peoples eat raw vegetables that have been irrigated with the polluted water. In several cases, increased use of municipal sewage sludge on soils has resulted in increased heavy metal content of soils and crop grown on there. This leads to the chromosomal abnormalities in plants. Different kinds of abnormalities are observed were clumping or stickiness (Darlington 1942), lagging chromosome, chromosome bridges and fragments, tropokinesis, nuclear lesions and micronuclei (Omanakumari et al., 2006).

There are number of toxic chemicals in the environment, they are mostly discharged by industries into water, air and land. Once they enter in our biological process, it is really difficult them to remove from the environment and it disturb various biochemical processes, leading to fatal results. Environmental biologists are presently concerned to safe guard the human beings by determining the magnitude of genetic risk to man by an environmental agents and chemicals under a specific level of exposure. Unfortunately, the direct assessment in human is not feasible because of ethic, logistic and practical considerations. So we are using cytogenetic test. In these circumstances, Allium cepa test are used to evaluate the genotoxicity of toxic and mutagenic substances in the environment.

2. Materials and methods

2.1. Plant materials and treatment

Root meristems of Allium cepa L. (2n=16) were used as the biological material for the present study. Sewage effluents were collected from the Kunnamkulam before their discharge into the water bodies. The sprouted onion bulbs were treated in the sewage effluent in different time durations such as 3, 6, 12, 24 and 48 h. The sewage effluent was taken in five test tubes and in each test tube the sprouted onion bulbs were placed. Test tubes are labeled. The bulbs were allowed to germinate in the test tubes at room temperature and were observed at 3, 6, 12, 24 and 48 h. Distilled water treated as a control. After, the root tips of treated and controlled were excised and fixed in the Carnoy’s fluid (3 alcohols: 1 acetic acid). The acetocarmine squash preparation was used for mitotic studies. Two slides were prepared for each treatment and scoring was done from five cites that were randomly selected. From these slides, numbers of cells identified in each stage of mitosis were counted and these data were used for statistical analysis in order to determine whether there is a significant deviation between treatments and control samples. The resulting data were pooled to calculate the mitotic index. For the estimation of mitotic index (M.I), the following formula, is used.

Mitotic index (M.I) = Number of Dividing Cells/Total Number of Cells X 100

Percentage of abnormality of each stage of mitosis (Prophase, Metaphase, Anaphase and Telophase) was counted for each slide.

Percentage of Abnormality = Number of Abnormal Cells/Number of Dividing Cells X 100

3. Results

Treatment with sewage effluent on the root tip cells of A. cepa L. induces chromosomal abnormalities. The mitotic index of the sewage effluent was decreased when the increase in the time durations. When decrease in the mitotic index proportional to the increase in the percentage of abnormalities. Mitotic
index was decreased when compared to the control. The mitotic index of the control was 49.65±3.35. The mitotic index of the sewage effluent in 3 h was 42.28±3.37. From this a gradual decrease in the mitotic index when the duration of time increased. The mitotic index 42.15±3.17, 32.27±3.04, 31.65±3.19 and 28.19±2.87 are 6hr, 12 h, 24 h and 48 h respectively (Table1). Percentage of abnormalities were increased when the duration of time increased (Table 2).

The chromosomal abnormalities were observed on prophase, metaphase, anaphase and telophase. When the time durations were increased proportional to the chromosomal abnormalities were increased. Chromosomal abnormalities such as sticky chromosomes, spindle abnormalities, C- metaphase, vagrant chromosomes, bridges, fragments and disorientation (Fig. 1). Chromosomal bridges were more frequently found in the sewage effluent treatment on root tips of Allium cepa L. (Fig. 2). Bridges were found in both anaphase and telophase. C- Metaphases, spindle abnormalities, vagrant chromosomes, fragments, and disoriented chromosomes were also prominent in this treatment (Fig.3).

Table 1. Mitotic index (M.I) on root tip cells of Allium cepa L. treated with sewage effluent.

<table>
<thead>
<tr>
<th>Treatment No</th>
<th>Duration of treatment (hr)</th>
<th>Total no. of cells (Mean±Sd)</th>
<th>No. of dividing cells (Mean±Sd)</th>
<th>Mitotic index (M.I) % (Mean±Sd)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>1025±4.08</td>
<td>509±3.96</td>
<td>49.65±2.08</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>1031±5.32</td>
<td>436±3.09</td>
<td>42.28±2.18</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>1032±4.87</td>
<td>435±3.56</td>
<td>42.15±2.74</td>
</tr>
<tr>
<td>4</td>
<td>12</td>
<td>1100±4.69</td>
<td>355±2.99</td>
<td>32.27±2.29</td>
</tr>
<tr>
<td>5</td>
<td>24</td>
<td>1030±5.76</td>
<td>326±3.13</td>
<td>31.65±2.48</td>
</tr>
<tr>
<td>6</td>
<td>48</td>
<td>1128±5.86</td>
<td>318±3.10</td>
<td>28.19±2.65</td>
</tr>
</tbody>
</table>

Table 2. Percentage of abnormalities on root tip cells of Allium cepaL. treated with sewage effluent.

<table>
<thead>
<tr>
<th>Treatment No</th>
<th>Duration of treatment (h)</th>
<th>No of dividing cells</th>
<th>No of abnormal cells</th>
<th>Percentage of abnormality</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>509±3.01</td>
<td>5±1.01</td>
<td>0.98±1.90</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>436±2.98</td>
<td>34±1.2</td>
<td>7.79±2.09</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>435±2.90</td>
<td>50±1.97</td>
<td>11.49±2.32</td>
</tr>
<tr>
<td>4</td>
<td>12</td>
<td>355±2.69</td>
<td>57±2.06</td>
<td>16.05±2.50</td>
</tr>
<tr>
<td>5</td>
<td>24</td>
<td>326±2.58</td>
<td>61±2.15</td>
<td>18.71±2.64</td>
</tr>
<tr>
<td>6</td>
<td>48</td>
<td>318±2.01</td>
<td>75±2.2</td>
<td>23.58±2.88</td>
</tr>
</tbody>
</table>
Fig. 3. Chromosomal abnormalities caused by sewage effluent.
4. Discussion

Cytotoxic effects of sewage effluent on root tip cells of *A. cepa* L. were conducted. Sewage effluent induces chromosomal abnormalities at different time durations. Treatment with sewage effluent caused a decrease in mitotic index and induction of chromosomal aberrations, which was directly proportional to the duration of time. Based on the results of the present investigation, it is quite clear that sewage effluent were capable of inducing cytological abnormalities such as stickiness, vagrant or lagging, bridges, fragments, spindle abnormalities, C- mitosis, disorientation etc. The most frequent abnormalities were due to chromatin dysfunction (stickiness, anaphase bridges) or spindle failure (C-mitosis). Chromosome bridges results from chromosome and or chromatid breaks indicating the clastogenic effect, whereas vagrant chromosomes and C- mitosis increase the risk of for aneuploidy mitosis, sticky and abnormal chromosome orientation also reported from the cytotoxic effect of sea water (Amin and Migahed 2002).

Sewage effluent induced chromosomal aberration through interactions with DNA and proteins leading to chromosomal stickiness, mitotic disturbances and cell damage. This result was similar to the findings of Odeigah et al. (1997a) in which roots of *Allium cepa* exposed to effluent from industrial waste water resulted to cells with chromosomal aberrations. Pronounced stickiness of the chromat mat matrix often resulted in a typical metaphase and anaphase. Darlington (1942) suggested that stickiness can result from the breakdown of DNA to a depolymerized and fluid condition. According to Odeigah et al. (1997b) sticky chromosomes are indicative of a highly toxic condition. Stickiness is considered a common sign of toxic effects on chromosomes probably leading to cell death (Fiskejo 1997).

Chromosome lagging or vagrant chromosomes was one of the most frequent abnormalities observed in sewage effluent. Lagging chromosomes during anaphase considered to be a sign of genetic material loss and closely associated with the aneuploidy formation. Similar condition was observed by Ivanova et al. (2008) in the effect of heavy metal and cyanide- contaminated waters. C- Mitosis, present in industrial and municipal waste water samples disturb the mitotic spindle, most likely the kinetochore function (Seth et al., 2008). When the treatment with sewage effluents the root tip cells of *Allium cepa*, during anaphase the chromosome moved apart to two opposite poles bridges are formed. Sometimes fragments were formed. Similar results were obtained by Omanakumari et al. (2006) in *Allium cepa* on treatment with Monosodium glutamate.

Pratibha in 1987 also reported the chromosome bridges and fragments in the genotoxicity of starch factory effluents. In the present study the spindle abnormalities were observed, when the orientation of spindle it shifted to the corners of the cell. A support of this observation could be found from Omanakumari et al., 1980 in *Allium cepa* with Monosodium glutamate. Positive results in *Allium test* should be considered a signal of warning as this may constitute risk to environment and human health. Therefore our study recommended that sewage water be treated before they are used for irrigation and also when the waste waters from the laboratories cannot directly discharged to the soil or other water bodies. So that safety of humans would be achieved use of treated waste water for irrigation farming.

4. Conclusion

Results obtained in this study showed that sewage effluent can induce chromosomal aberration through interactions with DNA and proteins. This leads to chromosomal abnormalities such as sticky chromosomes, spindle abnormalities, C- metaphase, vagrant chromosomes, bridges, fragments, disorientation, mitotic disturbances and cell damage. Heavy metals have toxic effects on living organisms. They can damage their cells and tissues inducing neurodegenerative diseases and cancer. Heavy metals and toxic chemicals are transferred to plants from sewage water. The results in the present
study revealed the cytotoxicity of sewage water. Therefore it is recommended that sewage water should be treated before they are used as fertilizer or for irrigation purpose, so that safety of humans would be achieved.

**Conflict of interest statement**

We declare that we have no conflict of interest.

**References**