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Cytotoxic effects of Paper mill Effluent on *Allium cepa* L.

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Abstract

Paper-mill effluents was examined for its cytotoxic nature using *Allium cepa* L. root meristems, Cytotoxicity of the effluent was investigated by treating onion root with different concentrations of effluent (25%, 50%, 75% and 100%) for 24, 42 and 72 hours. Simultaneously the physicochemical properties of such effluent's sample were also analyzed for common parameters. The effluents inhibited cell division at strong dosage effect was observed from a decline in mitotic index with increase in effluent concentrations and duration of treatment. The effluent was also induced various mode of mitotic anomalies, such as laggard, fragmentation, stickiness and bridge etc. were observed. Observations of the present investigation revealed that the Paper-mill effluent acts as a cytotoxic /mutagenic agent at high concentration.

Keywords: Paper-mill effluent, cytotoxicity, mitotic index, chromosomal abnormalities, *Allium cepa* L.

1. Introduction:

With rapid industrialization the problems of pollution is being further accentuated and threatening to human, animal as well as plants. Almost all industries discharge their effluents into lakes, rivers and canals or drains without proper treatment or untreated. Now-a-days effluent has used to support agricultural production and the area of land under irrigation with industrial effluent has increased significantly due to the scarcity of water supply. Effluent reuse can provide considerable social, economic and environmental benefits when used under controlled conditions to protection of plant and human health.

The effluents have significant amount of major and minor nutrient (N, P, Ca and Fe, Zn, Cu, Mn), heavy metals (Cd, Pb, Cr, Hg), total dissolved solids, chemical oxygen demand, Biological oxygen demand and certain chemical pollutants which might cause adverse effects on soil properties and plant growth. The wastewater from different industries is also known to induce chromosomal abnormalities in plant cells Ray, Malabika *et al.* and Somashekar *et al.* and Thangapandian V. ^[1, 2, 3]

Pulp and paper mill effluents are constant sources of discharge of a complex mixture of organic pollutants, which produce aquatic contamination and impact human health. Examples of this type of contamination are given by various authors Grant *et al.*, Blavk *et al.*, Garcia *et al.*, Bowron *et al.*, Mac Latchy *et al.* and Kulshreshtha *et al.* [4, 5, 6, 7, 8, 9, 10]. Many of these agents are mutagens, and some are carcinogenic for humans. Thus, scientific studies have detected both toxic and genotoxic effects caused by the liquid effluents dumped into the aquatic environment. The common onion, *Allium cepa* L. (2n = 16), constitutes a very convenient test system for estimating the harmful effects of chemicals on biological materials.

2. Materials and methods

Effluent sample was collected from Madhya Bharat Paper Industries Limited situated at Village Birgahani of Janjgir Champa district, Chhattisgarh in plastic jar, prior to the collection the samples jar were rinsed thoroughly with the sample. Temperature and pH of the samples were recorded immediately and then carried to the laboratory carefully. For analyzing the chemical parameter like Turbidity, Conductivity, Total alkalinity, Total acidity, DO, BOD, COD, chloride, Total Hardness, Calcium, Magnesium, Nitrate Nitrogen, Nitrite nitrogen, Total Phosphate, Total dissolved solids and Total suspended solids, Standard methods APHA ^[11] were carried out.

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Allium cepa bulbs were germinated primarily in glass jar filled with normal water, then after bulbs with well germinated roots were transferred into another glass pots irrigated with different concentrations (25, 50, 75, and 100 %) of effluents in three replicates and distilled water used as control. *Allium cepa* was grown at 20-22°C and after 24 hrs, 48 hrs. and 72 hrs. of treatment from germinated bulbs the 2 cm long root meristems were cut and treated with 2,4-para dichlorobenzene for 2-3 hr. then washed with running water. The washed root tips were fixed in 1:3 Aceto-alcohol, after a day slides were prepared by carmine squashing technique. All temporary slides were scored for total number of dividing cells and its different phases as well as various types of chromosomal abnormalities concerned the phase was observed. Cytotoxic nature of the effluent has been evaluated in relation to Mitotic Index. Mitotic index [(mitotic Index %) = (number of dividend cell/number of total cell) x 100]. The mitotic cell number, the % of anomaly cells, total anomaly cells and types of anomalies for each concentrations were counted in microscopic analysis.

3. Results & Discussion

The quality and the concentrations of potentially toxic elements in effluent are summarized in Table -1. The influence of paper mill effluent which was found to be slightly alkaline in nature has been evaluated on the cytogenetic system, as relation to mitotic index and chromosomal abnormalities.

3.1. Mitotic Index:

Observed mitotic index has been mentioned in Table- 2 that revealed decrease in MI among the treated populations as against untreated one to be directly proportional to the duration of treatment. Observed data has been shown by histogram in Fig. 1. It is apparent that variation in duration of soaking the root in distilled water has no effect on phase. Frequency of prophase and telophase at each treatment and is higher and lower respectively than rest of the other phases. Metaphase and anaphase follows within the frequency a prophase and telophase. It is shown that the effluent

produces variable effect on each phase of the cell division has been mentioned in the Table-3, observation has been shown by bar diagram in Fig. 2.

Table 1: Mean values of Different parameters of effluent of Orion Paper mill.

| Parameters | Mean values |
|---|----------------|
| Temperature (°C) | 33.75 ±5.40 |
| pH | 8.0 ±0.27 |
| Turbidity(NTU) | 28.39 ±4.49 |
| Electric Conductivity(dSm ⁻¹) | 1.12 ±0.13 |
| Total Alkalinity(mg L ⁻¹) | 257.8 ± 98.32 |
| Total Acidity(mg L ⁻¹) | 24.6±9.47 |
| DO(mg L ⁻¹) | 1.00 ±0.58 |
| BOD(mg L ⁻¹) | 310.28 ±46.75 |
| COD(mg L ⁻¹) | 572.6 ±99.32 |
| Chloride(mg L ⁻¹) | 461.6 ± 32.96 |
| Total Hardness(mg L ⁻¹) | 280.8 ±42.39 |
| Calcium(mg L ⁻¹) | 102.8 ± 24.75 |
| Magnesium(mg L ⁻¹) | 43.1 ±5.91 |
| Nitrate Nitrogen(mg L ⁻¹) | 67.8 ±8.27 |
| Nitrite Nitrogen(mg L ⁻¹) | 58.0 ±8.45 |
| Phosphate(mg L ⁻¹) | 35.7 ±3.54 |
| TDS(mg L ⁻¹) | 2525.8 ±422.38 |
| TSS(mg L ⁻¹) | 784.2±140.12 |

3.2. Chromosomal abnormalities: Roots of growing Onion bulb were treated with effluents for 12, 24 and 48 hours. Slide of mitotic plates were prepared adopting similar methods as mentioned in previous section. Through microscopic observation of cytological slide, Chromosomal anomalies were scored randomly at different phases of the cell cycle. The frequency of each type of aberration has been calculated as percentage of cells showing a particular kind of aberration in respect to the total number of aberrant cells. The observed data has been incorporated in Table – 4 shown in Fig. 1 - 6 (Plate –III). The commonest type of Chromosomal anomalies observed at different phases were – (i) Slight stickiness (ii) Laggard (iii) C – mitosis (iv) Bridges (v) Multipolarity / change in Spindale orientation (vi) Fragmentation.

Table 2: Mean Mitotic index of root meristem of *Allium cepa* following treatment for different periods (h) of time with different concentrations of Paper mill effluent. (Figures indicate mean values of 5 observations ±S.D.)

| Treatment Period | Effluent Concentration | | | | |
|------------------|------------------------|----------------|----------------|---------------|---------------|
| | 0 | 25 | 50 | 75 | 100 |
| 24 hr. | 23.41 ±4.12 | 18.06 ±4.67 | 14.04 ±2.04 | 9.27 ±2.36 | 7.61 ±2.06 |
| 48 hr. | 23.41 ±4.12 | 16.32 ±3.40 | 13.64 ±2.17 | 0.00 0.0 | 0.00 0.0 |
| 72 hr. | 23.41 ±4.12 | 13.89 ±2.05 | 11.37 ±2.45 | 0.00 0.0 | 0.00 0.0 |

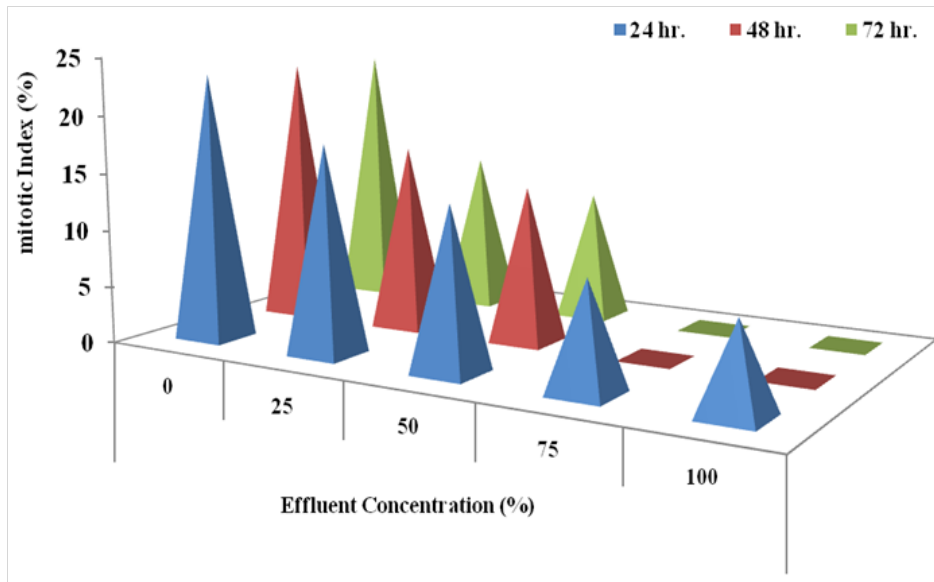


Fig. 1: Shows Mitotic index of root meristem of *Allium cepa* following treatment for different periods (hr) of time with different concentrations of effluent

Table 3: Mean Mitotic index of root meristem of *Allium cepa* following treatment for different periods (h) of time with different concentrations of Paper mill effluent. (Figures indicate mean values of 5 observations \pm S.D.)

| Treatment period (Hrs.) | Effluent concentration in (%) | | | | |
|--|-------------------------------|-------|-------|-------|-------|
| | 0 | 25 | 50 | 75 | 100 |
| PROPHASE (Frequency expressed in %) | | | | | |
| 24 | 36.50 | 42.52 | 47.53 | 56.92 | 69.84 |
| 48 | 36.50 | 48.03 | 56.48 | 0.00 | 0.00 |
| 72 | 36.50 | 56.85 | 66.52 | 0.00 | 0.00 |
| METAPHASE | | | | | |
| 24 | 28.26 | 22.69 | 20.55 | 18.45 | 16.04 |
| 48 | 28.26 | 21.04 | 17.45 | 0.00 | 0.00 |
| 72 | 28.26 | 16.82 | 13.17 | 0.00 | 0.00 |
| ANAPHASE | | | | | |
| 24 | 20.13 | 17.86 | 16.83 | 12.85 | 8.45 |
| 48 | 20.13 | 14.27 | 11.87 | 0.00 | 0.00 |
| 72 | 20.13 | 11.20 | 9.54 | 0.00 | 0.00 |
| TELOPHASE | | | | | |
| 24 | 15.14 | 16.90 | 15.09 | 11.78 | 5.67 |
| 48 | 15.14 | 16.66 | 14.20 | 0.00 | 0.00 |
| 72 | 15.14 | 15.13 | 10.77 | 0.00 | 0.00 |

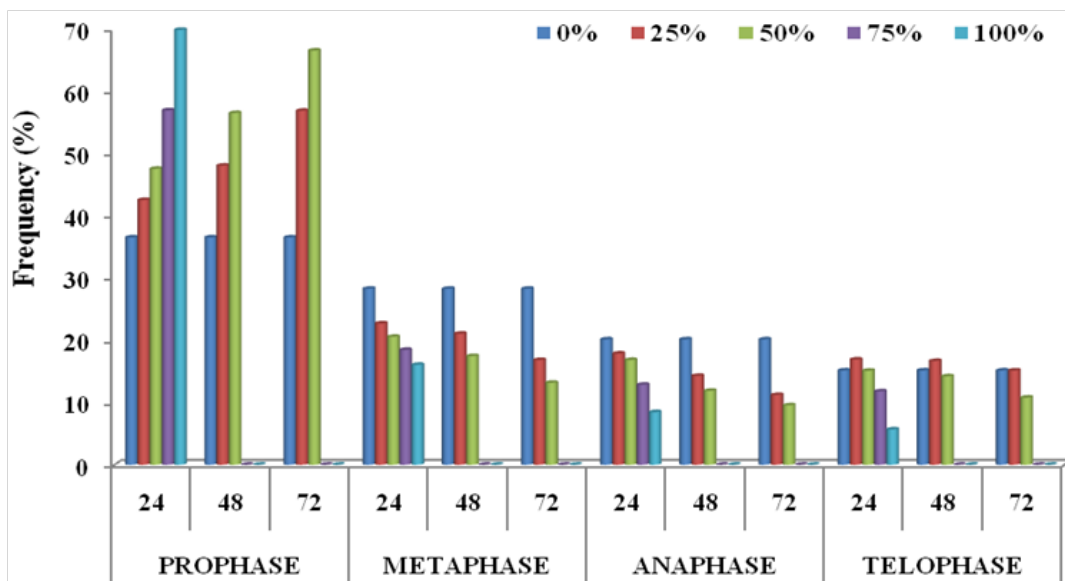


Fig. 2: Shows changes induced by Paper mill effluent in mitotic phase in Root meristem of *Allium cepa*.

Table 4: Frequencies (%) of chromosomal aberrations in root meristem of *Allium cepa* treated for different periods of time with effluent collected from Paper Mill. (Figure indicate mean values of 3 observations)

| Time (h) | Effluent concentration | Abnormalities % | | | | | | Total Abnormalities |
|----------|------------------------|-----------------|---------|-----------|---------|---------------|---------------|---------------------|
| | | Stickiness | Laggard | C-mitosis | Bridges | Multipolarity | Fragmentation | |
| 24 | 0 | 0 | 0 | 0.6 | 0 | 0 | 0 | 0.6 |
| | 25 | 8.06 | 6.07 | 10.23 | 5.65 | 32.06 | 6.55 | 68.62 |
| | 50 | 10.06 | 7.45 | 22.52 | 6.82 | 35.34 | 9.12 | 91.31 |
| | 75 | 15.62 | 10.5 | 28.34 | 9.18 | 29.76 | 19.04 | 96.82 |
| | 100 | 17.07 | 13.06 | 30.76 | 10.06 | 26.9 | 28.95 | 126.8 |
| 48 | 0 | 0 | 0 | 0.8 | 0 | 0 | 0 | 0.8 |
| | 25 | 12.67 | 10.78 | 15.36 | 9.38 | 25.54 | 15.35 | 89.08 |
| | 50 | 13.5 | 16.45 | 19.84 | 11.09 | 24.89 | 20.72 | 106.49 |
| | 75 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 72 | 0 | 0 | 0 | 0.4 | 0 | 0 | 0 | 0.4 |
| | 25 | 16.32 | 14.74 | 18.42 | 10.65 | 24.56 | 25.96 | 110.65 |
| | 50 | 18.97 | 19.45 | 24.05 | 11.88 | 22.72 | 33.32 | 130.39 |
| | 75 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

In present context the cytotoxicity has been studied using *Allium cepa* as a test material and treated with different concentration of effluents and its cytological study was performed. Several abnormalities laggard, fragmentation, stickiness, bridge were observed have been presented in figure and scored in table. Obviously the cytological or chromosomal anomalies were due to toxic action of chemicals/ metals present in the effluents. The occurrence of breaks at the same locus and their subsequent lateral fusion help the formation of di-centric chromosomes, which is pulled apart both the poles at anaphase leading to the formation of bridge. Stickiness has been induced by several heavy metals compounds, fungicides, and pesticides that are entering human environment. The frequency of stickiness by paper mill effluent may be attributing to chemical reaction between the effluent and pellicle protein of the chromosome resulting into stickiness of the chromosome.

Stickiness of chromosomes observed in the treated cells reveal the depolymerisation effect of effluent on the nucleic acid of the chromosomes Soheir E *et al.* [12] reported a high level of chromosomal stickiness in metaphase stage after herbicide treatment Patil B *et al.* [13] reported that stickiness is a type of physical adhesion involving mainly the proteinacious matrix of chromatin material. The formation of small fragments can be attributed to the chromosomal breakage due to the effect of effluents.

The effluent had induced c-mitosis configuration at metaphase and anaphase, similar to the action of colchicine. The presence of high frequency of cells showing c-mitosis as a result of treatment with effluent suggest that effluent is more toxic than colchicine which by its interference with –SH group can prevent organization of the spindle and also destroy already organized spindle, although its dose not exhibit onset of mitosis.

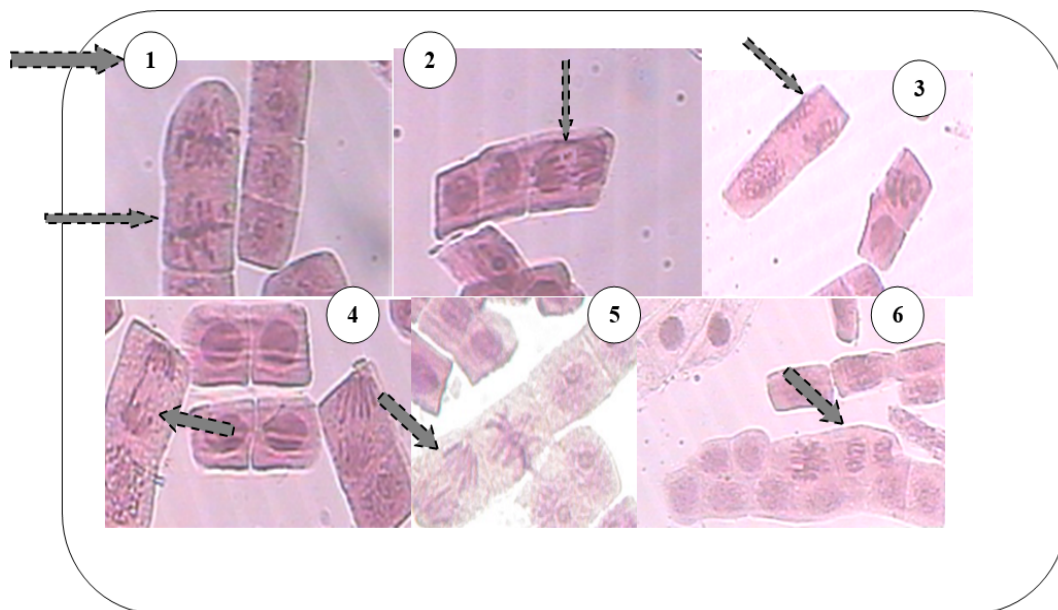


Fig 3: Shows Plate (1) Slight stickiness (2) Laggard (3) C – mitosis (4) Bridges (5) Multipolarity / change in Spindale orientation (6) Fragmentation.

4. Conclusion

The paper mill effluent in the present investigations has been found to induce lagging chromosome, which might constitute micronuclei suggesting genetic damage. If laggard chromosome per chance is included in any of the two nuclei, it results into the formation of aneuploid cells.

From the above observation it is evident that silk dyeing effluents are potential genotoxic agents in the environment. The abnormalities manifested may be due to the action of organic and inorganic traces present in the effluent and also might be exerted by the microorganisms whose secretion cause cytological damage Rangaswamy, V *et al.* ^[14]. Hence extensive pollution control measures are to be taken to prevent the lethal damage caused by the flow of untreated pollutants. Further the use of eco-friendly dyes should be encouraged in view of the safety of the future generations.

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