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Biomonitoring of cytotoxicity of industrial effluents

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Abstract
The harmful effects of industrial effluents are becoming a worldwide concern. The present work aims in studying the characteristics of paper industry effluent and its influence on cytogenetic characters. By evaluating the effects of different effluent concentration of 100%, 50% untreated, pre treated effluent and distilled water at 24 and 48 hours, observed a concentration exposure- depend manner in relation to cell death feature. The results showed that the toxic effect of effluent increases the frequency of chromosomal abnormalities, decrease the percentage of germination, inhibit root growth, and induction of cell death in effluent treated root tip. The percentage of germination, root length and mitotic index decreased on treatment with untreated factory effluent. The clastogenic aberrations observed are clumping, cell with budding, amitotic index, macronucleus, strap shaped nucleus, abnormal shaped cells, Chromosome Bridge and micronucleus. The pre treated effluent showed less chromosomal abnormalities, percentage of seed germination, inhibition of root growth, and induction of cell death. The untreated factory effluent was toxic on meristematic cells of plants. This plant bioassay can be used to evaluate the extent of environmental pollution caused by factory effluent, pesticides application, waste material dumping etc.

Keywords: Biomonitoring, cytotoxicity, micronucleus

1. Introduction
Healthy environment is a requisite for human life. Industrialization is considered as an index of modernization but rapid industrialization sometimes deteriorates the physical and chemical properties of water, air and soil. Industrial waste contain very large quantity of pollutants, especially the paper mills, textile and dye industries, chemical manufacturing plants; dumping organic and inorganic wastes usually in the rivers. The effluents released from such industries contain hazardous genotoxic chemicals, some of which will not undergo degradation during waste water treatment due to high degree of persistence (Rank and Nielson, 1998) [16]. This will cause several ecological problems and also harm organisms as well as human through food chain. Human exposure to the industrial waste have in the past cause a variety of genotoxic effect involving cancer, birth defect, heart disease and reproductive anomalies (Claston *et al.,* 1988) [4]. However the cytological effects of the industrial effluent are very little studied, especially from this part of the world. It also has public health significance, adversely affect fish diversity.

2. Materials and Methods
Seeds of *Trigonella foenum-graecum* (2n = 12) were used as plant material for bioassay. Two different concentrations of untreated (100% and 50%) and effluent after pretreatment are used. Distilled water is used as control. Genotoxic and bioassay studies were carried out by using seed germination studies, root growth analysis and cytogenetic analysis.

2.1 Germination test and root growth analysis
Seeds were socked in the effluent of different concentration of 100% and 50%, pretreated and distilled water at different time period of 6 and 12 hours. The treated seeds were randomly arranged on the blotting paper in a petri dish at room temperature for each concentration. The percentage of germination is recorded after 24 and 48 hours. The root lengths of at least 10 seeds are measured for each concentration. The same root was measured for each evaluation time.

2.2 Cytogenetic analysis

Seeds were socked in the effluent of different concentration of 100% and 50%, pretreated and distilled water at different time period of 6 and 12 hours. After exposure, the root tip ranging from 0.5 to 1.0 are collected from each treatment, washed in distilled water and fixed in ethyl alcohol: acetic acid (3:1 v/v) solution. Fixed root tips were hydrolyzed in 1 N HCL at 60^{O} for 5 minutes. Acetocarmin squash preparations are made and analyzed for cytological aberrations and mitotic index.

3. Results

3.1 Germination analysis

Percentage of seed germination decreases with the increasing the effluent concentration. In two types of treatments, 100% untreated effluent shows less percentage of germination. The significant differences were detected in pre treated effluent. The pretreated effluent shows more percentage of germination when compared to concentrated 100 and 50% untreated. The control shows high percentage of germination (Table 1). The seed of 6 hour pre socked shows more percentage of germination than the seeds of 12 hour pre socked.

3.2 Root growth analysis

The result present in the table 2 shows the root growth in the test plant was reduced by all the concentration applied; as compared with control value. The inhibition of root growth was greater with increasing concentration of effluent. The seed of 6 hour pre socked shows more root length than that of seeds of 12 hour pre socked.

3.3 Cytogenetic analysis

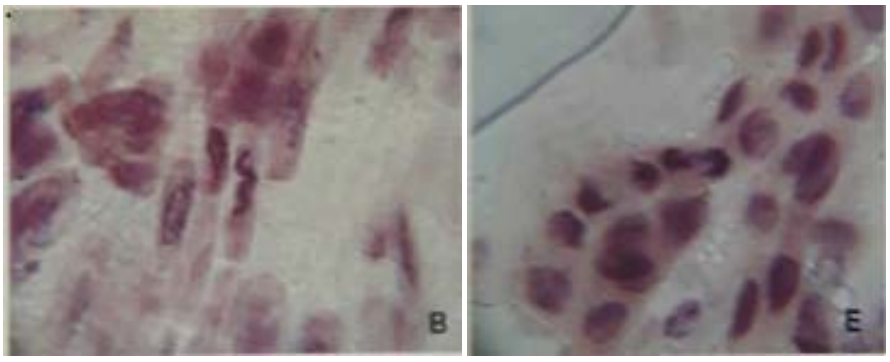
The effluent provoked strong inhibition of mitotic index, where significant difference in relation to control can be noticed. The cytological aberration include amitotic division, cell with budding, clumping, micronucleus, multinucleus, chromosome bridge, strap shaped nucleus, abnormal shaped nucleus.

Table 1: Table shows percentage of germination

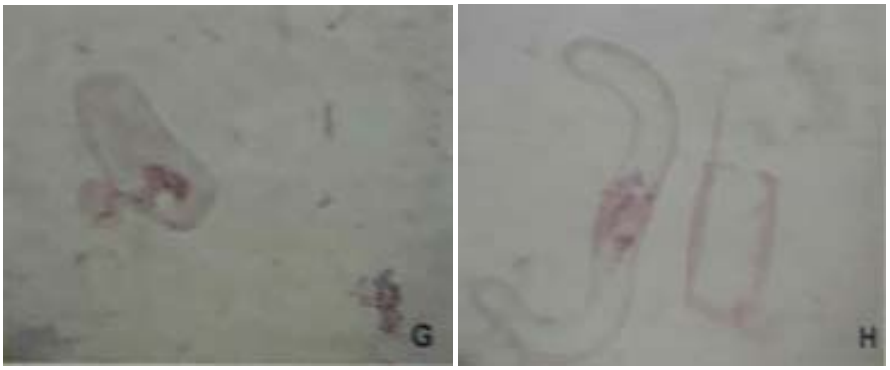
Sample	Time period	Concentration of effluent	Root length (in cms)
Effluent treatment 6 hrs	After 24 hrs	100% untreated	91.5%
		50% untreated	92.8%
		Pre treated	81.1%
		Distilled water	92.7%
	After 48 hrs	100% untreated	92.1%
		50% untreated	92.8%
		Pre treated	90.9%
		Distilled water	94.7%
Effluent treatment 24 hrs	After 24 hrs	100% untreated	85.6%
		50% untreated	85%
		Pre treated	89%
		Distilled water	92.7%
	After 48 hrs	100% untreated	89.4%
		50% untreated	88.2%
		Pre treated	92.2%
		Distilled water	94.7%

Table 2: Table shows Root length

Sample	Time period	Concentration of effluent	Root length (in cms)
Effluent treatment 6 hrs	After 24 hrs	100% untreated	0.91
		50% untreated	0.97
		Pre treated	1.00
		Distilled water	1.74
	After 48 hrs	100% untreated	3.51
		50% untreated	3.61
		Pre treated	3.73
		Distilled water	3.84
Effluent treatment 12 hrs	After 24 hrs	100% untreated	1.12
		50% untreated	1.31
		Pre treated	1.41
		Distilled water	1.74
	After 48 hrs	100% untreated	3.04
		50% untreated	3.07
		Pre treated	3.31
		Distilled water	3.38



a) Strap shaped nucleus b) Chromosome bridge



c) Cell with budding d) Abnormal shaped cell

Fig 1: Cytological aberration

4. Discussion

Plants are immobile organisms. Under the pollution stress roots are primarily in contaminants. The influence of environmental mutagens on a plant depends not only the type and dose of mutagen, exposure time and interaction with other factors, but also the plant species, genotype and stage of development (Gichner, 2000) [7]. The tests used in the present study are cytogenetic analysis; seed germination and root growth analysis.

It is obvious from the result of the investigation that the effluent is cytotoxic on meristramatic cell of the test plant, *Trigonella foenum graecum.* The cytotoxic effect has been evaluated at micro and macroscopic levels. Macroscopically, reduction of root growth, and a slight decrease in percentage of germination were observed. Microscopic observation of clastogenic alternation shows cell with budding, amitotic division, clumping, micronucleus, single bridges, multiple bridges, strap shaped nucleus. The result obtained shoes a decrease in mitotic index.

The present investigation on the effect of untreated and pretreated factory effluent shows a decrease in the percentage of germination, decrease in the root length and diminished mitotic index. The directly socked seeds for 6 and 12 hrs show less percentage of germination in untreated 100%, untreated 50% effluents than control. The seeds treated with pretreated effluents show almost equal to control. In the case f root length for all treatments the length observe after 24 hrs shows less than control, but for 48 hrs almost equal to control. The result obtained in the present study is similar to the result obtained by Ajibade (2007) [1].

The mitotic index exhibition is an evident effect of effluent on the plant test. The inhibition of mitotic index can also be attributed to the effect of environment chemicals on DNA or Protein synthesis of the biological system (Chauhan *et al*., 1998) [3]. These effects a manifested by induction of interphase arrest or cell death. By analyzing cells of the test plant exposed to effluent, the main cause of mitotic inhibition was verified to be the increase of cell death. The reduction in mitotic index may be due to the arrest of cells in G1 phase and retardation in pace of events during S or G2 phase (Linninmma *et al*., 1978) [10]. The cytogenetic analysis shows that the inhibition of root growth is due to toxicity of factory effluent, through disturbance of mitotic process and induction of chromosome aberration.

The sickness of clumping is one of the clastogenic aberration observed. Different views have been put forward to explain the cause of chromosome stickness. The metaphase with sticky chromosomes loss their normal appearance and they are seen with a sticky ‘surface’, causing chromosome agglomeration (Babich *et al*., 1997) [2]. The presence of this type of aberration reflects toxic effect of chromatin, which genetically leads irreversible to cell death (El-Ghamery *et al*., 2003) [6]. Stickness has been attributed to the effect of pollutants on the physical-chemical properties of DNA, Proteins or both. On the formation of complexes with phosphate groups in DNA or DNA condensation or on formation of inter and intra chromatid cross links (El-Ghamery *et al*., 2003) [6]. Darlington and Mc Leish (1951) [5] suggested that stickiness might be due to degradation or depolymrization of chromosomal DNA. Observed sticky chromosomes after ribonuclease treatment and have suggested that this is the result of dissociation of nucleoprotein. Verma (1959) [21] suggested that stickiness is due to interruption of protein metabolism caused by disturbance of RNA synthesis.

Klasterka *et al*, (1976) [9] and Mc-Gill *et al*, (1974) [12] interpreted that when the chromosome fiber fails to condense properly in preparation of mitosis they might get trapped and tangled with fibers of other chromosomes resulting in stickiness.

Other abnormalities observed in the present investigation are the cells with strap shaped nuclei and binucleate cells. Walum *et al*., (1990) [22] suggested that toxic substance might cause membrane structure alterations resulting in permeability changes by interference with lipid metabolism. Somashekhar and Gowda (1984) [19] have reported the suppression of cell plate formation was an essential step for the formation of binucleate cells.

Micronuclei are observed in the study. Micronuclei often result from the acentric fragments or lagging chromosomes that fails to incorporate into the daughter nuclei during telophase of the mitotic cell and can cause cell death due to the deletion of primary genes (Yi and Meng, 2003) [23]. reported the presence of micronuclei in the cytogenetic alteration induced by SPL meristematic cells of plant bioassay.

Chromosome Bridge is observer, in the present study. Sax (1940) is of opinion that Anaphase Bridge may be due to unequal exchange of dicentric chromosome. The occurrence of breaks at the same locus and their lateral fusion leads to the formation dicentric chromosome which is pulled equally to both the poles at anaphase to form a bridge (Nelson and Rank, 1994) [13]. According to Kabir and Alan (1980) [8] anaphase bridges might due to formation of dicentric chromosome as a result of breakage and reunion of broken chromosome. Chromosome bridges are formed by chromosome breakage and reunion (Tomkins and Grant, 1972; Shehab; 1985) [20, 18] and bridges devoid of acentric fragment might have arisen out of symmetrical recombinations among the broken ends of chromosome (Raj and Rao, 1972) [15]. Pronoti and Reghu Vanshi (1986) [14] also attributed bridge to the exchange in chromosome or chromatid fiber before metaphase although their manifestation is noted during anaphase or telophase.

Amitotic division observed in the presence study may be due to the stress condition produced by the factory effluent. The budding of the cell suggest amitotic division or unequal nuclear division of the cell.

The result of the present bioassay shows the untreated factory effluent is definitely cytotoxic on the meristramatic cells of the plants; causing alternation in percentage of seed germination, root growth, mitotic index. However the pretreated factory effluents have less genetoxic effect.

5. Conclusion

It can be concluded that untreated factory effluent (100%, 50%) is definitely cytotoxic on meristramatic cells of plants. The percentage of seed germination, root length and mitotic index was decreased in treatment with untreated factory effluent. The clastogenic aberration observed are clumping, cell with budding, amitotic index micronucleus and macronucleus, single bridge, multiple bridge and strap shaped nuclease. Pre treated factory effluent shows very less clastogenic aberration and percentage of seed germination, root growth and mitotic index is almost same as that of control. The above plant bioassay method can be used to evaluate the extent of environmental pollution caused by factory effluent, pesticide application and dumping of waste materials.

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