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Cytotoxicity and growth effects of industrial effluent on *Allium sativum*

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ABSTRACT

Industrial wastes play a major role in the environmental pollutants at least in major cities. The objective of the present work was to study the cytotoxic effects of common industrial effluent from PETL, Hyderabad on *Allium sativum*. As these effluents inhibited the growth of the roots which may be due to the inhibition of the mitotic cell divisions taking place in the root tips, the present investigation was focused to study the cytotoxicity in the root tip cells of *Allium sativum*. Mitosis division frequency, chromosomal aberrations, micronuclei formation and mitotic index were also considered for evaluation of cytotoxicity. The results showed a clear decline in the mitotic index with the increase in effluent concentration and the duration of treatment. The results also demonstrated other mitotic abnormalities like change in nuclear morphology, nuclear death, perforation, formation of micronuclei, fragmentation of chromosomes, bridge formations, nuclear bursting, and polyploidy.

KEYWORDS

Environmental pollution;
Industrial waste;
Mitotic index;
Allium sativum;
Cytotoxicity;
Growth retardation.

INTRODUCTION

Environmental pollution is the contamination of the physical and biological components of the earth systems where normal environmental processes are adversely affected. The increase of pollution by the release of genotoxic chemicals and the increase of radiation levels have affected the ecosystem and the health of organisms, including humans. Industries share a major portion of such pollutants at least in the major cities. There are many such industries in Hyderabad releasing the toxic chemicals into the environment which adversely affects the biota. Patancheru is one such area where there are more than 50 small and large scale industries located. These industries which includes chemical, pharmaceutical and steel plants send their organic and inorganic effluents to a common effluent treatment plant which is known as PETL [Patancheru Effuent Treatment Limited]. As there are different types of industrial effluents with various types of pollutants, the strength of the effluents in PETL is very high at any point of time.

There is a need for quick and precise methods for the detection and evaluation of air, water and soil contamination and their effects on organisms. As plants comprise a highly conserved structure of the genetic material, it is possible to use a broad variety of species in geno-toxicity tests. The most widespread methods are based on the use of bacterial indicator species, yeasts, fungi, insects and mammalian cells or laboratory rodents. Several higher plant bioassays for screening and monitoring industrial effluents have been established. The influence of industrial effluents on a plant depends not

only on the type of mutagen, exposure time, dose and interaction with other factors, but also on the plant species, genotype, and stage of development. Plant response to effluent treatment can be considered on different levels of organization from DNA, chromosome, and genome to the whole organism.

The problems affecting the environment are diverse, and approaches to find solutions are often intimately connected with modern and classical methods of biotechnology. The common industrial effluent from PETL was collected and physicochemical analysis was carried out. Several studies with *Allium sativum* are conducted to asses the toxicity of water bodies receiving industrial and domestic effluents[1-7]. Cytotoxic effects of industrial effluents, for the detection of abnormalities and chromosomal aberrations on root mitosis of *Allium sativum* is simple, less time taking and economically feasible.

The present work project therefore aims in studying the cytotoxic effects of industrial effluent collected from PETL, Hyderabad on root-tip cells of *Allium sativum* during a time-course. The endpoints considered are changes in the mitotic stages, mitotic index and frequencies of aberrant cells and micronuclei. The exposure to industrial effluents induced mitotic depression of abnormal cells to a degree directly proportional to the concentration used and the period of treatment up to 24 hrs, 48hrs, 72hrs, and 96hrs. The objective of the project is to study the effect of industrial effluents on the cytotoxicity with respect to cell cycle events, micro nuclei, and chromosomal aberrations in *Allium sativum*.

MATERIALS AND METHODS

All the reagents used in the experiment are of analytical grade. Standard approved methods were followed for the analysis of physico-chemical parameters of PETL effluents and cytotoxic studies.

Collection of industrial effluent and analysis

The effluent samples were collected from the PETL common effluent. The effluent samples were brought to the laboratory and were analyzed for physico-chemical parameters. The parameters considered were pH, Conductivity, Total Dissolved Solids, Suspended Solids, Chlorides as Cl^- Sulphates as SO_4, COD, BOD, Sulphides as S, Dissolved phosphates as P, % Sodium, and Oil & Grease. Standard protocols (APHA Standard Methods of Water and Waste Water Analysis) were followed for the analysis of physico-chemical parameters of the PETL effluents.

Collection of allium sativum cloves

Fresh healthy cloves were purchased from the local market. Medium, equal sized bulbs were selected and used for the study. Each group contained 4 such cloves.

Eexperimental setup and methodology for cytological studies

The common industrial effluents from PETL were taken for the cytotoxic studies. 25%, 50%, and 100% concentrations of the above industrial effluent were prepared and used for the experimentation. The test was carried out at room temperature and the garlic cloves were kept away from direct sunlight during the experiment. 200ml beakers were filled with the sample of various concentrations and cloves of *Allium sativum* were kept on the sample with the help of tooth picks. Potable tap water served as control to compare the results with PETL effluents. The growths of the roots were observed and the root lengths were taken for 24, 48 and 72 hrs. The cells were observed for mitotic division and for chromosomal aberrations. Simultaneously, controls were also maintained under identical conditions. For every 24 h the test solutions were replaced by fresh solutions and maintained at room temperature to observe root growth. Root lengths were measured with the help of a thread and scale and the results tabulated. After sufficient growth, the roots were cut off and placed in a test tube containing aceto-alcohol. The last 2mm from several young vigorously growing root tips were removed with the help of scissors. These root tips of both the treated and controls were taken and fixed in aceto-alcohol for cytological preparation. The observations were recorded from 5000 cells selected from each concentration and cytological observations were made using aceto-orcein stain and squash technique. Mitotic index for each slide examined (the mitotic index is the fraction or percentage of cells containing condensed chromosomes) was calculated using the following formula.

Mitotic index = number of cells containing chromosomes / Total number of cells.

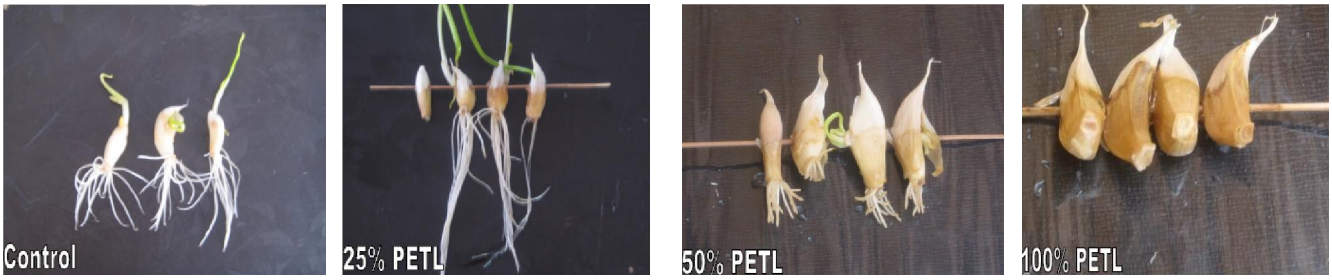

Figure 1 : Photograph showing the growth of the roots with various concentrations of PETL. Tap water served as control

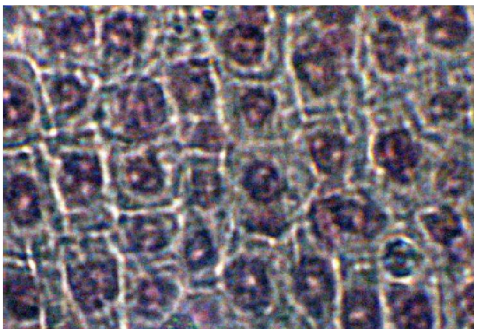

Figure 2 : Photograph showing microscopic view of root tip cells of *Allium sativum* after 24 h growth in 50% PETL effluent with a few mitotic divisions

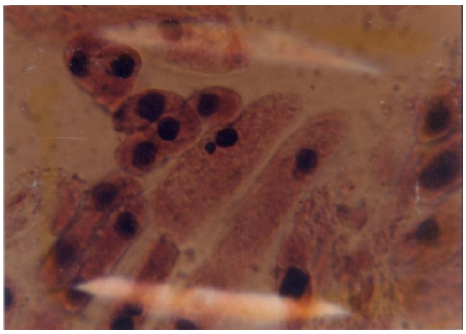

Figure 3 : Photograph showing the microscopic view of micro nuclei formation in the root tip cells of *Allium sativum* after 24h growth in 50% PETL effluent

RESULTS AND DISCUSSION

The effluents collected from PETL were initially analyzed for physicochemical parameters. TABLE 1 shows the results of various parameters analyzed to tabulate toxicity in PETL. The BOD and COD levels were high enough to affect the cell division and cause cytotoxicity. The levels were very much higher than the standard limits (TABLE 1) given by pollution control board. The effluent BOD was 3810mg/lit, where it should be 30 mg/lit according to the Pollution Control Board. The COD was 6730mg/lit, against 250mg/lit according to the Pollution Control Board. The remaining parameters considered were also higher than the standard limits, but not much higher as BOD and COD. As the strength of the effluent was high it was decided to study the effect on cytological parameters.

The results of experiments conducted to study the cytotoxic effects under pollutant was very good showing reduction in the growth of root lengths probably due to a wide range of mitotic abnormalities which include change in nuclear morphology, nuclear death, perforation, micronuclei, fragments, bridges, nuclear bursting and polyploidy. The results showed that there is a drastic inhibition in root growth with increasing concentrations of the effluent. Very little or no growth was observed in 50% and 100% effluent (Figure 1). Surprisingly, with 25% effluent concentration the growth was higher than control (Figure 1). Probable reason behind this would be the ratio of organic content to inorganic content in the effluent. It is proved that effluent waste could be utilized in plant growth for their organic contents[8]. Organic content present in the effluent might have helped in the growth of the *Allium sativum* where the inorganic strength might have been less to inhibit the growth.

It is recognized that environmental chemicals could damage the genetic integrity of plants, animals and humans. Unfortunately, different agents produce variable proportions of different types of genetic injury, such as base substitutions, frame shift mutations, translocations and chromosomal deletions. Thus it is not surprising that there is no single method available for the screening of all geno-toxic substances. Somatic cells are easier to analyze, but since the type of event scored is generally not heritable, one must extrapolate to the gonadal tissues (meiotic cells) in order to judge genetic risk and other tissues to judge carcinogenic risk. In general, more relevant the aberrations are to genetic risk, the more effort is required to detect them. The use of micronuclei to assay chromosomal damage is consistent with this pattern. Chromosomal aberrations produced in a target cell population can be measured more easily by monitoring micronuclei appearing in a descendent cell

TABLE 1 : Physico-chemical analysis of the industrial effluent

Sr.No	Parameters	Amount in mg/lit
1	pH	8.3
2	Conductivity	4.3
3	Total dissolved solids	2940
4	Suspended solids	320
5	Chlorides	720
6	Sulphates	360
7	COD	6730
8	BOD	3810
9	Sulphides	0.9
10	Dissolved Phosphates	1.1
11	%Sodium	91%
12	Oil & Grease	9

population rather than by direct metaphase analysis[9]. Thus although the measure of genetic risk is not direct, less effort is required to measure it. To date micronuclei assays have been used with a variety of target organisms and cells including plants. Micronuclei have been recognized as indicators of genotoxic damage. It is generally accepted that micronuclei arise primarily from chromosome fragments that are not incorporated into daughter nuclei at the time of cellular division[10]. They may also be generated from whole chromosomes that are excluded from the telophase nucleus although the loss of whole chromosome is much a less common result of chemicals than in chromosome breakage[11]. The principal mechanism by which micronuclei are thought to be formed is presented below.

Abberations induced by clastrogens during interphase may include an accentric chromosomal fragment. Centromere is the organelle of chromosome movements during anaphase. If this fragment is not caught up in the cytoplasmic flow when the chromosomes are drawn to the poles of the daughter cells, they appear in the cytoplasm as a micronucleus i.e. a small, round chromatin positive body which stains like the nucleus. In *Allium sativum* (Garlic cloves), root meristamatic cells are a suitable cell population in which the frequency of micronuclei can be monitored. *Allium sativum* offers the best condition for this experiment first on account of their small size yet with many roots and secondly one garlic bulb yields several cloves. This makes the entire material of the same genetic origin. The root meristems of *Allium sativum* are employed for assaying micronuclei, and mitotic indices.

In the current study, root meristems were immersed in various concentrations of PETL industrial effluents from 25%, 50%, and 100%. Duration of the treatment was 24 and 48 h. Root tips were randomly processed following standard aceto-orcine technique after fixing them in 1:3 aceto-alcohol and 5000 to 5700 cells were scored to determine mitotic index. Mitotic index was severely reduced in 50% concentration of the effluent (Figure 2). In 100% PETL effluent treated bulb showed complete root growth inhibition (Figure 1). Tests conducted showed a decrease in the cell division frequency with increasing concentrations of the effluent at 50% (Figure 3). The reason for this change would have been due to micronuclei formed as a reason of chromosomal breakage and consequently forming a cluster. This was clearly observed in the study (Figure 2 & 3).The mitotic index also indicates the decrease in mitotic divisions.

In conclusion, the present work indicates a cost effective method to assess the cytotoxic effects of industrial effluents using the *Allium sativum*. These effluents inhibited the growth of the roots which may be due to the inhibition of the mitotic cell divisions happening in the root tips. The results also showed concomitant decline in the mitotic index with the increase in effluent concentration and duration of treatment time.

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