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Research Article

Assessment of Cytotoxic Effects of Coal-fired Thermal Power Plant Effluents on *Allium cepa* L.

Bikash Kalita[1], Dikshit Goswami[1], Mohesh Gogoi[2], Prantik Sharma Baruah[3]

[1] *Department of Botany, Gauhati University, Guwahati 781014, Assam, India*
[2] *Department of Botany, Science College, Kokrajhar 783370, Assam, India*
[3] *Department of Botany, Nowgong College (Autonomous), Nagaon 782001, Assam, India*

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Abstract

Rapid industrialization and the consequent release of effluents containing heavy metals pose a significant threat to ecosystems. This research aimed to evaluate the cytotoxic effects of effluents from a coal-fired thermal power plant on *Allium cepa* L. as a representative organism. Water samples collected from the plant's effluent outlets were analyzed for heavy metal content, revealing the presence of lead (Pb), manganese (Mn), and zinc (Zn). *A. cepa* bulbs were subjected to different concentrations of industrial water samples, and cytological analyses were conducted to assess mitotic index (MI) and chromosomal aberrations (CA). The study observed a notable reduction in MI and an increase in CA in response to higher effluent concentrations, indicating cytotoxicity. Additionally, root growth was stunted in a concentration-dependent manner. These findings highlight the potential environmental hazards associated with coal-fired thermal power plant effluents, emphasizing the importance of stringent pollution control measures and sustainable industrial practices to mitigate adverse impacts on ecosystems.

INTRODUCTION

The rising human population and increasing demand for essentials and commodities have led to rapid industrialization. Apart from meeting the demands, the industries release effluents containing heavy metals, which alter the natural state of different components of the ecosystem. Some industries have inadequate sewerage systems and thus discharge their waste into natural vegetation, agricultural fields, and aquatic habitats (Barthakur, 1995). Most industrial wastewater is characterized as mixtures that are very complex and contain various inorganic and organic compounds (Rank and Nielsen, 1994). A crucial review of the literature revealed that a good number of reports are available on the effect of industrial effluents on the productivity of different crops (Thangapandian, 1995; Chhonkar et al., 2000; Raia and Khan, 2010; Hossain et al., 2010; Arjun et al., 2013).

Thermal power plants, the principal source of electricity generation for any developing country, meet about 60 percent of our country's electricity generation. Thermal power stations are now regarded as the biggest global concern, as they are responsible for generating several environmental issues that are associated with using coal as a fuel in thermal power plants, leading to potential degradation of the surrounding environment. National Thermal Power Corporation (NTPC) is a major industry in the BTR region of Assam, India, which generates electricity by using coal and produces a considerable number of effluents. The thermal power industry has used a large amount of water to cool the steam turbine condenser. It returns water to the environment at a temperature that may be markedly higher than the actual temperature. During the rainy season, overflowing chemical oil and industrial effluents flowing from the NTPC canals in Salakati, Kokrajhar of Assam affect croplands along with aquatic organisms and reduce soil fertility (Basumatary, 2018).

Various test systems have been used to determine the cytotoxic effects of contaminated water.

Evaluation of chromosomal aberration in plants is one of the few direct ways to assess the damage to the living systems exposed to a toxic environment. Higher plants are known to be a good cytotoxicity indicator in the biological system, both because of their high sensitivity to detect toxicity in diverse conditions and because of their ability to evaluate such effects through several genetic endpoints, from point mutation to chromosome aberration. *Allium cepa* L. (commonly known as onion) is known as one of the best model plants to assess the cytotoxicity effect with fast response time, good correlation with an easy-to-handle test system, low cost, and large chromosomes with reduced number (2n=16) (Fiskesjö, 1985; Leme and Marin-Morales, 2009). To evaluate the genotoxicity and cytotoxicity of effluents released by industries *A. cepa* test has been used by several researchers (Chauhan et al., 1999; Grover and Kaur, 1999; El-Shahaby et al., 2002; Babatunde and Bakare, 2006; Júnior et al., 2007; Sik et al., 2009; Samuel et al., 2010). The assessment of cytotoxic effects of coal-fired thermal power plant effluents on *Allium cepa* L. is an essential aspect of environmental monitoring. *A. cepa* has been widely used as a bioindicator in ecotoxicological studies due to its sensitivity to various environmental pollutants, including heavy metals and chemicals (Düsman et al., 2014; Geremias et al., 2011; García-Medina et al., 2020).

Assessing the cytotoxic effects of industrial wastewater in India is crucial for understanding the potential impact on the environment and human health. Therefore, the present study aimed to investigate the cytotoxic effect of the effluent of a coal–fired thermal power plant on the nearby rice fields studied by using *A. cepa* as a test organism along with the analyses of heavy metals present in effluents.

MATERIALS AND METHODS

Collection and analysis of water samples

The water sample was collected near the effluent outlets of NTPC and filtered off the sample to remove particulate matter. Samples were analyzed for the presence of potentially mutagenic heavy metals- Lead (Pb), Iron (Fe), Manganese (Mn), Chromium (Cr), Zinc (Zn), and Magnesium (Mg) with the help of an Atomic Absorption Spectrophotometer (Russell et al., 1957). The pH of the water sample was recorded with a pH meter in the laboratory.

Preparation of plant samples

Allium cepa L. has been used as a model plant to evaluate the mitotic index (MI) and chromosomal aberrations (CA). Healthy bulbs of *A. cepa* were collected from the market and washed thoroughly to clean under running tap water. The outer surfaces of the disc were scraped with a scalpel to remove the remnants of old roots and were placed in distilled water to initiate rooting.

Preparation of different concentrations of industrial water sample

Filtered samples were diluted with the appropriate amount of distilled water to prepare different concentrations for testing, such as 25%, 50%, and 75%. The undiluted effluent is taken as 100%. The distilled water was used as a control for comparison.

Transfer of onion bulb from distilled water to industrial water samples

When the new roots in the distilled water reached a length of 1.5-2.0 cm, the onion bulbs were transferred into beakers containing different concentrations to grow (25%, 50%, 75%, and 100%) of the effluent samples. To see CA, a single duration of exposure (48 h) was applied based on Tripathy et al. (2013), who reported that in the case of 48 h of exposure, the result was more prominent.

Cytological analysis

The root tips were excised (~5 mm) from each concentration as well as from the control and washed with distilled water. The washed root tips were finally kept in a saturated aqueous solution of para-dichlorobenzene (pDCB) for 4 hours, followed by fixation in acetic acid: ethanol (1:3) solution for about 20-24 hours, washed in 70% alcohol before storing in 70% ethanol for further use.

Figure 1. Onion bulbs in different effluent concentrations with control. (A) control, (B-E) treatments at 25%, 50%, 75%, and 100%)

The fixed root tips were transferred in aceto-orcein solution and heated for a few seconds on a spirit lamp, and placed in a clean and grease-free slide (SP and Talukdar, 2002; Sarma et al., 2017). The tip portion (~2 mm) is cut, covered with a cover slide, and squashed by applying uniform pressure to disperse the cell and observed under a microscope (100X) to count the mitotic cells. Thereafter, MI was evaluated by analyzing at least 1000 cells per treatment (Tanti *et al*., 2009). The mitotic index was calculated and expressed as

$$\text{Mitotic Index (MI)\%} = \frac{No.of\ dividing\ cells}{Total\ no.of\ cells\ observed} \times 100$$

Some photographs of selected abnormal cells were taken to represent the chromosomal aberrations found. CA was calculated for each concentration and the values were expressed as

$$\text{Chromosomal Abnormalities (CA)\%} = \frac{No.of\ aberrant\ cells}{No.of\ dividing\ cells} \times 100$$

RESULTS

Analysis of the water samples

Atomic Absorption Spectrophotometric analysis of the water samples collected from the site showed the presence of heavy metals such as Pb, Mn, and Zn, and the pH was found to be around 7.6 (Table 1). The recovery R (%) of Mn was found to be 0.79%, whereas Pb and Zn have an R% of 0.79 and 0.078, respectively (Table 1).

Table 1. pH and recovery R (%) of heavy metals analysis of NTPC industrial effluent.

Collection Site	pH	Pb	Fe	Mn	Cr	Zn	Mg
NTPC	7.6	0.706	-	0.79	-	0.078	-

Effect of effluent on the mitotic index (MI) and chromosomal aberration (CA)

The mitotic index (MI) is characterized by the total number of dividing cells upon the number of cells observed in percentage. It has been used as a parameter to assess the cytotoxicity of several agents. The control shows the highest MI (15%). The MI is greatly affected by the various concentrations of the water sample. Higher concentration (100%) of the effluent shows lower MI (2.7%) (Table 2). The chromosomal aberration (CA%) of the 100% concentration of effluent was found to be the highest (66.7%), while a gradient of CA% was seen from lower to higher of the different concentrations accordingly (Table 2).

Table 2.: MI% and CA% of different concentrations at 48 h.

Concentration	No. of cells scored	No. of dividing cells	No. of aberrant cells	Mitotic Index (MI) %	Chromosomal aberration (CA) %
Control	1026	154	4	15	6.25
25%	1012	96	36	9.4	24.4
50%	1016	47	21	4.6	36.1
75%	1013	34	19	3.4	51.9
100%	1021	28	16	2.7	66.7

Various chromosomal aberrations were seen in the aberrant cells, which include C-metaphase, nuclear disintegration, vagrant chromosomes, chromosome bridge at anaphase, multipolar anaphase, sticky metaphase, binucleate cells, etc. C-metaphase, nuclear disintegration, binucleate cells, and sticky metaphase were found to be in higher frequency in all concentrations. Photos collected during the observation of the aberrant cells show the chromosomal aberrations distinctly, which are given below (Figure 3).

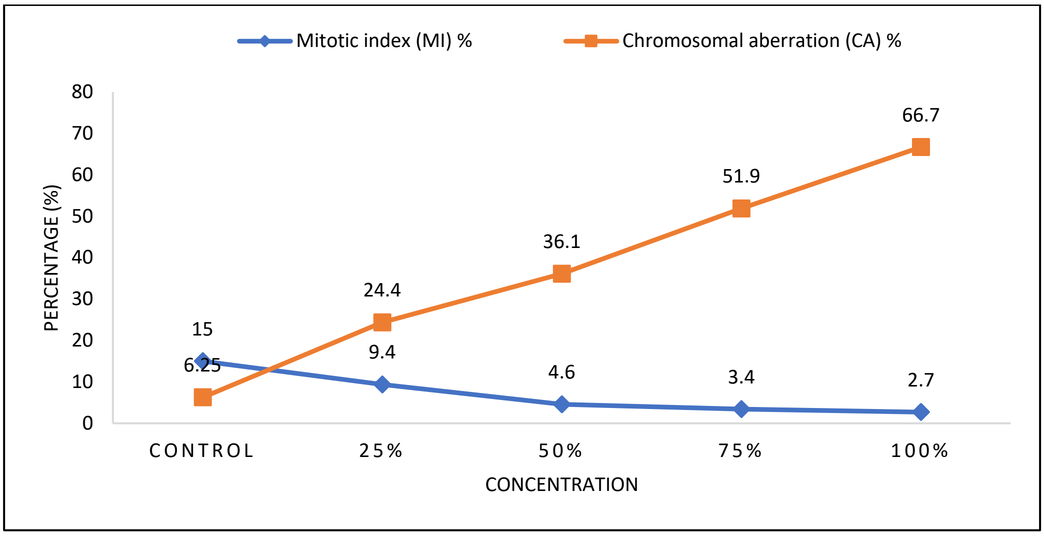

Figure 2. Scatter plot showing Mitotic index (MI)% and Chromosomal aberration (CA)% at different concentrations of effluents.

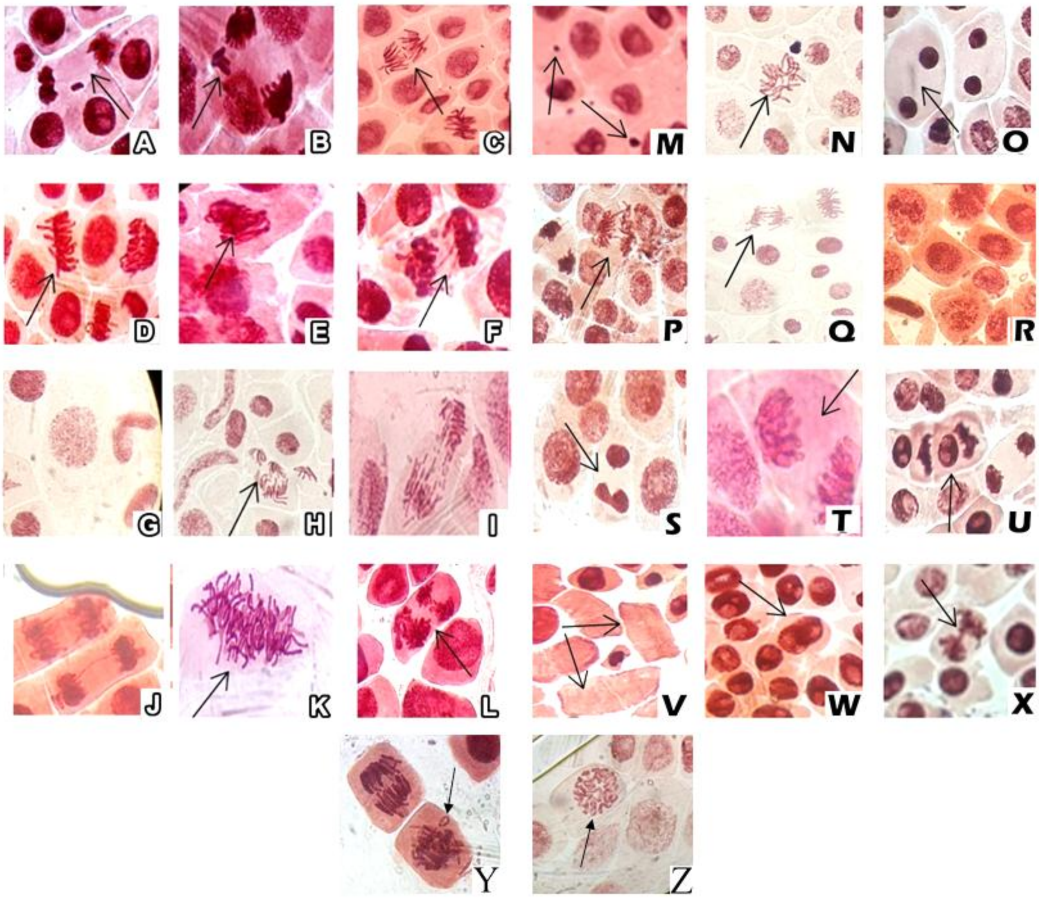

Figure 3. Chromosomal aberrations found in the root tips of *A. cepa* under the treated concentrations of effluents- (A) Laggard chromosome at telophase, (B) Large fragment of chromosome Loss, (C) Disturbed anaphase with fragments, (D-E) Sticky metaphase, (F) Anaphase with double bridge, (G) Lobulated nucleus, (H, L) Multipolar anaphase, (I) Anaphase with break and vagrant chromosome, (J) Anaphase with single bridge, (K) Polyploidy, (M) Small and Large micronucleus, (N) C-Metaphase, (O) Binucleate Chromosome, (P) Nuclear disintegration, (Q) Nuclear Lesson, (R) Fragmented Prophase, (S) Nuclear bud with a nuclear, (T) Diagonal metaphase, (U). Disturbed anaphase, (V) Ghost Cell, (W) Resident nucleus at prophase, (X) Sticky anaphase, (Y-Z) Chromosomal ring.

Moreover, the root growth was seen stunted gradually from the lower concentration to the higher one. The root length of the control was found to be around 11.4 cm, whereas it was found to be 9.9 cm, 8.7 cm, 5.1 cm, and 3.8 cm for 25%, 50%, 75% and 100% concentration of effluent, respectively (Table 3)

Table 3. Root length under different concentrations of effluent expressed as mean ± SD.

Concentration	Control	25%	50%	75%	100%
Root length (cm)	11.4 ± 0.3	9.9 ± 0.5	8.7 ± 0.3	5.1 ± 0.4	3.8 ± 0.5

DISCUSSION

Chromosomal aberration is a change in the structure or number of chromosomes due to various physical and chemical treatments. In the present study, a cytotoxic study on *A. cepa* was carried out using collected effluent from the thermal power plant based on changes in mitotic index and other induced abnormalities. The result showed positive responses under the chromosomal aberration assay when compared with the control. The analysis of the thermal power plant effluent contaminated water sample showed the presence of heavy metals such as Pb, Mn, and Zn. The presence of heavy metals in thermal power plant effluent was also reported by Javed and Usmani (2016), which were biomagnified in the human body through the consumption of freshwater fish inhabiting the thermal power plant effluent-loaded water body. The cytotoxicity level of an agent can be determined by the increase or decrease in the mitotic index (Leme and Marin-Morales, 2009). These heavy metals decrease mitotic index and induce chromosomal aberration in plants (Sabeen et al., 2020). Smaka-Kincl et al. (1996) showed that the decrease in the MI of *A. cepa* meristematic cells can be considered a reliable method to determine the presence of cytotoxic agents in the environment and, thus, can be regarded as a sensitive test to estimate pollution levels. In this study, we also found a decrease in the mitotic index and several chromosomal aberrations in the mitotic cells, such as C-metaphase, nuclear disintegration, binucleate cells, and sticky metaphase. Truta et al. (2011) reported lead-induced genotoxicity in wheat with similar chromosomal aberrations. Doroftei et al. (2010) reported smaller mitotic index and chromosomal aberrations in the case of manganese and lead treated *A. cepa.*

The genotoxic effect of zinc and lead treatment in Bambara groundnut was reported by Oladele et al. (2013). Rank and Nielsen (1994) evaluated the suitability of the *Allium* test in the screening of wastewater for genotoxicity. Grover and Kaur (1999) have assessed the genotoxicity of wastewater samples from sewage and industrial effluent using *Allium* root anaphase aberration, while Migid et al. (2007) evaluated the efficiency of algal biofilters in bioremediation of toxic industrial effluent using *Allium* genotoxicity bioassay. We also found reduced root growth and a decrease in root length due to the effluents, which indicate the treated effluent is harmful to all the essential aspects of plant life. The 100% concentration of effluent showed the most petite root length. Reduction of root growth was reported by Ivanov et al. (2003) of *Zea mays* L. in the presence of heavy metals.

CONCLUSION

The study demonstrates that effluents from a coal-fired thermal power plant, particularly those containing heavy metals, have significant cytotoxic effects on root meristem of *Allium cepa* L. The observed reduction in mitotic index, increase in chromosomal aberrations, and stunted root growth indicate potential environmental hazards. Industries must adopt proper wastewater treatment processes to minimize the impact on the ecosystem and agricultural lands before discharging effluents into the environment. These findings emphasize the importance of stringent pollution control measures and sustainable industrial practices to mitigate the adverse effects on ecosystems and human health.

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