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Research Article

Evaluation of the Phytotoxic and Genotoxic Potential of Pulp and Paper Mill Effluent Using *Vigna radiata* and *Allium cepa*

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Pulp and paper mill effluent induced phytotoxicity and genotoxicity in mung bean (*Vigna radiata* L.) and root tip cells of onion (*Allium cepa* L.) were investigated. Physicochemical characteristics such as electrical conductivity (EC), biological oxygen demand (BOD_5), chemical oxygen demand (COD), and total phenols of the pulp and paper mill effluent were beyond the permissible limit specified for the discharge of effluent in inland water bodies. Compared to control plants, seedling exposed to 100% effluent concentration showed a reduction in root and shoot length and biomass by 65%, 67%, and 84%, respectively, after 5 days of treatment. *A. cepa* root tip cells exposed to effluent concentrations ranging from 25 to 100% v/v showed a significant decrease in mitotic index (MI) from 32 to 11% with respect to control root tip cells (69%) indicating effluent induced cytotoxicity. Further, the effluent induced DNA damage as evidenced by the presence of various chromosomal aberrations like stickiness, chromosome loss, anaphase bridge, c-mitosis, tripolar anaphase, vagrant chromosome, and telophase bridge and micronucleated and binucleated cell in *A. cepa*. Findings of the present study indicate that pulp and paper mill effluents may act as genotoxic and phytotoxic agents in plant model system.

1. Introduction

The pulp and paper mill uses large amounts of water and different chemicals during production processes of cellulose pulp from plant materials and produces large quantities of toxic and intensely coloured effluents. The effluents from pulping, bleaching, and washing processes are often characterized by their high colour, BOD, and COD and also consisting of potentially toxic chlorinated compounds, suspended solids, tannins, resin acids, and sulphur compounds along with lignins [1].

There are near about 759 pulp and paper mills in India and mostly using chlorine compounds in their bleaching sequences. The Indian pulp and paper industry is highly water intensive, consuming 100–250 m^3 freshwater/ton paper and generating a corresponding 75–225 m^3 wastewater/ton paper [2]. The effluents are often treated by biological treatment processes such as activated sludge process or aerated lagoon. However, compounds like lignin and chlorinated organic compounds are often not removed sufficiently by conventional methods due to their toxicity and low biodegradability and pose a threat to aquatic receiving environments [3].

Most of the Indian paper mills are small scale and do not have adequate treatment facilities. Thus, treated or allegedly treated wastewater from these mills contains toxic elements such as magnesium, sodium, chlorides, and sulphur as well as toxic organic compounds such as chlorinated lignins and phenolic derivatives, which can cause toxic

effects on living organisms. In aquatic system, it blocks the photosynthesis and decreases the dissolved oxygen (DO) level, which adversely affects flora and fauna and causes toxicity to aquatic ecosystem [4, 5]. In the contaminated soil it causes accumulation of toxic pollutants and metals. The chlorinated compounds are highly toxic and cause carcinogenic, mutagenic, clastogenic, and endocrinic effects [6].

Several studies confirm the toxic and genotoxic effects of pulp and paper mill effluents in living organisms. Most of this work has been carried out in Canada and Europe, but not enough data are available from Asia including India [7]. It is thus necessary to assess the toxic and genotoxic effects of effluents discharged by Indian pulp and paper mills.

The sensitivity of plants to different compounds can be used in toxicity tests to identify toxicants. Plant bioassays are well-established systems used for screening and monitoring of environmental pollutants. Phytotoxicity is the impact of various compounds or pollutants on seed germination and subsequent growth, while plant genotoxicity tests detect a wide range of genetic damage, including gene mutations and chromosomal aberrations [8].

In the present work, toxic and genotoxic effect of pulp and paper mill effluent was investigated on mung bean (*V. radiata* L.) and onion (*A. cepa* L.), respectively. *V. radiata* is one of the important test species used to evaluate the phytotoxicity of environmental pollutants [9, 10]. *A. cepa* ($2n = 16$) is used for chemical screening and in situ monitoring of the genotoxicity of heavy metals, effluents, and herbicides [11–14].

2. Materials and Methods

2.1. Effluent Samples. Studies were conducted with effluents collected from the Star Paper Mill in Saharanpur, Uttar Pradesh (India). It is one of the large scale paper mills in India and is equipped with a Kraft pulping process and a three-stage bleaching process (chlorination, alkali extraction, and hypochlorination). It has a soda recovery plant which processes about 1700 m^3/day of black liquor from the pulping process. All wastewater from the mill is channeled through a single drain to the effluent treatment plant, where it is treated by activated sludge after primary physical treatment and finally released into the river Hindon through open drain. Effluent samples were collected monthly over a three-month period (from February to April 2014). Samples were collected from effluent drain in plastic containers of 5 liters. All the samples were transported to the laboratory on ice. The samples were filtered with Whatman filter paper No. 1 and stored at 4°C until analysis.

2.2. Physicochemical Analysis. The effluent samples were analyzed for various physicochemical parameters (Table 1) according to APHA [15]. Colour unit (CU) and lignin content were estimated by established [16, 17] methods. The pH was measured with a digital pH meter (Metrohm, USA). Electrical conductivity (EC) was determined by a conductivity meter (Thermo Orion, model 162A, USA). Heavy metals were determined with an atomic absorption spectrometer (AAS) (GBC, Avanta Sigma, Australia).

Table 1: Physicochemical characteristics of pulp and paper mill effluent. Values are mean ± SD of three samples.

Parameters	Values (mg/L)	
	Effluent samples ($n = 3$)	Effluent standard*
pH	8.2 ± 0.1	5.5–9.0
TDS	1080 ± 54	2100
EC (μS/cm)	1640 ± 82	1000
BOD	165 ± 8	30
COD	384 ± 19	250
Colour (CU)	1460 ± 73	NS
Lignin	249 ± 12	NS
Total phenol	44 ± 2	1.0
Sulphate	157 ± 37	1000
Heavy metals		
Zn	0.051 ± 0.00	5.0
Fe	0.229 ± 0.01	3.0
Ni	0.02 ± 0.00	3.0
Mn	0.01 ± 0.00	2.0
Cu	0.006 ± 0.0	3.0
Cr	0.118 ± 0.01	2.0

*Indian Standard Institute No. 2490 (1974); NS: not specified.

2.3. Gas Chromatography-Mass Spectroscopy (GC-MS) Analysis. Effluent samples (50 mL) were acidified to pH 1-2 using 1 N HCl and then extracted thrice with an equal volume of ethyl acetate. The ethyl acetate extract was vacuum dried. The ethyl acetate extract was derivatized using trimethyl silyl (BSTFA (N, O-bis (trimethylsilyl) trifluoroacetamide) TMCS) [18]. An aliquot of 1 μL of silylated compounds was injected in the injector port of a PE Autosystem XL gas chromatograph interfaced with a Turbomass mass spectrometric mass selective detector (Perkin Elmer, Waltham, MA, USA). The analytical column connected to the system was a PE-5MS capillary column (20 m × 0.18 mm internal diameter, 0.18 μm film thickness). Helium gas with a flow rate of 1 mL/min was used as the carrier gas. The column temperature program was 50°C for 5 min and then 50–300°C at 10°C/min, with a final hold time of 5 min. The transfer line and ion source temperatures were maintained at 200 and 250°C. A solvent delay of 3.0 min was selected. In the full-scan mode, electron ionization (EI) mass spectra in the range of 30–550 (m/z) were recorded at electron energy of 70 eV. Identification of compounds was done by comparing their mass spectra with those at the National Institute of Standards and Technology (NIST) library available with instrument.

2.4. V. radiata Phytotoxicity Test. V. radiata L. Wilczek (var. K-851) was used in this study. Mung bean seeds were purchased from a local certified shop and surface-sterilized with 0.1% (w/v) $HgCl_2$ and then washed thrice with distilled water to remove all the traces of mercury. The seed germination test was conducted on filter paper in petri dishes

(20 mm × 120 mm) with two layers of filter paper (125 mm in diameter, Whatman No. 1) followed by a layer of cotton on the bottom. Five test solutions (12.5, 25, 50, 75, and 100% v/v) of the effluent were prepared using dechlorinated tap water. Each dish contained 10 mL of test solutions or tap water (control). Ten seeds were kept on filter paper in each petri dish soaked with the respective test solution. The plates were incubated at 28 ± 1°C in the dark and the number of seeds germinated was observed after 48 h. The experiment was conducted for three samples collected from different locations. After 5 days, five randomly selected seedlings were taken from each petri dish to measure root and shoot length. For biomass estimation, 10 seedlings from each petri dish were wrapped with aluminum foil and weighed the fresh weight first and then they were dried in oven at 70°C for 48 h and weighed the dry weight. Biomass was calculated by subtraction of dry weight from fresh and expressed in gram.

2.5. A. cepa Phytotoxicity Test. *A. cepa* phytotoxicity test was carried out as per the basic protocol [14] for the toxicity bioassay of the pulp and paper mill effluents. *A. cepa* bulbs used in this study were purchased from a local market. The test was conducted using healthy and equal-sized onion bulbs. Five onion bulbs were placed over 50 mL Falcon tubes filled with the different concentration of paper mill effluent (25, 50, 75, and 100% v/v). Tubes were kept in an incubator at 23°C for 5 days. Dechlorinated tap water was used as control. The test solutions stored at 4°C were refilled morning and evening to ensure the contact between onion bulbs and samples present in the tube. After 5 days, the root length of onion bulbs at each concentration was measured. The experiment was conducted in five replicates of each dose for three samples collected from different locations. Inhibition in the growth of *A. cepa* roots was correlated with an index of the degree of toxicity [14].

2.6. Genotoxicity Test. Five onion bulbs per effluent concentration were initially rooted in tap water for 48 h as above until roots were 1-2 cm long and then transferred to test solutions for 24 h, as a complete cell cycle of *A. cepa* root meristematic occurs within 24 h [19]. The root tips were fixed in absolute alcohol and glacial acetic acid (3 : 1) for 12 h and then washed a few times with distilled water. After this root tips were hydrolyzed with 1 N HCl at 60–70°C for 5 min and washed with distilled water. This was followed by slide preparation using haemotoxylin as the stain [14, 19]. All the slides were analysed microscopically to calculate the mitotic index and the chromosomal aberrations present by the established procedure [20].

2.7. Data Analysis. Statistical analysis was performed using graph pad prism 5, version 5.1 (San Diego, California, USA). The data was analysed using one way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test to confirm the variability of the data and validity of results. The criterion of significance was $p < 0.05$. The values of experiments are reported as mean ± SD ($n = 3$).

3. Results and Discussion

3.1. Physicochemical Characteristics of Effluents. The measured physicochemical parameters of the effluent samples and ISI standard for tolerance limits of industrial wastewaters discharge into inland surface waters are given in Table 1. The effluent pH was measured to be pH = 8.2 ± 0.1 which is alkaline. It was within the tolerance limits. The average values of EC, BOD, COD, and phenols in the effluent were found to be significantly higher than the ISI standard [21]. EC which bears direct relation with the salinity and total salt content of the effluents was recorded as 1640 ± 82 μS/cm. The increased EC in irrigation water leads to lower crops production [22]. BOD which indicates the pollution strength of the waste waters was recorded as 165±8.25 mg/L. High BOD is harmful to aquatic animals like fish and microorganisms [23]. The COD value of the effluent samples was recorded as 384 ± 19.2 mg/L. High COD levels indicate the toxic state of the waste water along with the presence of biologically resistant organic substances [24].

Further, effluents were found to be containing high level of colour unit (1460 ± 73 CU) and lignin (249 ± 12.4 mg/L). Colour is usually the first contaminant to be recognized in wastewater/effluent that affects the aesthetics, water transparency, and gas solubility of water bodies. The colour of the effluent is mainly due to the presence of lignin and degradation products which are produced during different process stages such as pulping, bleaching, and alkali extraction [25]. Colour derived from lignin is an indicator of the presence of potentially inhibitory compounds and in addition may have direct inhibitory effects on some of the lower organisms in the food chain. The phenol content of the effluent was recorded as 44 ± 2.2 mg/L. Phenols are the major part of plant cell walls that are solubilized during industrial pulping and bleaching processes. Phenol and its derivatives induce genotoxic, carcinogenic, immunotoxic, hematological, and physiological effects in fish in receiving waters and have a high bioaccumulation rate along the food chain due to their lipophilicity [26]. The source of sulphate ions in effluent might be sodium sulphite, which is used during the pulping process [27]. Metals in the effluent might be due to their bioaccumulation by plants which are used as raw material as well as from various chemicals used during the paper making process. Pulp and paper mill effluent containing high colour, BOD, COD, and phenol has harmful effects on aquatic flora and fauna [28].

GC-MS is a powerful tool to identify compounds in wastewaters that has been used in a number of previous studies to identify compounds present in pulp and paper mill effluent [29, 30]. The compounds detected by GC-MS in ethyl acetate extracts of effluent are shown in Figure 1 and Table 2. The effluent was contaminated with hydrocarbons (3-octadecene RT = 23.28, 1-octadecene RT = 26.62), phenolics ((+)-5-hydroxy-6-(1-hydroxyethyl)-2,7-dimethoxynaphthoquinone, RT = 18.42), extractives (D-fructose, 1,3,4,5,6-pentakis-o-(trimethyl silyl)-o,methyloxime RT = 24.53, N-tetracosanol-1 RT = 32.13), and plant-derived organic and fatty acids (Table 2 and Figure 1). GC-MS analysis indicates that treated effluent still contains various

Table 2: Compounds identified in ethyl acetate extract from pulp and paper mill effluent sample as given in Figure 1.

Retention time (in min)	Compounds
7.99	Propanoic acid
12.82	Phosphoric acid
18.42	(+)-5-Hydroxy-6-(1-hydroxyethyl)-2,7-dimethoxynaphthoquinone
20.37	Tartaric acid
23.28	3-Octadecene
24.53	D-Fructose, 1,3,4,5,6-pentakis-o-(trimethyl silyl)-o,methyloxime
26.62	1-Octadecene
27.42	Hexadecanoic acid
29.68	1-Heneicosanol
30.33	Octadecanoic acid
32.13	Tetracosanoic acid
34.07	1-Octacosanol
42.20	α-D-Galactopyranoside, methyl 2,3-bis-o-(trimethyl silyl)-, cyclic methylbronate
44.00	2′-4′-6′-Trinitro-5′-phenyl-1,1′: 3′,1″-terphenyl
48.88	N,N′-Dicyclohexyl-1-cyano-7-pyrrolidinylperylene-3,4:9,10-tetracarboxylic acid

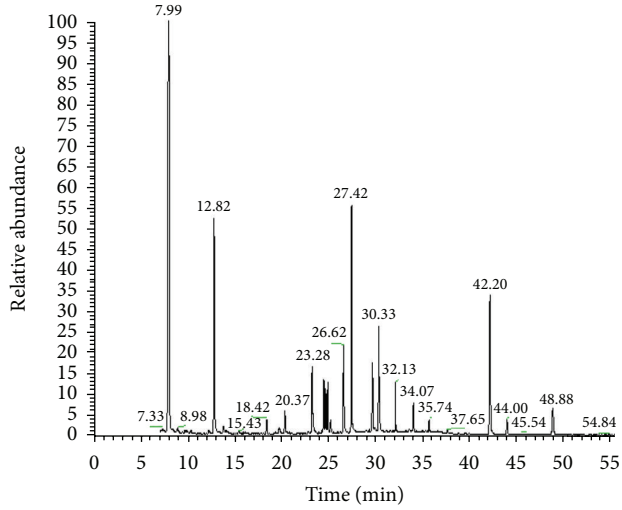


Figure 1: Typical chromatographic profile obtained by GC-MS of compounds extracted with ethyl acetate from pulp and paper mill effluent. MS-identified compounds with respect to their retention times are listed in Table 2.

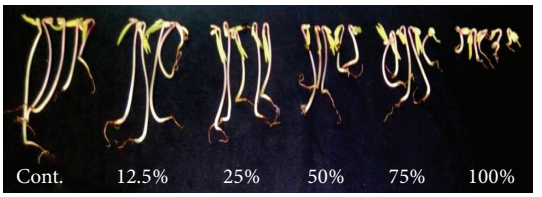


Figure 2: Effect of different concentrations of pulp and paper mill effluent on early seedling growth of mung bean.

persistent organic pollutants even after secondary biological treatment. The detected compounds had previously been reported in pulp and paper mill effluents and during lignin biodegradation [29–32].

3.2. Phytotoxicity Evaluation. Environmental toxicity assessment of industrial wastewater using a plant seed germination test is considered one of the simplest short-term methods [33]. Seed germination is a very sensitive process likely to be disturbed by inhibitory substances in the growing environment. In the present study mung bean seeds were germinated in different concentration of pulp and paper mill effluents and number of seeds germinated in each concentration was observed on 48 h. The result indicates (Table 3) that no seed germination inhibition was observed up to 50% (v/v) effluent concentration. Only 10 and 30% seed germination inhibition was observed at 75 and 100% (v/v) effluent concentrations, respectively. The result indicates that diluted effluents did not affect *V. radiata* seed germination significantly.

The effect of different concentration of effluent on early seeding growth (5 days) is apparent in Figure 2. Compared to controls, root lengths of 5-day-old seedling were highest at 12.5% (v/v) effluent and then gradually decreased with increasing effluent concentrations (Table 3). Compared to controls, shoot lengths of seedlings were gradually decreased with increasing effluent concentrations. Significant reduction in root length (65%) and shoot length (67%) was observed at 100% (v/v) effluent concentration. On the other hand, significant reduction in biomass (68%) was observed at 12.5% (v/v) and this reduction was almost stable at 25 and 50% (v/v) effluent concentration. At 100% effluent concentration, there was an 84% reduction in biomass compared to controls. The significant reduction in root length, shoot length, and biomass of seedling at 100% effluent may be correlated with the cumulative effect of excess amount of EC, BOD, COD, and phenols in the effluent. This observation confirms the results obtained by others working on various crop plants [34–36].

Root growth inhibition of *A. cepa* root is considered a toxicity indicator since it may result from inhibition of the cell division [14, 37]. The effect of different concentrations

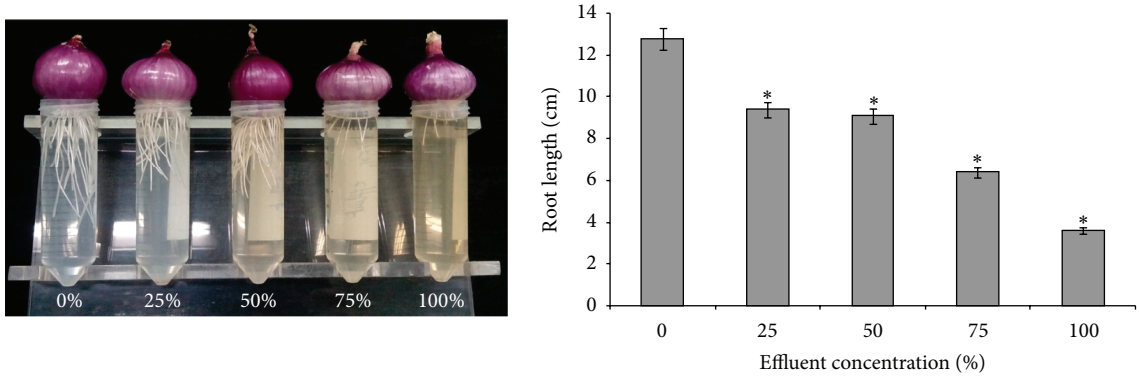


Figure 3: Root growth (a) and root length (b) of *A. cepa* after treatment with different concentrations of effluent. Values are mean ± SD of three samples. $^{*}p < 0.05$, significant when compared to control using ANOVA.

Table 3: Effect of different concentrations of pulp and paper mill effluent on seed germination and root length, shoot length, and biomass of early seedling of mung bean plant.

Effluent (%)	Germination (%)	Root length (cm)	Shoot length (cm)	Biomass (gm)
0	100 ± 0	1.7 ± 0.6	5.1 ± 1.4	1.9 ± 0.03
12.5	100 ± 0	1.9 ± 0.2	4.7 ± 1.7	0.6 ± 0.07*
25	100 ± 0	1.6 ± 0.4	4.3 ± 1.1	0.6 ± 0.05*
50	100 ± 10	1.4 ± 0.2	4.2 ± 1.4	0.6 ± 0.03*
75	90 ± 10	1.4 ± 0.2	4.1 ± 1.4	0.5 ± 0.04*
100	70 ± 10	0.6 ± 0.3*	1.7 ± 0.5*	0.3 ± 0.03*

Values are mean ± SD of three samples. $^{*}p < 0.05$, significant when compared to control using ANOVA.

of pulp and paper mill effluent on the root growth and length of *A. cepa* is shown in Figures 3(a) and 3(b). Onions grown in pulp and paper mill effluent showed decreased root growth and root length when compared with controls. Root growth and root length inhibition was more pronounced at 75% and 100% (v/v) effluent concentrations. Following 5 days of treatment, the mean root lengths were 3.9, 6.6, 9.0, 9.1, and 11.8 cm when grown in 100%, 75%, 50%, 25%, and 0%, respectively (Table 4). Inhibition of *A. cepa* root growth and length observed in the present study following treatment with pulp and paper mill effluents was in agreement with the previous studies [12, 38].

3.3. Mitotic Index. Mitotic index (MI) is a good method in biomonitoring to assess the effect of number of pollutants on cell division [39]. MI measures the proportion of the cells in the mitotic phase of the cell cycle and its inhibition could be interpreted as cellular death [40]. The effect of pulp and paper mill effluent on percent MI in *A. cepa* is shown in Table 4. The percentage of MI was lower than the control at all tested effluent concentrations and it decreased progressively with increasing effluent concentrations. This conforms well to the above-mentioned effects of effluent on root growth. Compared to a control value of 69, mean MI (%) values of 32, 27, 22, and 11 were recorded for 25%, 50%, 75%, and 100% effluent concentrations, respectively. The results indicate cytotoxic effect of the pollutants present in the pulp and paper mill effluent. These pollutants may interfere with the normal process of mitosis, thus preventing a number of cells from entering the prophase and blocking the mitosis cycle during interphase [41]. The inhibition of MI can also be attributed to be an effect of environmental chemicals on DNA/protein synthesis of the biological system [42, 43]. Declined MI following treatment with paper mill effluents have been reported in earlier studies [38, 41].

3.4. Chromosomal Aberrations. Chromosomal aberrations analysis in meristematic root tip cells of *A. cepa* is considered an efficient test to investigate the genotoxic potential of chemical agents, sewage, and industrial wastewaters [39, 44, 45]. Chromosomal aberrations are characterized by changes in either total number of chromosomes or chromosomal structure which can occur both spontaneously and as a result of the exposure to physical or chemical agents [46]. To evaluate the different chromosomal abnormalities, several types of chromosome aberrations are considered over the four stages of the cell cycle (prophase, metaphase, anaphase, and telophase). Chromosomal aberrations analysis not only allows estimation of genotoxic effects, but also enables evaluation of their clastogenic and aneugenic actions [20].

Chromosomal aberrations during mitotic stages in root tip cells of *A. cepa* following treatments are shown in Table 5 and Figures 4 and 5. No chromosomal aberrations and nuclear abnormalities were observed in control cells

Table 4: Showing inhibition of root length and MI% in root tip cells of *A. cepa* following treatment with different concentrations of pulp and paper mill effluents.

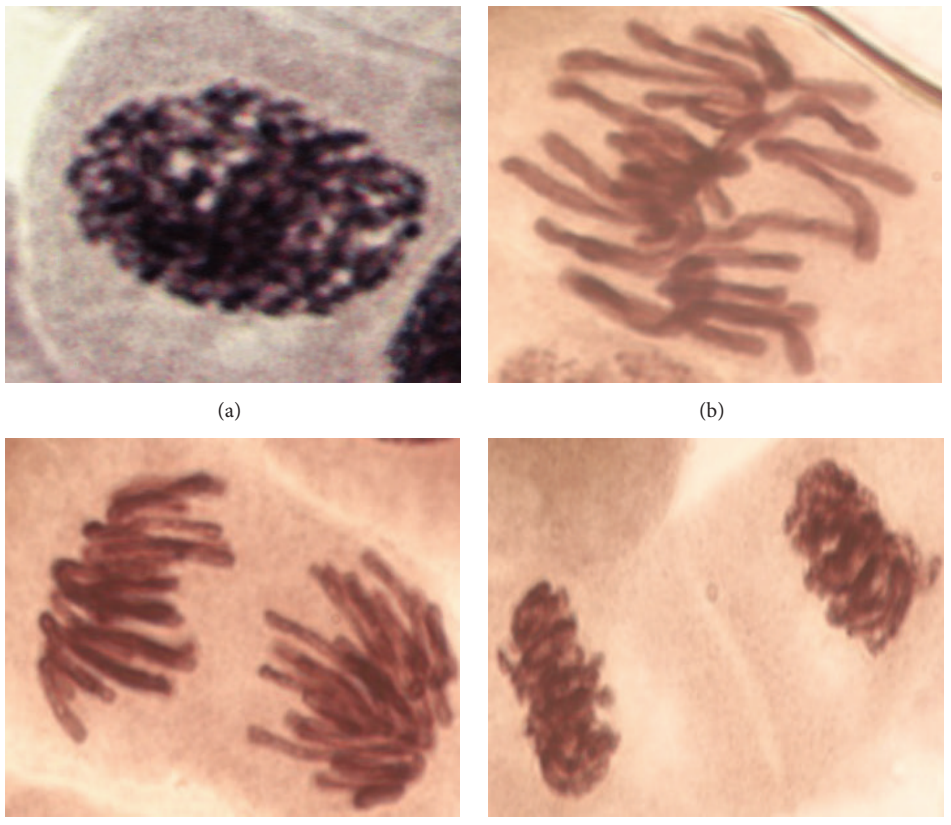
Concentration (%)	Root length (cm)	Total cells	Dividing cells	MI%
0	11.8 ± 0.9	540 ± 20	372 ± 10	69 ± 0.9
25	9.1 ± 0.6*	776 ± 41	244 ± 12	32 ± 0.4*
50	9.0 ± 0.4*	775 ± 36	206 ± 10	27 ± 0.5*
75	6.6 ± 0.5*	842 ± 20	186 ± 8	22 ± 0.7*
100	3.9 ± 0.3*	801 ± 20	91 ± 7	11 ± 0.5*

Values are mean ± SD of three samples. * $p < 0.05$, significant when compared to control using ANOVA. MI was calculated as number of dividing cells × 100/number of total observed cells.

Table 5: Frequency of chromosomal aberrations and nuclear abnormalities in root tip cells of *A. cepa* following treatments with different concentrations of pulp and paper mill effluents.

Concentration (%)	Chromosomal aberrations						Nuclear abnormalities		Aberrant cells (%)
	Stickiness	Bridge	Vagrant	Tripolar	Chromosomal break	c-metaphase	Binucleated cells	Micronuclei	
0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
25	1.3 ± 0.6	2.0 ± 1.0	3.0 ± 1.0	2.0 ± 1.0	0.3 ± 0.6	4.0 ± 1.0	1.3 ± 0.6	0.0 ± 0.0	2.6 ± 1.0*
50	7.0 ± 1.0	9.0 ± 1.0	9.0 ± 2.0	2.7 ± 1.2	3.0 ± 1.0	14.3 ± 2.1	2.7 ± 0.6	1.3 ± 0.6	9.1 ± 1.7*
75	4.3 ± 0.6	3.0 ± 1.0	6.3 ± 1.5	1.3 ± 0.6	2.7 ± 0.6	13.0 ± 2.0	2.7 ± 1.2	2.3 ± 0.6	6.6 ± 1.4*
100	3.7 ± 1.2	4.7 ± 1.5	2.7 ± 1.2	3.0 ± 1.0	2.7 ± 0.6	13.3 ± 1.5	3.0 ± 1.0	1.7 ± 0.6	6.4 ± 1.0*

Values are mean ± SD of three samples. * $p < 0.05$, significant when compared to control using ANOVA. Chromosomal aberrations were scored on 100–500 cells per slide.



(a) (b) (c) (d)

Figure 4: Various stages of mitosis show normal prophase (a), metaphase (b), anaphase (c), and telophase (d) in root tip cells of *A. cepa* treated with tap water (control).

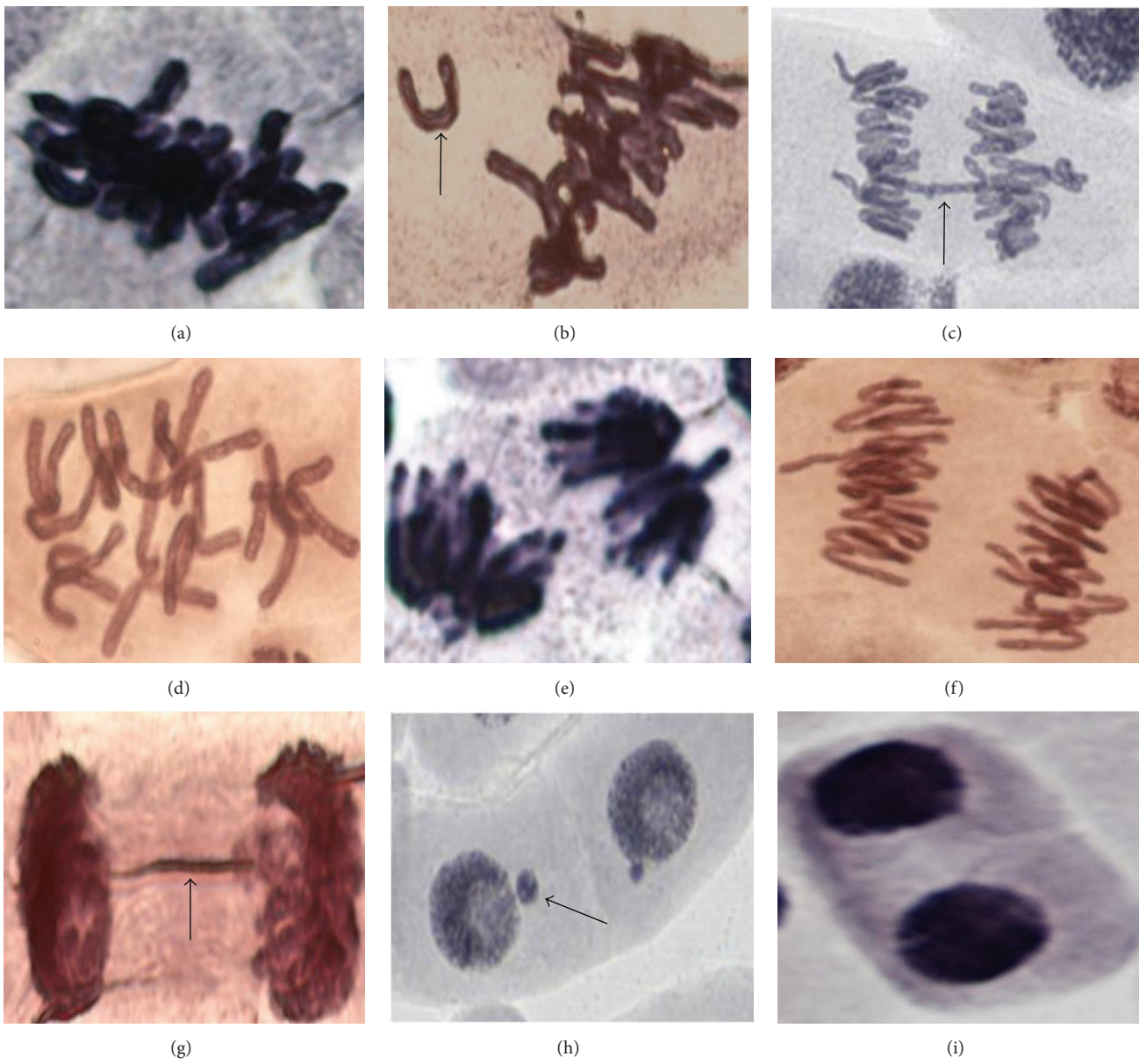


Figure 5: Chromosomal aberrations and nuclear irregularities observed in root tip cells of *A. cepa* following treatments with different concentrations of pulp and paper mill effluent. (a) Sticky metaphase, (b) chromosome loss, (c) anaphase bridge, (d) c-mitosis, (e) tripolar anaphase, (f) vagrant chromosome, (g) bridge at telophase, (h) micronucleated cell, and (i) binucleated cell.

treated with tap water (Figure 4). On the other hand, treatment with different concentrations of pulp and paper mill effluent induced significant chromosomal aberrations and nuclear abnormalities (Figure 5). All three effluents induced similar type of abnormalities. The observed aberrations were stickiness (Figure 5(a)), chromosome loss (Figure 5(b)), anaphase bridge (Figure 5(c)), c-mitosis (Figure 5(d)), tripolar anaphase (Figure 5(e)), vagrant chromosome (Figure 5(f)), and telophase bridge (Figure 5(g)). The nuclear abnormalities included micronucleated cell (Figure 5(h)) and binucleated cell (Figure 5(i)) in all treatments. The most frequent aberrations were c-mitosis, vagrant, and bridge chromosomes at all tested effluents concentration. The percentage of aberrant cell was recorded highest at 50% effluent concentration and thereafter declined. Induction of different types of chromosomal aberrations in *A. cepa* as a result of exposure to industrial wastewaters has been well documented [44, 45, 47].

Stickiness is considered a common sign of toxic effects on chromosomes probably leading to cell death [48]. Stickiness of chromosomes may occur due to either increased chromosomal contraction and condensation or depolymerization of DNA and partial dissolution of nucleoproteins [49, 50]. Sticky chromosomes have been reported in *A. cepa* after treatment with industrial wastewaters, surface water, and heavy metals [45, 48, 51]. Chromosome bridges may be caused

by stickiness of chromosomes preventing their complete separation during anaphase indicating a likely mutagenic event in the cell [52]. Colchicine mitosis (c-mitosis) is defined as an inactivation of the spindle followed by random scattering of the chromosomes around the cell. The effluent induced a high frequency of c-mitosis, which has been also shown by other studies [38, 41], indicating that effluent is comparable in toxicity to colchicine. Occurrence of anaphase tripolar and chromosomal break indicates failure of the spindle apparatus to organize and function in a normal way [53].

Observation of nuclear abnormalities such as binucleated cells and micronuclei in *A. cepa* root tip cells is a clear indication of genotoxicity [54]. Binucleated cells may arise as a result of an incomplete process of cell division, that is, karyokinesis with incomplete cytokinesis. These cells also may arise due to the suppression of cell plate formation between cells in early telophase. Micronuclei are formed as a consequence of chromosome breakage (clastogenic agent) or whole chromosomes (aneugenic agent) that were not incorporated to the main nucleus during the cell division cycle [55]. Moreover, micronuclei also derive as processes of polyploidization, in which they originate from the elimination of exceeding DNA of the main nucleus in an attempt to restore the normal conditions of ploidy [56]. The induction of chromosomal aberrations and nuclear abnormalities in *A. cepa* root tip cells may be associated with the cumulative effect of various pollutants such as phenols, heavy metals, and other persistent organic pollutants detected in GC-MS. This result is similar to the previous findings in which roots of *A. cepa* exposed to pulp and paper mill effluents resulted in cells with chromosomal aberrations and nuclear abnormalities [38, 41].

4. Conclusion

Pulp and paper mill effluents were found to be containing higher EC, BOD, COD, and phenol along with various persistent organic pollutants. The effluents were phytotoxic, as they inhibited root length, shoot length, and biomass of *V. radiata* seedlings. Inhibition of *A. cepa* root growth and mitotic index were also observed following effluent treatments. The effluent induced DNA damage as evidenced by the presence of various chromosomal aberrations like stickiness, chromosome loss, anaphase bridge, c-mitosis, tripolar anaphase, vagrant chromosome and telophase bridge, micronucleated cell, and binucleated cell in root tip cells of *A. cepa*. From this study, presence of toxic and genotoxic agents in pulp and paper mill effluent after secondary treatment was proved. Thus, there is need for the adoption of proper treatment and bioremediation strategies to alleviate the pollution hazards caused by these wastewaters.

Competing Interests

The authors declare no competing interests.

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