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Mutagenicity and genotoxicity evaluation of textile industry wastewater using bacterial and plant bioassays

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ABSTRACT

Textile industrial wastewater samples were taken from the Panki site 5 industrial area of Kanpur city, India. Atomic Absorption spectrophotometer and Gas Chromatography-Mass spectrometry techniques have shown that the wastewater contained several heavy metals and organic pollutants (Khan and Malik, 2017) [1]. Further, in order to explore the potential toxicity of these pollutants present in the effluent, a battery of short-term biological assays (Ames test, DNA repair defective mutation assay and *Allium cepa* chromosomal aberration test) were used. Wastewater samples were concentrated with XAD-4/8 resins and liquid-liquid extraction procedure. XAD-concentrated samples were more mutagenic than the liquid-liquid extracted samples. Ames TA98 and *pol*A (SOS defective) strains were the most responsive strains. The wastewater also resulted in significant decline in mitotic index and induced chromosomal aberrations in *A. cepa* roots. The findings thus showed that the combination of physico-chemical analysis alongwith the toxicity assessment (using short term biological assays) would provide valuable and more realistic information about the joint toxicity of chemical pollutants present in the textile effluent.

1. Introduction

In almost all developing and in many of the highly developed nations, water pollution due to discharge of inadequately treated wastewater into the environment is a major issue of concern [2]. The wastewater generation is mainly attributed to the rapid increase in industrial sector, which is growing substantially for economic development of a country. Among the various industries, textile industry is considered as one of the major contributors of the water pollution, since the waste generated is very complex in nature containing color content and toxic components [3]. Textile wastewater constitutes a large number of chemicals like acids, bases, salts, dispersants, etc. [4–6], and most importantly dyes that altogether are responsible for high biological oxygen demand (BOD), chemical oxygen demand (COD) and total organic carbon (TOC) [7]. Dyes therefore are one of the most important constituents of the textile industry and are major polluters of water reservoirs [8].

The direct discharge of the effluent in rivers not only adversely affects flora and fauna but is the cause of various human illnesses. In spite of the harmful consequences, textile effluent is being continuously released into water streams without any prior treatment or sometimes after partial treatment which deteriorates the quality of receiving water. The increasing discharge of these hazardous chemicals into the environment severely affect the natural ecosystems [9], and have adverse effects on human and environmental health [10].

In order to minimize the environmental deterioration due to exposure of textile wastewater, the effluent should be monitored carefully using multidisciplinary approach that involves a combination of chemical and biological methods. The wastewater can be assessed by means of various chemical methods, which require the standardization of thousands of organic pollutants present in the environment, making them tedious and time consuming processes. Moreover, the methods are not sufficient to assess the joint toxicity of the pollutants present in a mixture at low concentrations. Therefore, biological assessment method is required in order to detect the combined effects of chemical pollutants present as a mixture in the environment [11]. Biological method of assessment employs both prokaryotic and eukaryotic systems and can detect DNA damage from point mutations to chromosomal alterations [12].

Among the prokaryotic system, Ames test is one of the most widely used, short-term mutagenicity bioassay involving specifically designed *Salmonella typhimurium* strains with different pre-existing mutations in the histidine operon, making them unable to synthesize histidine amino acid [13–17]. Any chemical substance that may cause mutations at or near the histidine operon restores the "*his* gene" function and results in growth of the bacteria in the absence of histidine. The Ames strains can

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not only detect mutagenic potential of the substance capable of producing DNA damage, but also the mechanism by which it causes mutation [16,18].

DNA repair defective mutation assay is another important parameter for genotoxicity testing [19], involving *Escherichia coli* K-12 strains with defects in their SOS repair mechanism. SOS response depends on a repressor (LexA protein), an inducer (RecA protein) and an effector molecule (single stranded DNA molecules, produced as a result of damage) [20], and it involves more than 40 genes, which express simultaneously when the cells are exposed with a DNA damaging substance [21–24]. The assay involves the estimation of DNA damage caused by chemicals/ pollutants thus showing their mutagenic/ genotoxic potential [25].

Among the eukaryotic systems, *A. cepa* chromosomal aberration assay is a low cost, easy to handle short term assay which has been extensively used for environmental monitoring and genotoxicity assessment of various types of wastewater [26–28]. The *A. cepa* test does not only provide information about genotoxic potential of chemicals but also its mechanism of action on genetic material.

Our previous study has demonstrated that the textile wastewater (collected from textile industries located at Industrial area, Panki site 5, Kanpur, India.) contained several heavy metals with concentrations of Cr, Pb, Cd exceeding the permissible limits (assigned by WHO and US EPA) and a large number of organic compounds viz. alkanes, aromatic amines, azobenzene, alcohols, ketones, phenols, amides, ethers, esters, carboxylic acids, fatty acids and triaozle [1]. Therefore, the present study was conducted to get a comprehensive idea about the toxicity exerted by these chemicals present in the textile effluent, using a combination of the bacterial and plant bioassays i.e., Ames *Salmonella*/ mammalian microsome test, *E. coli* DNA repair defective mutation assay and *A. cepa* chromosomal aberration assay. As these assays have different genetic end points, they would give a more realistic information about genotoxicity and mutagenicity of the test samples.

2. Materials and methods

2.1. Sampling

Wastewater samples were collected from open channels, receiving effluent from textile industries located at Industrial area, Panki site 5, Kanpur, India. A total of 16 wastewater samples were collected from Jan 2012 to Dec 2015 and transported to the laboratory as described in standard methods [29]. The samples were composited by mixing 2 L of wastewater collected at five different points at the sampling site to make 10 L composite sample.

2.2. XAD-concentration of wastewater

For the concentration of organic constituents, a sample of 1 L of wastewater was used. Before concentration, wastewater was filtered through Whatman filter paper grade 1 (pore size 11 μm) and membrane filter (pore size 0.45 μm) (Axiva, India). The column adsorbent was prepared by mixing equal amount of XAD-4/8 [30]. Adsorption of organic compounds of the wastewater on the XAD resins was done by the method of Wilcox and Williamson [31]. The organic compounds thus adsorbed were eluted by using 20 mL of acetone (HPLC grade) and the eluate was evaporated at room temperature (25 °C) under reduced pressure to dryness and reconstituted in Dimethyl sulfoxide (DMSO, HPLC grade, SRL India). The sample was filter sterilized through 0.22 μm membrane filter and stored at −20 °C until it was used for the testing.

2.3. Liquid-liquid extraction

Textile wastewater was extracted using different solvents separately (Dichloromethane and n-Hexane, HPLC grade, SRL, India) as described in standard methods [29]. 1 L of wastewater was filtered through Whatman filter paper grade 1 (pore size 11 μm) and membrane filter (pore size 0.45 μm) (Axiva, India). Filtered wastewater was shaken vigorously with the extraction solvent in a separatory funnel and was allowed to withstand until the organic (solvent) and aqueous (water) phases were separated. Organic layer was collected in a beaker and evaporated at room temperature (25 °C) under reduced pressure to make a volume of 5 mL. The extract was filter sterilized through 0.22 μm membrane filter and stored at −20 °C until it was used for the testing [11].

2.4. Bacterial test systems

Salmonella typhimurium strains were kindly provided by Prof. T. Nohmi (National Institute of Hygienic Sciences, Division of Genetics and Mutagenesis, Japan). The strains were maintained in frozen stocks and grown as described by Maron and Ames [32]. Each strain was tested on the basis of associated genetic markers raising it from a single colony from the master plate [32]. *Escherichia coli* K-12 strains were received from B. J. Barbara (*E. coli* Genetic Stock Centre, Department of Biology, Yale University, New Haven, USA). The bacterial strains of *E. coli* K-12 were always plated on nutrient broth containing 1.5% (w/v) agar.

2.5. Ames mutagenicity testing

Mutagenicity testing was performed by preincubation procedure as described by Maron and Ames [32], with some modifications [33]. Five different doses of the wastewater extract i.e., 2.5, 5, 10, 20, and 40 μL per plate (0.5, 1.0, 2.0, 4.0 and 8.0 m L-equivalent wastewater per plate, respectively) were incubated separately with 0.1 mL of bacterial culture at 37 °C for 30 min. After incubation, 2 mL of top agar containing trace amounts of histidine and biotin was added and poured onto minimal glucose agar plates. The plates were incubated at 37 °C for 48–72 h. Negative control (containing bacteria and DMSO) and positive control (containing bacteria and methyl methane sulfonate) were also included in each test. In order to determine the presence of any promutagens in the samples, similar experiments were also conducted in the presence of (S9) microsomal fraction, which contained 20 μL of S9 liver homogenate mix per plate. S9 fraction was prepared as described by Garner et al. [34], and Maron and Ames [32].

2.6. Survival of E. coli K-12 mutants

Both isogenic wild type and SOS defective mutants (*recA*, *lexA* and *polA*) of *E. coli* K-12 strains were grown in nutrient broth at 37 °C and exponentially growing culture (1–3 × 10^8 viable counts/mL) was harvested by centrifugation. The pellet thus obtained was resuspended in 0.01 M $MgSO_4$ solution and treated with 20 μL of liquid-liquid extracted and XAD-concentrated samples separately. The samples were withdrawn at different time intervals, diluted suitably and spreaded on nutrient agar plates to obtain colony forming units (CFUs). The plates were incubated overnight at 37 °C and CFUs were calculated. Solvent control was also run simultaneously.

2.7. Allium cepa anaphase-telophase test

The cytogenotoxic effect of textile wastewater was evaluated with root tips of *A. cepa* as described by Fiskesjö [35]. Small onion bulbs (1.5–2.0 cm in diameter) were used for the analysis. Before use, the outer dead scales and dry bottom plates of the bulbs were removed carefully. The bulbs were then placed in beakers, containing distilled water, in a position that the basal parts of the bulbs remained dipped in the water and allowed to germinate at room temperature for 2–3 days. Freshly emerged roots (1.0–2.0 cm in length) were used in the test. Roots of *A. cepa* were treated with a series of wastewater

concentrations, i.e., 5, 10, 25, 50, and 100% for 72 h. Positive control (methyl methane sulfonate, 10 mg-mL^{-1}) and negative control (double distilled water) were also included in each assay. After 72 h of exposure of the test and control samples, the root tips were randomly selected and fixed in 3:1 ethanol: glacial acetic acid (v/v) and kept at 4 °C for overnight. The fixed root tips were then rinsed in distilled water and heated in 1 N HCl for 2–3 minutes. After washing with distilled water, it was stained with acetocarmine and examined microscopically (Light microscope, Olympus BX60). Mitotic index was calculated by observing about 6000 cells (2000 cells per slide) as follows:

$$\text{Mitotic index (\%)} = \frac{\text{Total Number of Dividing Cells}}{\text{Total Number of Cells Examined}} \times 100$$

Chromosomal aberrations were analysed by observing about 300 dividing cells (100 cells per slide). Different types of the aberrant cells were observed and recorded.

2.8. Statistical analysis

Mutagenic Index, induction factor (Mi) and mutagenic potential (*m*) were calculated as described by Ansari and Malik [36].

$$\textit{Mutagenic Index} = \frac{\textit{Number of his}^{+}\textit{ revertants induced in the sample}}{\textit{Number of his}^{+}\textit{revertants induced in the negative control}}$$

$$\textit{Induction factor (Mi)} = \frac{\ln\,(n-c)}{c}$$

Where 'n' is the number of revertant colonies in the sample and 'c' is the number of revertants in solvent control. The mutagenic potential of the test samples was calculated by the initial linear portion of the dose-response curve with tester strains. The slope (m) was obtained by the least square regression of the initial linear portion of the curve of initial dose-response.

The significance of the number of *his*$^{+}$ revertants compared to the control was statistically established by one way analysis of variance (ANOVA) at $P \leq 0.05$. Further, for *Allium cepa* test, the data were presented in terms of percent mitotic index and percent aberrant cells. Duncan Multiple Range Test (DMRT) was used to determine significance in different treatment groups and against control (positive and negative) values.

3. Results

Our previous study has demonstrated that the textile wastewater was dark colored, slightly alkaline (pH of 8.04) having high concentration of total dissolved solids (TDS of ~9 g L^{-1}). Atomic absorption spectrophotometric analysis revealed that the wastewater contained several heavy metals viz. Ni (0.124 mg L^{-1}), Cu (0.151 mg L^{-1}), Cr (1.533 mg L^{-1}), Pb (0.199 mg L^{-1}), Cd (0.088 mg L^{-1}) and Zn (2.694 mg L^{-1}), with concentrations of Cr, Pb and Cd were significantly higher than the permissible limits as assigned by WHO (World Health Organization) and US EPA (Unites States Environmental Protection Agency). Moreover, a large number organic compounds (alkanes, aromatic amines, azobenzene, alcohols, ketones, phenols, amides, ethers, esters, carboxylic acids, fatty acids and triaozle) have also been detected in the wastewater [1].

Mutagenicity testing of wastewater samples were evaluated using *S. typhimurium* strains. XAD-concentrated and liquid-liquid extracted (hexane and DCM extracted) samples were analysed both in the absence and presence of S9 fraction. In case of XAD-concentrated sample (Table 1), number of revertant colonies for all the tester strains increased upto the concentration of 20 µL/plate and then declined at the dose of 40 µL/plate. Among all the tester strains tested, maximum number of revertants were observed for TA98, which showed mutagenic index of 15.42 (without S9 fraction) and 15.60 (with S9 fraction). TA98 strain exhibited maximum response both in terms of induction factor (Mi 2.67 and 2.68 without and with S9 fraction, respectively) and mutagenic potential/slope (m 7.7 and 8.2 without and with S9 fraction, respectively) of the initial linear dose-response curve (Table 1). The order of responsiveness of the tester strains on the basis of the mutagenic index and induction factor both in the absence and presence of S9 fraction for XAD concentrated sample was as follows:

TA98 > TA97a > TA100 > TA102 > TA104

However, the order of responsiveness of *S. typhimurium* strains on the basis of mutagenic potential/ slope was in the following order:

TA98 > TA97a > TA100 > TA104 > TA102

For hexane extracted wastewater sample, the number of revertant colonies increased with increasing concentration up to 20 µL/plate and then decreased at the dose of 40 µL/plate (Table 2). TA98 exhibited maximum response in terms of mutagenic index (13.20 without S9 and 13.26 with S9 fraction); induction factor (2.50 without S9 and 2.51 with S9 fraction); and mutagenic potential (4.7 without S9 and 6.2 with S9 fraction) (Table 2). The response of tester strains, in terms of mutagenic index and induction factor, followed the similar trend as was observed in case of XAD-concentrated sample, i.e.,

TA98 > TA97a > TA100 > TA102 > TA104

Reversion of the tester strains treated with the basic and acidic fractions of dichloromethane extracted samples are presented in Tables 3 and 4. Basic fraction of dichloromethane extract displayed maximum response of 12.90 (without S9 fraction) and 13.09 (with S9 fraction) in terms of mutagenic index; 2.48 (without S9 fraction) and 2.49 (with S9 fraction) in terms of induction factor; and 6.0 (without S9 fraction) and 6.2 (with S9 fraction) in terms of mutagenic potential for TA98 strain (Table 3). Also this strain i.e., TA98 showed maximum response in terms of mutagenic index (9.15 and 9.21 without and with S9 fraction, respectively), induction factor (2.10 and 2.11 without and with S9 fraction, respectively) and mutagenic potential (5.1 and 5.6 without and with S9 fraction, respectively) when treated with acidic fraction of dichloromethane extract (Table 4). Furthermore, it was found that the increase in mutagenic index, induction factor and mutagenic potential followed the similar trend as for the XAD-concentrated and hexane extracted samples, except for the low number of revertants in case of basic and acidic fractions of DCM extracts.

The XAD-concentrated sample was found to be more mutagenic than the liquid-liquid extracted samples as evidenced by the highest values of mutagenic index, induction factor and mutagenic potential observed at the dose level of 20 µL/plate. On the basis of toxicity, wastewater extracts can be arranged as follows:

XAD-concentrated sample > Hexane extracted sample > DCM extracted samples

For all the samples tested, there was an increase in the number of revertant colonies of the tester strains up to dose level of 20 µL/plate and further decline at a dose of 40 µL/ plate. Moreover, TA98 showed maximum response in the presence as well as in the absence of S9 fraction. It was also observed that number of revertants were significantly higher in comparison to the control in all the tester strains, indicating dose-dependent mutagenicity. In order to determine the significance of the reversion of the tester strains, one way ANOVA was performed, which indicated concentration dependent increase in number of revertants compared with the negative control.

The survival pattern of the SOS defective *recA*, *lexA* and *polA* mutants and their isogenic wild type *E coli* K-12 strains is shown in Fig. 1. The damage to the cell by XAD-concentrated sample was found to be high as compared with liquid-liquid extracted samples at the dose of 20 µL-mL^{-1} culture. The survival was 18% in *polA*, 31% in *lexA* and 42% in *recA* mutants when treated with XAD-concentrated extract after 6 h of treatment (Fig. 1a), whereas the hexane extract of the wastewater

Table 1
Reversion of *Salmonella* tester strains in the presence of XAD concentrated wastewater.

Strain	S9	Wastewater extract (μL/plate) Control	2.5	5	10	20	40	Mi	m	LSD P ≤ 0.05
TA97a	–	93 ± 7	287 ± 14 (3.09)	375 ± 10 (4.03)	459 ± 12 (4.94)	512 ± 11 (5.51)	442 ± 9 (4.75)	1.51	6.3	4.46
	+	97 ± 10	302 ± 11 (3.11)	392 ± 15 (4.04)	481 ± 10 (4.96)	539 ± 13 (5.56)	463 ± 12 (4.77)	1.52	6.6	3.64
TA98	–	33 ± 4	265 ± 20 (8.03)	318 ± 11 (9.64)	468 ± 16 (14.18)	509 ± 12 (15.42)	450 ± 13 (13.64)	2.67	7.7	9.80
	+	35 ± 7	287 ± 15 (8.20)	342 ± 14 (9.77)	499 ± 12 (14.26)	546 ± 10 (15.60)	479 ± 15 (13.69)	2.68	8.2	5.75
TA100	–	120 ± 8	251 ± 10 (2.09)	321 ± 11 (2.68)	411 ± 9 (3.43)	487 ± 12 (4.06)	394 ± 11 (3.28)	1.11	5.5	2.57
	+	135 ± 14	283 ± 14 (2.10)	365 ± 14 (2.70)	469 ± 17 (3.47)	549 ± 16 (4.07)	447 ± 13 (3.31)	1.12	6.2	2.57
TA102	–	236 ± 12	321 ± 14 (1.36)	389 ± 12 (1.65)	470 ± 11 (1.99)	559 ± 14 (2.37)	419 ± 17 (1.78)	0.31	4.0	2.57
	+	251 ± 18	364 ± 9 (1.45)	420 ± 16 (1.67)	499 ± 13 (1.99)	597 ± 12 (2.38)	452 ± 12 (1.80)	0.32	4.1	5.75
TA104	–	322 ± 14	493 ± 12 (1.53)	537 ± 11 (1.67)	611 ± 12 (1.90)	698 ± 20 (2.17)	566 ± 13 (1.76)	0.16	4.4	20.17
	+	338 ± 21	516 ± 18 (1.53)	584 ± 13 (1.73)	654 ± 9 (1.93)	739 ± 15 (2.19)	598 ± 11 (1.77)	0.17	4.6	8.14

Values in parentheses are mutagenic index; Mi = induction factor; m = mutagenic potential; LSD = least significant difference.

Table 2
Reversion of *Salmonella* tester strains in the presence of hexane extracted wastewater.

Strain	S9	Wastewater extract (μL/plate) Control	2.5	5	10	20	40	Mi	m	LSD P ≤ 0.05
TA97a	–	92 ± 8	273 ± 15 (2.97)	363 ± 11 (3.95)	432 ± 8 (4.70)	492 ± 9 (5.35)	389 ± 8 (4.23)	1.47	5.2	5.15
	+	96 ± 7	292 ± 12 (3.04)	384 ± 13 (4.00)	458 ± 11 (4.77)	515 ± 15 (5.36)	412 ± 13 (4.29)	1.47	5.4	4.81
TA98	–	30 ± 6	198 ± 14 (6.60)	250 ± 6 (8.33)	342 ± 12 (11.40)	396 ± 14 (13.20)	291 ± 16 (9.70)	2.50	4.7	7.93
	+	38 ± 14	254 ± 10 (6.68)	322 ± 11 (8.47)	439 ± 9 (11.55)	504 ± 15 (13.26)	384 ± 12 (10.11)	2.51	6.2	16.27
TA100	–	130 ± 12	254 ± 14 (1.95)	302 ± 12 (2.32)	370 ± 16 (2.85)	419 ± 17 (3.22)	362 ± 14 (2.78)	0.80	4.4	4.81
	+	156 ± 21	311 ± 19 (1.99)	362 ± 9 (2.32)	449 ± 11 (2.88)	512 ± 13 (3.28)	436 ± 10 (2.79)	0.83	5.3	9.10
TA102	–	222 ± 11	301 ± 20 (1.35)	361 ± 13 (1.62)	436 ± 16 (1.96)	514 ± 11 (2.32)	392 ± 14 (1.76)	0.27	3.6	6.30
	+	236 ± 8	327 ± 11 (1.38)	396 ± 10 (1.68)	465 ± 12 (1.97)	550 ± 9 (2.33)	425 ± 16 (1.80)	0.29	3.9	5.15
TA104	–	318 ± 16	376 ± 14 (1.18)	484 ± 11 (1.52)	544 ± 21 (1.71)	685 ± 14 (2.15)	524 ± 12 (1.65)	0.14	5.0	6.56
	+	327 ± 22	398 ± 20 (1.22)	499 ± 17 (1.53)	562 ± 16 (1.72)	705 ± 12 (2.16)	546 ± 17 (1.67)	0.14	5.2	6.30

Values in parentheses are mutagenic index; Mi = induction factor; m = mutagenic potential; LSD = least significant difference.

exhibited survival of 39% in *polA*, 42% in *lexA* and 48% in *recA* mutants (Fig. 1b). When the strains were treated with the basic and acidic fractions of dichloromethane, they also showed decline in their growth and the survival was 44% in *polA*, 46% in *lexA*, and 51% in *recA* mutants in case of basic fraction; and 48% in *polA*, 51% in *lexA* and 55% in *recA* mutants in case of acidic fraction under similar experimental conditions (Fig. 1c and d).

The effect of the wastewater on the frequency of mitotic phases and mitotic index (MI) of *A. cepa* root meristem cells is given in Table 5, which showed that mitotic index decreased with increasing wastewater concentrations (MI of 11.23% at 5% and 6.38% at 100% wastewater concentrations). Negative control (i.e., distilled water) was found to exhibit the highest MI (23.45%), whereas the positive control (i.e., MMS) displayed the lowest MI (6.2%). Moreover it was observed that the frequency of mitotic phases were affected by the treatment, as the percentage of prophase cells decreased and metaphase cells increased gradually with increasing wastewater concentrations upto 100%, whereas no uniform pattern in anaphase-telophase stages was observed. Furthermore, root meristem cells, treated with wastewater also exhibited different types of abnormalities like C-mitosis, anaphase-bridge, disturbed anaphase-telophase, stickiness and vagrant chromosomes (Table 6, Fig. 2) and percentage of aberrant cells increased with increasing wastewater concentrations. Positive control (MMS) had maximum number of aberrant cells, while cells treated with distilled water exhibited few abnormalities. Statistical analysis of the test indicated that the MI and percentage of aberrant cells caused by the treatments with the wastewater samples were significant ($P < 0.05$) and quite different from that of control samples (positive and negative) using Duncan multiple range test.

Table 3
Reversion of *Salmonella* tester strains in the presence of basic fraction of dichloromethane extracted wastewater.

Strain	S9	Wastewater extract (μL/plate) Control	2.5	5	10	20	40	Mi	m	LSD P ≤ 0.05
TA97a	–	91 ± 9	213 ± 12 (2.34)	275 ± 14 (3.02)	334 ± 21 (3.67)	397 ± 15 (4.36)	304 ± 17 (3.34)	1.21	4.0	7.50
	+	96 ± 14	253 ± 15 (2.64)	291 ± 18 (3.03)	365 ± 11 (3.80)	420 ± 14 (4.38)	362 ± 12 (3.77)	1.22	4.9	4.46
TA98	–	31 ± 6	126 ± 9 (4.06)	212 ± 14 (6.83)	345 ± 11 (11.13)	401 ± 17 (12.90)	307 ± 9 (9.90)	2.48	6.0	7.28
	+	33 ± 8	149 ± 12 (4.52)	239 ± 11 (7.24)	374 ± 20 (11.33)	432 ± 14 (13.09)	328 ± 16 (9.94)	2.49	6.2	7.50
TA100	–	123 ± 17	220 ± 14 (1.79)	262 ± 21 (2.13)	343 ± 12 (2.78)	394 ± 16 (3.20)	320 ± 11 (2.60)	0.79	4.0	6.56
	+	131 ± 24	249 ± 11 (1.90)	286 ± 13 (2.18)	372 ± 18 (2.84)	422 ± 12 (3.22)	353 ± 15 (2.69)	0.80	4.3	20.01
TA102	–	238 ± 15	324 ± 13 (1.36)	377 ± 15 (1.58)	420 ± 17 (1.76)	489 ± 20 (2.05)	406 ± 12 (1.71)	0.05	3.4	5.15
	+	252 ± 21	351 ± 12 (1.39)	402 ± 11 (1.60)	458 ± 13 (1.82)	518 ± 16 (2.05)	433 ± 11 (1.72)	0.05	3.5	7.05
TA104	–	328 ± 22	362 ± 17 (1.10)	448 ± 15 (1.37)	512 ± 10 (1.56)	556 ± 14 (1.70)	509 ± 9 (1.55)	−0.36	4.1	19.59
	+	335 ± 25	380 ± 12 (1.13)	460 ± 11 (1.37)	535 ± 14 (1.60)	581 ± 13 (1.73)	537 ± 15 (1.60)	−0.31	4.6	9.28

Values in parentheses are mutagenic index; Mi = induction factor; m = mutagenic potential; LSD = least significant difference.

Table 4
Reversion of *Salmonella* tester strains in the presence of acidic fraction of dichloromethane extracted wastewater.

Strain	S9	Wastewater extract (μL/plate) Control	2.5	5	10	20	40	Mi	m	LSD P ≤ 0.05
TA97a	–	95 ± 9	208 ± 18 (2.19)	245 ± 11 (2.58)	321 ± 14 (3.38)	388 ± 14 (4.08)	278 ± 13 (2.93)	1.13	3.5	5.46
	+	99 ± 12	238 ± 13 (2.40)	268 ± 16 (2.71)	352 ± 18 (3.56)	410 ± 12 (4.14)	315 ± 16 (3.18)	1.14	4.0	4.46
TA98	–	34 ± 4	96 ± 12 (2.82)	136 ± 14 (4.00)	227 ± 13 (6.68)	311 ± 13 (9.15)	247 ± 20 (7.26)	2.10	5.1	14.67
	+	38 ± 9	124 ± 18 (3.26)	152 ± 11 (4.05)	258 ± 12 (6.78)	350 ± 17 (9.21)	279 ± 13 (7.34)	2.11	5.6	6.30
TA100	–	126 ± 7	204 ± 14 (1.62)	255 ± 14 (2.02)	315 ± 17 (2.50)	398 ± 15 (3.16)	287 ± 13 (2.28)	0.77	3.5	6.03
	+	138 ± 13	231 ± 17 (1.67)	280 ± 10 (2.03)	352 ± 14 (2.55)	442 ± 20 (3.20)	318 ± 14 (2.30)	0.79	3.8	6.30
TA102	–	257 ± 13	297 ± 14 (1.16)	367 ± 20 (1.43)	411 ± 16 (1.60)	472 ± 13 (1.84)	384 ± 17 (1.49)	−0.18	2.9	4.81
	+	263 ± 20	312 ± 13 (1.19)	405 ± 14 (1.54)	431 ± 12 (1.64)	488 ± 15 (1.89)	406 ± 21 (1.54)	−0.16	3.0	6.80
TA104	–	322 ± 21	345 ± 14 (1.07)	412 ± 11 (1.28)	495 ± 17 (1.54)	542 ± 18 (1.68)	448 ± 21 (1.39)	−0.38	3.1	7.28
	+	343 ± 15	371 ± 16 (1.08)	438 ± 17 (1.28)	528 ± 15 (1.54)	578 ± 11 (1.69)	479 ± 15 (1.40)	−0.38	3.3	3.64

Values in parentheses are mutagenic index; Mi = induction factor; m = mutagenic potential; LSD = least significant difference.

4. Discussion

Kanpur city is one of the major industrial centres in North India, situated in the Indo-Gangetic plains between the parallels of 26°28′N and 80°24′E. The city is the main hub of a large number of textile industries which generate enormous amount of wastes that are consistently discharged into the Ganges River, flowing across the city.

Although the measurement of various physico-chemical parameters gives an indication of pollution load of the wastewater, but the analysis alone does not reflect the actual hazards associated with the toxic chemicals [29]. Therefore, in addition to physico-chemical characterization, toxicity testing of the wastewater is of practical significance as it would help in predicting the combined effects of different chemicals in a mixture and the long-term harmful effects of these pollutants on the living organisms.

The dark color and alkaline pH of the wastewater are due to use of various types of chemicals like dyes, anionic stabilizers, detergents, sodium hydroxide and hydrogen peroxide etc. during textile manufacturing. Very high TDS is also an indicator of pollution which affects the self-purification process of the wastewater. Moreover, various metals present in the wastewater have a negative impact on the environment and human health. It causes various illnesses like hemorrhage, nausea, skin irritation, ulceration and dermatitis [10]. Textile wastewater possessed different types of organic compounds, many of them are highly toxic to living organisms like azobenzene and benzidine have been reported to be mutagenic and carcinogenic [37–40].

Present study was conducted to ascertain the mutagenic, genotoxic and cytotoxic potential of the textile wastewater. For this purpose, a combination of genotoxicity assays, involving both bacterial and plant systems, were performed to get a comprehensive idea about the effects of the textile effluents on the environment. Genotoxicity assays are very important for evaluation of hazardous wastes and risk assessments associated with the pollutants [41,42]. Since, a large number of known mutagens are organic compounds (aromatic amines, polycyclic

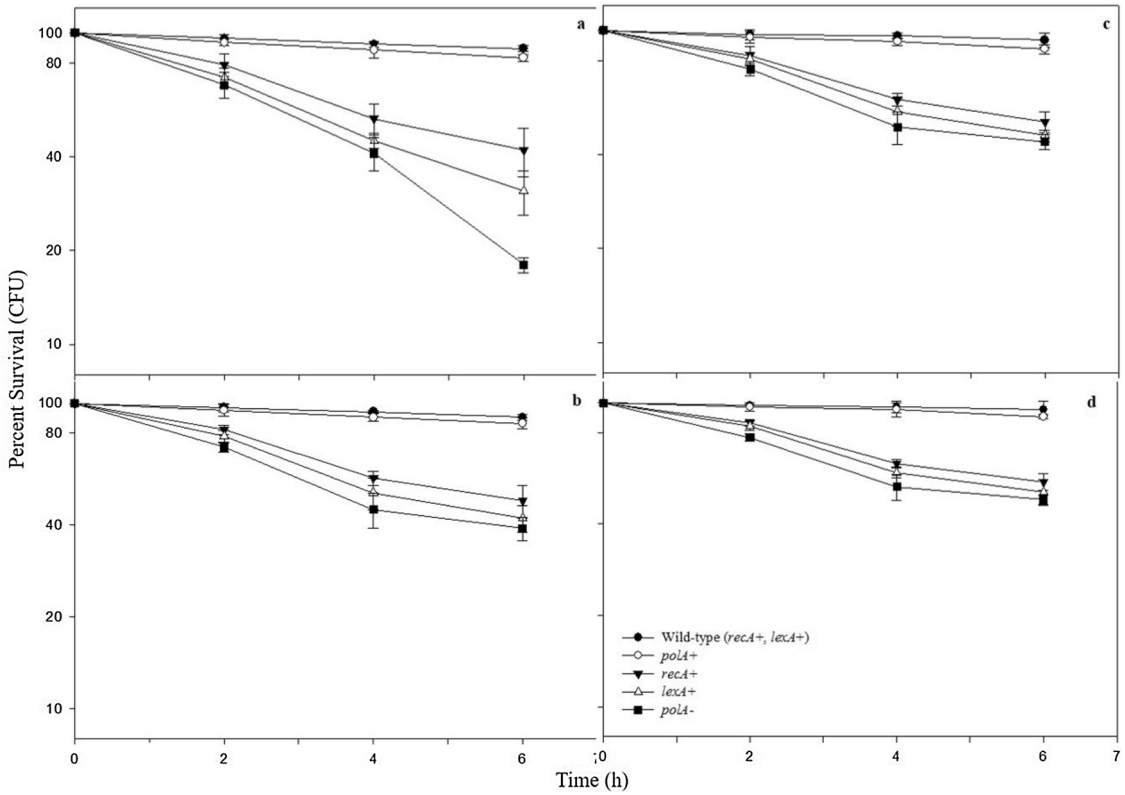

Fig. 1. Survival of *E. coli* K-12 strains treated with XAD-concentrated wastewater (a); hexane extracted wastewater (b); basic fraction of dichloromethane extract (c), and acidic fraction of dichloromethane extract (d).

Table 5
Effect of different concentrations of wastewater on mitotic index and mitotic phase of *Allium cepa* root meristematic cells.

Samples	Concentration (% v/v)	Mitotic Phases (%)			Mitotic index (% ± SD)
		Prophase	Metaphase	Anaphase-Telophase	
Wastewater	5	22.22	30.04	47.74	11.23 ± 1.0[b]
	10	19.35	32.25	48.39	9.73 ± 3.7[ab]
	25	11.00	34.00	35.00	7.65 ± 0.6[ab]
	50	10.34	41.38	48.28	7.54 ± 1.4[ab]
	100	6.00	51.14	42.86	6.38 ± 1.7[a]
Positive control		5.10	54.36	40.54	6.20 ± 1.6[a]
Negative control		28.52	43.20	28.28	23.45 ± 2.4[c]

Means with the same letters do not significantly differ at 0.05 level (Duncan multiple range test); ± Standard deviation.

aromatics, polychlorinated compounds etc.), it is preferable to extract and concentrate the samples using organic solvents prior to mutagenicity testing [18]. Using a range of organic solvents offers the advantage of trapping a variety of intermediates of unknown nature which are produced as a result of constant microbial transformations taking place in the environment [43]. Textile wastewater containing different chemicals has been found to be genotoxic causing different levels of DNA damage to aquatic organisms and consequently to the ecosystem [44].

Each tester strain possesses a specific type of mutation in its '*his* operon' (i.e., TA97a/ TA98 frameshift mutations, TA100 base pair substitution/ missense mutations and TA102/ TA104 transitions/ transversions) and can therefore detect a specific type of mutagen [32,45]. The results of the Ames test clearly showed that all the tester strains, particularly TA97a, TA98 and TA100 exhibited significant increase in the number of revertant colonies for all the extracts tested, TA98 being the most responsive strain in terms of mutagenic index, induction factor and mutagenic potential in the absence and presence of S9 fraction (Tables 1–4). Therefore, it can be suggested that the textile effluent contained large amount of frameshift and missense mutagens. Akhtar et al. [46] determined the mutagenic, genotoxic and cytotoxic potential of textile effluent, collected from a textile industry located at Kasur road, Lahore using Ames test (with or without S9), *in vitro* comet assay and MTT (methyl thiazole tetrazolium) assay and found positive response of Ames *Salmonella* strains, when treated with the effluent.

Although dyes are an essential part of textile industry, but they have proven to be mutagenic and genotoxic as indicated by various mutagenicity assays reported previously [47,48]. de Aragaˆo Umbuzeiro et al. [49], characterized the mutagenic activity of Cristais River, Saˆo Paulo, Brazil, that was receiving discharge of a dye processing plant by studying different samples viz. the treated industrial effluent, raw and treated water, and the sludge produced by a Drinking Water Treatment Plant (DWTP) located about 6 km from the industrial discharge. Their results have shown that the textile dyes contributed significantly to the mutagenic activity as indicated by the positive response in Ames *Salmonella* strain YG1041 (derived from the *Salmonella* strain TA98) in the presence of S9 fraction. Similarly, mutagenicity and carcinogenicity of a textile dye processing plant industrial effluent, that was being discharged into the Cristais River, has been evaluated by de Lima et al. [50] using Ames *Salmonella*/ microsome assay (strains TA98 and YG1041) and Aberrant crypt foci assay (Wistar rats). They found that the exposure of the effluent has given positive results in Ames test and also increased the number of preneoplastic lesions in the rat colon.

Moreover it was observed that the wastewater extracts exhibited mutagenicity both in the absence and presence of S9 fraction, however, the mutant strains gave higher mutagenic response when S9 fraction was added, thus indicating the presence of pro-mutagens (that required metabolic activation system) in addition to the direct acting mutagens in the samples. Furthermore, it was noticed that the tester strains gave significantly higher mutagenic response when treated with XAD-concentrated sample than the liquid-liquid extracts. This may be due to the fact that XAD adsorbs and concentrates various polar and nonpolar compounds present in the wastewater that may be genotoxic/ mutagenic [11].

E. coli K-12 survival assay, in which mutant strains are unable to induce SOS repair genes when exposed with any mutagen, is an important parameter for genotoxicity testing [25]. In the present study, all the DNA repair defective mutants showed significant decline in their colony forming units in the presence of test samples as compared to their isogenic wild type counterparts (Fig. 1). Fazili and Ahmad [51] reported the significant decline in DNA repair defective *E. coli* K-12 strains when treated with industrial wastewaters. Moreover, it was also observed that the damage brought about in the DNA repair-defective mutants were maximum when they were treated with XAD-concentrated sample, thus supporting the results obtained by Ames mutagenicity testing. Present findings demonstrated an important contribution of *recA*, *lexA* and *polA* genes in repair process that help bacteria in tolerating pollutant stress [11,21,52].

Presence of large sized chromosomes in less number (2n = 16) and short cell cycle (about 20 h) in root meristematic cells, make *A. cepa* a favourable eukaryotic system for assessing chromosomal damages [35]. Reduction in mitotic index and induction of chromosomal aberrations are two important parameters for evaluating cytotoxicity and

Table 6
Chromosomal aberrations in the root meristematic cells of *Allium cepa* exposed to different concentrations of wastewater for 72 h.

Sample	Concentration (% v/v)	Types of aberrations								Total aberrant cells (% ± SD)
		CM	AB	L	BN	S	DM	DAT	V	
Wastewater	5	1	–	–	–	1	–	–	–	3.08 ± 0.52[e]
	10	2	2	–	–	1	–	3	4	14.46 ± 2.10[d]
	25	4	1	–	–	–	–	5	3	24.07 ± 1.65[c]
	50	2	–	–	–	–	–	6	7	28.30 ± 4.30[c]
	100	10	1	–	–	2	1	10	7	50.88 ± 2.98[b]
Positive control		17	9	21	7	24	19	11	27	62.75 ± 3.66[a]
Negative control		1	–	1	–	–	–	1	2	1.8 ± 0.87[e]

CM: C-mitosis, AB: anaphase bridge, L: laggard, BN: binucleated cell, S: stickiness, DM: disturbed metaphase DAT: disturbed anaphase-telophase, V: vagrant; Means with the same letters do not significantly differ at 0.05 level (Duncan multiple range test); ± Standard deviation.

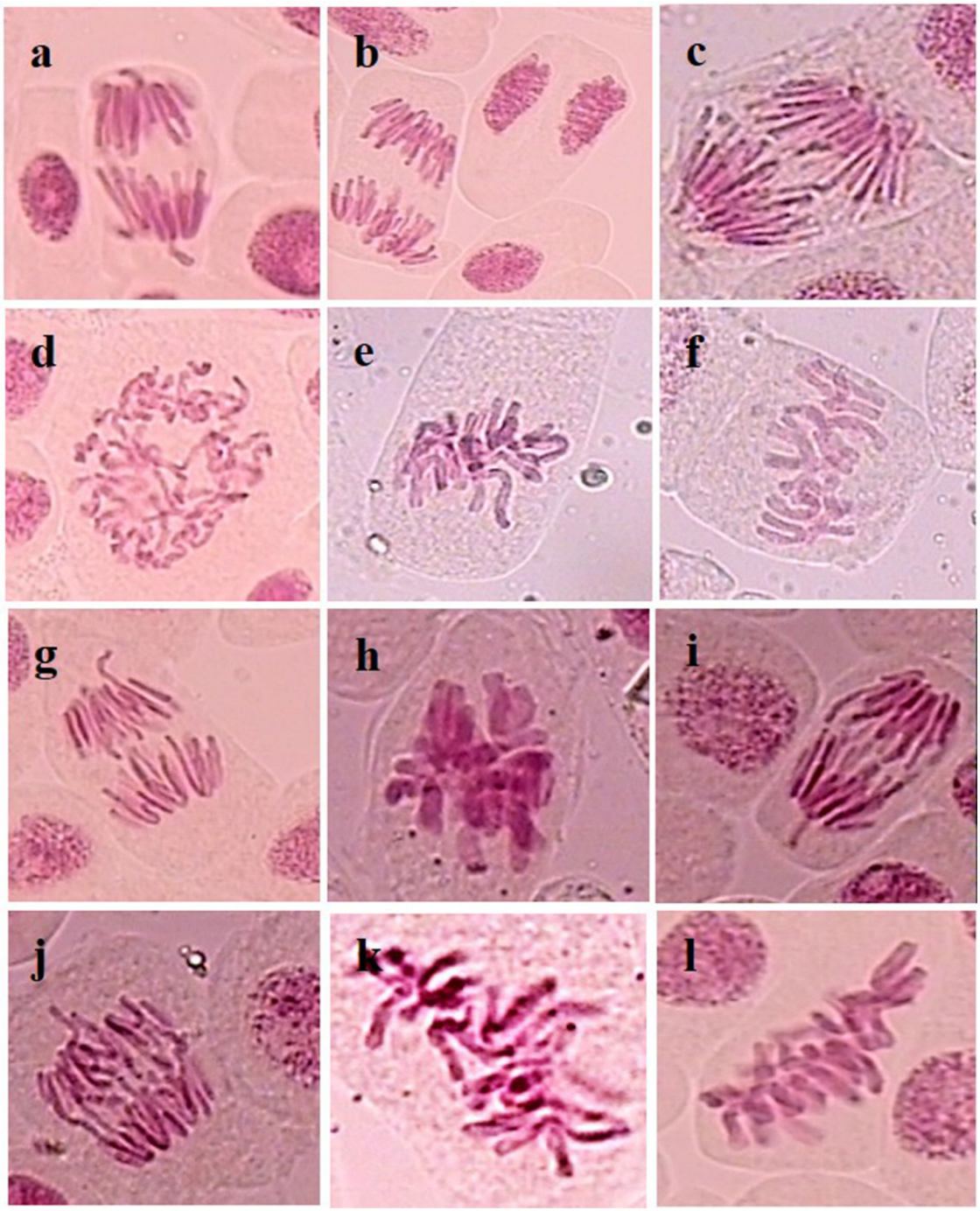

Fig. 2. Different types of chromosomal aberrations induced by textile wastewater in *Allium cepa* root tips: Vagrant chromosome (a), Multipolar anaphase and binucleated cell (b); Anaphase bridge (c); Spindle disturbance in anaphase (d); Disturbed metaphase (e, f); Multipolar anaphase with vagrant chromosome (g); Stickiness (h); Anaphase bridge with vagrant chromosome (i); Anaphase with micro bridge (j); C-Mitosis (k, l).

genotoxicity of various chemicals present in wastewaters [9]. Many previous reports have demonstrated that there is a good correlation between *A. cepa* and other mammalian and human test systems [9,53–55].

In the present study it was observed that the textile wastewater resulted in reduction of mitotic index in a dose dependent manner and the reduction was significant when compared with control (Table 5). The reduction in mitotic index can be ascribed to the presence of a large number of toxic chemicals having DNA damaging activity. Many chemicals present in the wastewater have been reported to affect the process of DNA synthesis and protein synthesis, thus ultimately result in the decline of MI [56]. Grover and Kaur [57] analysed the genotoxicity of sewage water and textile and paper mill effluents using *A. cepa* test and found that the industrial effluents have caused the development of micronuclei and aberrant cells in the root tip cells of *A. cepa*. Vijayalakshmidevi and Muthukumar [58] demonstrated the genotoxicity of untreated and treated textile wastewater employing *A. cepa* plant bioassay and observed the decrease in mitotic index and root length as well as the occurrence of various types of aberrant cells in the plant cells exposed with the effluent.

Chromosomal aberrations are the result of DNA breakage that could not be repaired at all or improperly repaired [59]. In the present study,

various types of chromosomal aberrations were observed, with C-mitosis, disturbed anaphase-telophase and vagrant chromosomes being the most prominent (Table 6, Fig. 2). C-mitosis is due to the disturbances in the mitotic spindles [60]. Vagrant indicates the unequal distribution of chromosomes due to failure of chromosomal separations in anaphase [61]. Thus, the presence of different types of aberrations (C-mitosis, anaphase bridges, stickiness, disturbed anaphase-telophase and vagrant) in root meristematic cells of *A. cepa* could be attributed to the combined effects of clastogenic and aneugenic actions of various chemicals present in textile wastewater [62].

Sudhakar et al. [63] studied the genotoxicity of a silk dyeing industry effluents using *A. cepa* assay and reported inhibition of mitotic index and induction of chromosomal abnormalities (like binucleated cells, bridges, fragments, laggards, stickiness and vacuolated nuclei) in root tips cells treated with the effluent. Caritá and Marin-Morales [48] also observed the induction of chromosomal aberrations, nuclear abnormalities and development of micronuclei in growing seeds of *A. cepa* exposed to increasing concentrations of textile effluent. Pathiratne et al. [62] used *A. cepa* test for toxicity assessment of industrial effluents, including the textile wastewater and demonstrated the presence of cytotoxic and genotoxic compounds in the textile effluent. Hemachandra and Pathiratne [64] assessed the cytotoxic and genotoxic effect of treated textile wastewater, which got discharged into a major river (Kelani River) in Sri Lanka, using *in vivo* systems i.e., *A. cepa* and *Oreochromis niloticus*. They observed significant reduction in root growth, induction of chromosomal aberrations and mito-depression in the root tip cells of *A. cepa*, when exposed to the treated textile effluent, thus demonstrating the inefficiency of the treatment process.

5. Conclusion

The physico-chemical analysis showed that the textile wastewater contained both inorganic and organic pollutants. Complementary to the analysis, a set of biological tests revealed that these pollutants present in the wastewater are mutagenic and genotoxic in nature, which resulted in increase in number of revertants of Ames *Salmonella* strains (i.e., TA97a, TA98, TA100, TA102 and TA104) and also initiate the SOS response and thus bring about mutation in bacterial DNA. Present findings suggested that most of the mutagens caused frameshift mutations, since TA98 strain gave the maximum response in terms of mutagenic index, induction factor and mutagenic potential. The wastewater also contained cytotoxic chemicals that resulted in reduction of mitotic index and induced various types of chromosomal aberrations in the root meristematic cells of *A. cepa*. Therefore, the study demonstrate the importance of performing environmental monitoring of water systems receiving textile effluents using both physico-chemical and biological methods in order to assess its harmful effects on ecosystems and public health.

Conflict of interest

The author(s) declared no conflicts of interest with respect to the authorship and/or publication of the article.

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