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Assessment of cytotoxic and genotoxic potential of refinery waste effluent using plant, animal and bacterial systems

Amit Kumar Gupta, Masood Ahmad *

Department of Biochemistry, Faculty of Life Sciences, Aligarh Muslim University, Aligarh, 202002, U.P., India

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ABSTRACT

The work described here presents the toxic effect of Mathura refinery wastewater (MRWW) in plant (*Allium cepa*), bacterial (*E. coli* K12) and human (blood) system. The samples were collected from adjoining area of Mathura refinery, Dist. Mathura, U.P. (India). Chromosomal aberration test and micronucleus assay in (*A. cepa*) system, *E. coli* K12 survival assay as well as hemolysis assay in human blood were employed to assess the toxicity of MRWW. MRWW exposure resulted in the formation of micronuclei and bridges in chromosomes of *A. cepa* cells. A significant decline occurred in survival of DNA repair defective mutants of *E. coli* K12 exposed to MRWW. On incubation with MRWW, calf thymus DNA–EtBr fluorescence intensity decreased and percent hemolysis of human blood cells increased. An induction in the MDA levels of MRWW treated *A. cepa* roots indicated lipid peroxidation also. Collectively, the results demonstrate a significant genotoxic and cytotoxic potential of MRWW.

1. Introduction

Biosphere components, air, water, soil are frequently reported to be contaminated with mutagens and carcinogens, and their interaction with humans increases carcinogenic hazards. For this reason, the monitoring of genotoxic compounds in the environment has become an important objective of public health, with the intention of avoiding or minimizing direct and indirect human exposure to these toxic substances [1].

Petroleum is one of the most important sources of energy on this planet. However, the petroleum industry activities, related to different stages of production (from oil to grease) have been leading to several environmental impacts, mainly the release of pollutants into water systems [2]. The refinery effluents consists of compounds from original crude oil stock as well as metallic (Zn, Cr, Va, Ni, Pb, Cu) and non-metallic constituents. Phenols are also a major component of refinery wastewaters [3]. Moreover, among the hydrocarbons present in crude oil, the polycyclic aromatic hydrocarbons (PAHs) are some of the most dangerous environmental contaminants due to their toxic, carcinogenic, and mutagenic effects [4].

Biomarkers refer to certain changes in living cells induced by environmental contaminants. They also serve as indicators of toxicant(s) that enter the organism [5] and, as such, provide information about the bioavailability of the toxicant(s). Moreover, they indicate that the toxicant(s) has/have been distributed within the organism and initiated a toxicological effect at certain critical targets.

Plant tests have been widely used for detecting the genotoxicity of chemical compounds and for in situ monitoring of environmental genotoxic contaminants [6]. Among them, *Allium cepa* root chromosomal aberration (AL-RAA), micronuclei (AL-MCN) and root inhibition tests are widely used to evaluate the genotoxicity of chemical compounds and environmental contaminants [7].

DNA repair assay takes a prominent position for the detection of genotoxic potential [8]. The estimate of the extent of DNA damage based on the expression of SOS genes in the bacterial cells [9], definitely serves as a better biomarker of genotoxicity of the test samples. However, the cytotoxic offence of the wastewater has been usually estimated by hemolysis assay [10,11].

Water samples from varied sources, including natural (rivers and lakes), domestic and industrial origins, have been analyzed for their toxicity by *A. cepa* test [12]. The advantages of the *A. cepa* test are that it is a fast and inexpensive method, easy to handle, gives reliable results, comparable with other tests performed in mammalian systems, e.g. with high concordance with the chromosomal aberration assay in bone marrow cells in rats [13],

* Corresponding author. Tel.: +91 571 2700741/9897459786; fax: +91 571 2706002.
E-mail address: masood_amua@yahoo.co.in (M. Ahmad).

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in human lymphocytes, in V79 cell line of Chinese hamster and in other organisms tests such as fish and unicellular algae [14].

The changes in the structure and properties of DNA upon exposure to unknown compounds can also be used as a screening tool for genotoxic potential. Methods that have been used to detect chemical-induced DNA damage include, comet assay, random oligonucleotide-primed synthesis assay, etc. [15]. Although, sensitive and specific, these methods are typically complex and require extensive training [15]. Hence, a fluorescence-based screening assay for DNA damage having sensitivity, specificity and ease of technique has been used lately.

Some reports of multibiomarker studies in freshwater environments with particular reference to their genotoxicants have been published [16]. We conducted the present study to gain an insight whether the above mentioned battery of toxicity assays can yield a simple genotoxic biomarker to the list of biomarkers presently used for monitoring the refinery wastewater toxicity. Moreover, this work was also carried out to evaluate the toxicity in general and genotoxicity in particular, of Mathura refinery wastewater.

2. Materials and methods

2.1. Reagents

Acetocarmine, Tris–HCl, $MgCl_2$, NaOH, glycine, nicotinamide adenine dinucleotide phosphate (NADP) were obtained from SRL, Chemicals, Mumbai, India. Tri-carboxylic acid was collected from Qualigens Fine Chemical (Mumbai). All other chemicals and reagents were of analytical grade. The wastewater samples were collected from the surrounding area of Mathura refinery, Mathura, U.P. (India) in sterile glass bottles as per the method described by APHA [17] and fresh sample was used every time for experiments. Prior to use, the particulate matter was removed by means of filtration using Whatman No. 1 filter paper.

2.2. Tests carried out in A. cepa

2.2.1. Root inhibition test

The basic protocol of Fiskesjo [14] was followed with slight modification using a sharp knife the yellowish brown scales and bottom plates of the onion bulbs were removed. Test tubes (60 ml capacity) filled with serially diluted MRWW (0.25×, 0.50×, 0.75×, 1.0×) were taken and on each test tube one onion bulb was placed. Aquaguard (India Ltd) purified water was used as negative control in all experiments. The treatment was continued for 2 days in a dark chamber at room temperature. After 2 days, all the onion bulbs were taken out and roots were collected for length measurement.

2.2.2. Quantification of lipid peroxidation

Lipid peroxidation was determined by measuring the amount of malondialdehyde (MDA) according to Unyayar et al. [18]. About 5 g of root tissues from control and MRWW treated onion were cut into small pieces and homogenized by the addition of 5 ml of 5% trichloroacetic acid (TCA) solution. The homogenates were then transferred into fresh tubes and centrifuged at 12,000 rpm for 15 min at room temperature. Equal volumes of supernatant and 0.5% thiobarbituric acid (TBA) in 20% TCA solution were added into a new tube and boiled at 96 °C for 25 min. The tubes were transferred into ice-bath and then centrifuged at 10,000 rpm for 5 min. The absorbance of the supernatant was measured at 532 nm and corrected for non-specific turbidity by subtracting the absorbance at 600 nm. 0.5% TBA in 20% TCA solution was used as the blank. MDA contents were calculated using the extinction coefficient of 155 m^{-1} cm^{-1}.

2.2.3. Chromosomal aberration test

Chromosomal aberration assay was conducted according to the method of Asita and Matobole [19] with slight modification. At the end of the 18 h exposure, root tips from onions were collected at random and assessed. Root tips (1–2 cm long) were cut from each treated onion and placed in a small glass specimen bottle and fixed in acetic alcohol (ethanol:glacial acetic acid in 3:1 ratio) for 24 h at 4–6 °C. The root tips were washed twice with ice cold water for 10 min each and allowed to dry in a watch glass. A solution of 1 N HCl pre-heated to 60 °C was added to the root tips in the watch glass for 10 min and HCl was discarded. The HCl treatment was repeated a second time. The root tips were transferred to clean microscope slides and cut into 2 mm slices from the growing tip. Acetocarmine stain was added to each slide to cover the root tip for about 10 min. A glass cover slip was placed on the root tip and tapped gently with a pencil eraser to spread the cells evenly to form a monolayer in order to facilitate the scoring process for normal and aberrant cells in the different stages of the cell cycle. The slides were viewed under the light microscope (Olympus CX21) using the 100× objective lens with oil immersion. On one slide for each treatment, a total of 5000 cells were scored and recorded as dividing (metaphase, anaphase) cell to determine the MI. MI was expressed as the number of dividing cells per 1000 cells scored.

2.2.4. Micronucleus assay

Micronucleus assay was conducted according to the method of Cavusoglu et al. [20] with slight modification. At the end of 18 h exposure with the test sample, root tips were collected and fixed for 6 h in a Clarke's fixator (3:1, i.e. acetic acid glacial and distilled water), washed for 15 min in ethanol (96%) and stored in ethanol (70%) at 4 °C until making the microscope slides. The root tips were hydrolyzed in 1 N HCl at 60 °C for 17 min, treated with 45% acetic acid solution for 30 min and stained for 24 h in acetocarmine. After staining, the root meristems were separated and squashed in 45% acetic acid solution. For the MN analysis, 5000 cells were obtained from the portion of root tip (1000 cells/slide). Micronucleated cells were scored under a binocular light microscope (Japan, Olympus BX51) at 100× magnification.

2.3. E. coli survival assay

The survival pattern of DNA repair defective single and double mutants along with isogenic wild type strain of *E. coli* K12 was determined following the procedure of Rehana et al. [21]. The bacterial cells were harvested by centrifugation from exponentially growing culture (1×10^8 viable counts/ml). The pellets so obtained were suspended in $MgSO_4$ (0.01 M) solution and treated with an equal volume of MRWW. Aliquots were withdrawn at regular intervals of 2 h for a maximum period of 6 h, suitably diluted and plated to assay the colony forming ability of the cells.

2.4. Hemolysis assay

2.4.1. Isolation of erythrocytes from human blood

Heparinized fresh human blood (self donor) was taken from young (28 years) healthy non-smoking individual. It was centrifuged at 1500 rpm for 10 min at 4 °C in a clinical centrifuge and the plasma and buffy coat were removed by aspiration. The erythrocyte pellet was washed thrice with phosphate buffered saline (PBS) (0.01 M sodium phosphate buffer, 0.9% NaCl, pH 7.2) and resuspended in PBS to give a 5% hematocrit.

2.4.2. Treatment of erythrocytes with MRWW and preparation of lysates

Erythrocytes were incubated with different concentrations of MRWW (0.2×, 0.4×, 0.6×, 0.8×) for 1 h at 37 °C. The treated RBCs

were centrifuged at 2500 rpm for 10 min at 4 °C. Supernatants were collected and their absorbance recorded at 540 nm.

2.4.3. Scanning electron microscopy study

Red blood cells (RBCs) were prepared from fresh human blood (self donor) collected in acid citrate dextrose by centrifugation at 1500 rpm for 10 min at 4 °C. The cells were washed thrice with 5 ml of isotonic NaCl solution. The packed RBCs were suspended in 3 ml of 10 mM Tris–HCl, pH 7.4 containing 0.15 M NaCl to give 0.5% hematocrit. The reaction mixture was incubated with 0.8× concentration of MRWW for 1 h. After incubation, RBCs were applied on glass slides washed with alcohol and dried. The glass slides were then coated with gold by a sputter coater and the micrographs were taken using scanning electron microscope (Philips, Japan).

2.5. Fluorescence measurements

Ethidium bromide displacement assay was performed by the method of Rahban et al. [22]. At first, DNA (0.1% in Tri-buffer, pH 7.5) was added to aqueous ethidium bromide solution (0.1 mg/ml), and the wavelength selected as the excitation radiation for samples at 37 °C was in the range 480–720 nm. To this solution (containing ethidium bromide and DNA), different concentrations of MRWW (20 μl, 25 μl, 30 μl, 35 μl) were added. Measurements were done in spectrofluorometer (Shimadzu, Japan) using a 1 cm path length fluorescence cuvette.

3. Statistical evaluation

Data were expressed as mean ± S.D. of six values and analyzed by one-way ANOVA. Differences among controls and treatment groups were determined using Student's *t*-test. *p* values of less than 0.05 were considered statistically significant. All comparisons were made with control.

4. Results

4.1. Root inhibition, lipid peroxidation, chromosomal aberration and micronucleus assays in A. cepa exposed to MRWW

Fig. 1a and b shows the metaphase stage of cell cycle with sticky chromosomes in onion cell. Fig. 1c depicts the changes occurred in *A. cepa* chromosomes after exposure to MRWW. Anaphase stage with laggard formation is shown in this figure, was observed in some cases, after the treatment with MRWW. Fig. 1d illustrates the normal late anaphase in *A. cepa* cell. The toxic effect of MRWW also results in the formation of bridge at anaphase stage with the presence of fragment in *A. cepa* cells (Fig. 1e). Fig. 1f shows the presence of double bridge in *A. cepa* cells upon treatment with MRWW. Fig. 1g presents the formation of single bridge with outwarded chromosome, and Fig. 1h illustrates the late anaphase stage with the formation of two bridges after the treatment with MRWW. Incubation with MRWW also caused generation of binucleated cells in *A. cepa* (Fig. 2a). Presence of micronuclei was also noticeable consequent upon MRWW treatment (Fig. 2b). Refinery wastewater treatment significantly reduced the *A. cepa* root length (Table 1). The highest root length was observed to be 3.7 cm in control onion bulbs whereas minimum root growth was 1.2 cm grown in onions exposed to 1× concentration of MRWW. Lipid peroxidation measured as MDA levels showed a significant increase in the roots exposed to wastewater (Table 1). The maximum induction in lipid peroxidation was recorded to be 28.40 μM compared to control value as 11.42 μM in onion roots exposed to 1× concentration of MRWW.

Table 1
The effect of test refinery wastewater samples on *Allium cepa* root length formation and MDA accumulation.

Sample concentration	*Allium cepa* root length (cm)	Malondialdehyde content (μM)
None (control)	3.7 ± 0.16	11.42 ± 2.10
0.25×	3.1 ± 0.13	13.40 ± 2.24
0.5×	2.5 ± 0.14	18.10 ± 2.76
0.75×	1.9 ± 0.11	22.10 ± 2.65
1.0×	1.2 ± 0.10	28.40 ± 3.01

An appreciable reduction in MI (mitotic index) was observed following MRWW treatment (30 units) to onion bulbs as compared to control (50 units) (Table 2). The total number of anaphase aberrations was also relatively much higher in MRWW treated bulbs (14 units) compared with control (0.4 units). Table 2 also summarizes the data on the formation of binucleated *A. cepa* cells and those containing micronuclei as a result of MRWW treatment. A comparatively higher number of binucleated as well as micronuclei containing cells was observed in case of MRWW treated onion bulbs compared to untreated control.

4.2. Fluorescence measurements

Fig. 3a depicts the changes in relative fluorescence of ethidium bromide bound to calf thymus initiated on incubation with DNA with increasing concentrations of MRWW (20–35 μl). A 30% reduction in relative fluorescence was observed as a result of exposure to MRWW. Fig. 3b presents the fluorescence emission spectra of intercalated ethidium bromide incubated with calf thymus DNA on treatment with increasing concentrations of MRWW at 37 °C. A significant reduction in the intensity of ethidium bromide at different concentrations of MRWW at physiologic temperature (37 °C) was observed.

4.3. E. coli survival assay in the presence of MRWW

Genotoxic nature of MRWW was also investigated by employing *E. coli* survival assay. The results are summarized in Table 3. AB2480 (*recAuvrA*) double mutant was the most sensitive to killing action by the test sample with the percent survival reducing to zero (approximately) after 6 h exposure. AB2463 (*recA*) and AB1186 (*uvrA*) mutants were the next in sensitivity with survival of 3% with the test sample. AB 2494 (*lexA*) strain exhibited a survival of 19% whereas AB3027 (*polA*) showed the highest survival (38%) with MRWW treatment.

Table 2
Summary of chromosomal aberrations, micronuclei and mitotic indices in *Allium cepa* cells exposed to Mathura refinery waste water, mineral water (negative control) and methyl methane sulphonate (10 mg/L) (positive control).

Sample	Mitotic Index (MI)	No. of chromosomal aberrations and micronuclei in the observed cells (1000)					
		Stickiness	Fragments	Laggards	Bridges	Micronuclei	Total Aberration
Negative control	49.2 ± 3.8	–	1	1	–	1.1 ± 0.5	0.4 ± 0.3
Positive control	43.3 ± 4.2	9	17	5	15	10.3 ± 1.1	11.3 ± 3.6
MRWW (1×)	37 ± 2.8	7	16	9	29	6.1 ± 0.9	14 ± 3.8

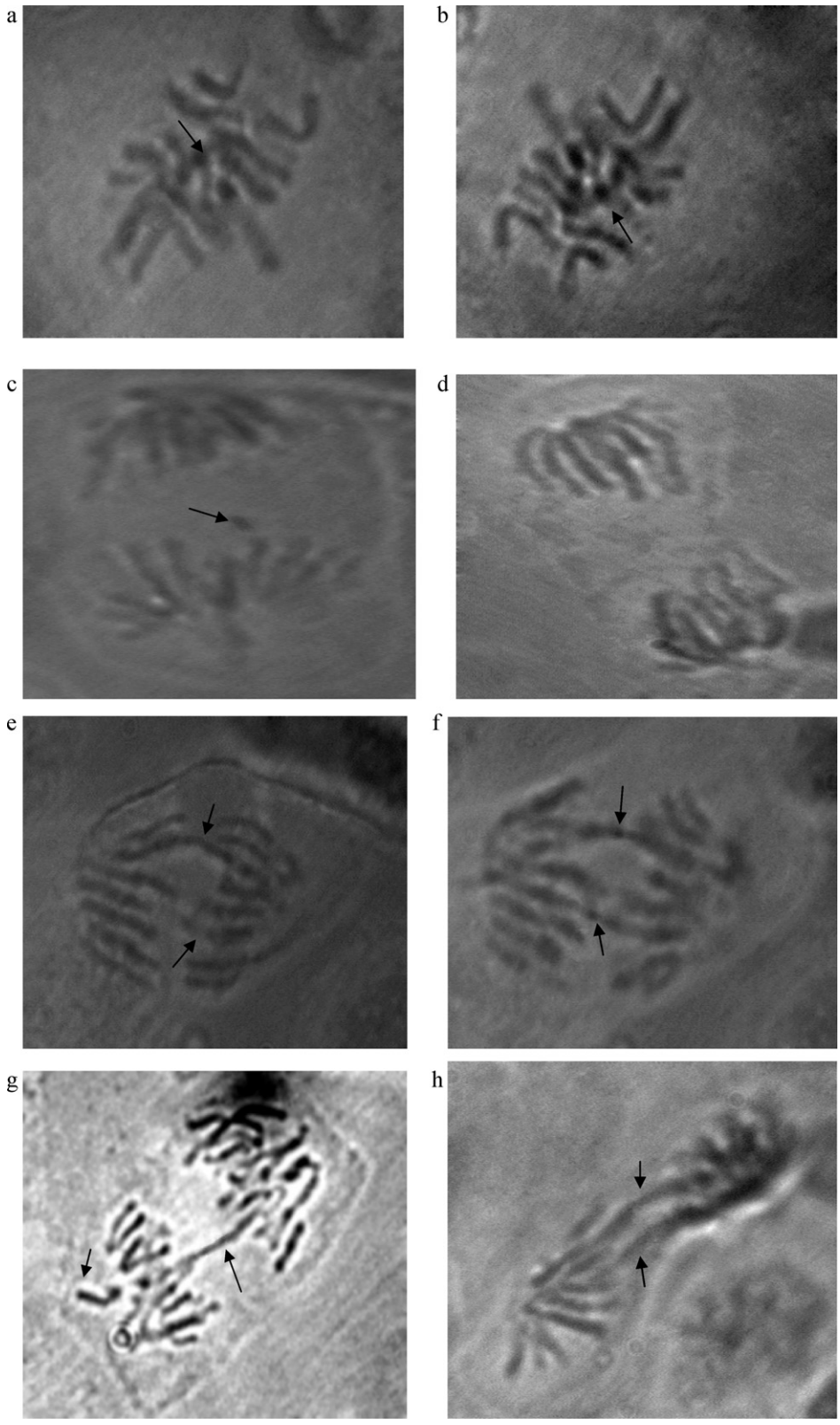

Fig. 1. a, b. Metaphase stage with sticky chromosomes in onion cell as a result of Mathura refinery wastewater treatment. c. Anaphase stage with laggard in onion cell as a result of Mathura refinery wastewater exposure. d. Late anaphase stage in onion cell. e. Anaphase stage with bridge and fragment in onion cell as a result of Mathura refinery wastewater treatment. f. Anaphase stage with double bridge in onion cell as a result of Mathura refinery wastewater treatment. g. Anaphase stage with bridge and out warded movement of chromosome as a result of Mathura refinery wastewater treatment. h. Late anaphase stage with double bridge as a result of Mathura refinery wastewater treatment.

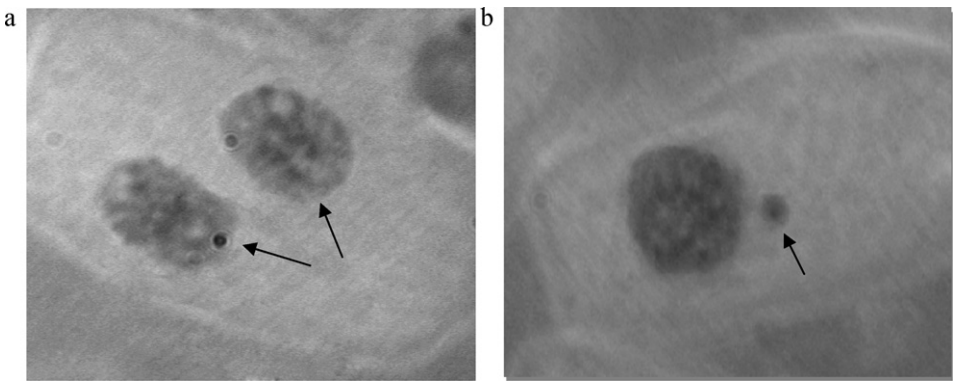

Fig. 2. a. Binucleated onion cell as a result of Mathura refinery wastewater exposure. b. Onion cell with micronuclei as a result of Mathura refinery wastewater exposure.

4.4. Hemolysis assay in the presence of MRWW

Fig. 4 presents the change in % hemolysis with increasing concentrations of MRWW (0.2×, 0.4×, 0.6×, 0.8×). Maximum % hemolysis was observed at 0.8× concentration of MRWW. An increase up to 52% in percent hemolysis was noticed after the treatment of RBCs with 0.8× concentration of MRWW as compared to control. Fig. 5a shows the SEM image of untreated RBC, and Fig. 5b presents the morphological changes in RBCs after treatment with MRWW.

5. Discussion

Pollution of the aquatic environment occurs from multitude of sources including from oil refineries. Oil refinery effluents contain various chemicals at different concentrations including heavy metals, phenol and hydrocarbons [23]. The exact composition cannot however be generalized as it depends on the refinery and which units are in operation at any specific time. It is therefore difficult to predict what effects the effluent may have on the environment [23].

The visual non-specific symptoms of toxicity on plants are inhibition of the root growth [20]. In the present study, we found a concentration dependent inhibition in the root growth in onion exposed to the test wastewater (Table 1). Cavusoglu et al. [20] have also reported a decrease in the root length in *Vicia faba* plant exposed to refinery effluent. The observed root growth inhibition can be attributed to the interference of toxicants with processes associated with root elongation in the zone contiguous to the meristems [24].

Measurement of MDA levels is routinely used as an indicator of lipid peroxidation under stress conditions [18]. There was a significant increase in the MDA levels in the roots exposed to MRWW (Table 1). These observations are in agreement with the results reported by Unyayar et al. [18] and Cavusoglu et al. [25]. The induction in MDA level might be due to the generation of ROS and/or other free radicals by the toxicants present in MRWW, which can cause peroxidation of lipid membrane leading to increased permeability and oxidative stress to the plants. ROS generation by the toxicants in various wastewaters have been reported earlier [26]. The accumulation of ROS results in significant functional alterations in lipid, protein and DNA molecules [27]. The capacity to scavenge the free radicals and repair of oxidatively modified macromolecules may also decrease in such cases [28].

Chromosomal aberration and micronucleus assays were performed to check the genotoxic effect of MRWW on *A. cepa* system. Their results were unambiguously positive at all concentrations of the MRWW under our experimental conditions. A remarkable breakage in *A. cepa* chromosomes in terms of chromosomal

Table 3
Survival pattern of *E. coli* K12 strains exposed to Mathura refinery waste water.

E. coli K12 strain	0 h	2 h		4 h		6 h	
	No of colonies	No of colonies	% Mean survival	No of colonies	% Mean survival	No of colonies	% Mean survival
AB1157	302	274		260		253	
	310	284	94	255	86	239	81
	289	287		263		240	
AB2494	105	54		35		18	
	98	49	48	31	31	22	19
	101	40		28		17	
AB2463	91	25		13		3	
	103	29	25	15	13	5	3
	97	22		10		3	
AB2480	51	5		2		0	
	45	6	10	1	4	0	0
	43	4		3		0	
AB3027	110	69		47		39	
	104	77	70	53	51	36	38
	97	71		58		42	
AB1186	106	72		33		4	
	109	77	66	27	27	2	3
	111	65		25		5	

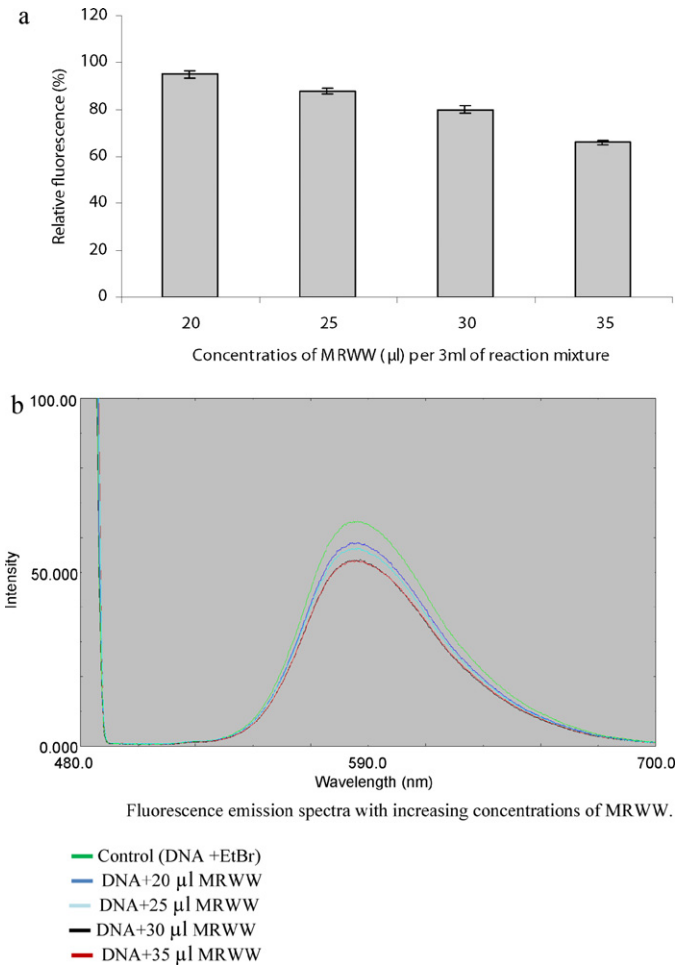

Fig. 3. a. The successive bars represent the change in relative fluorescence of intercalated ethidium bromide incubated with calf thymus DNA by increasing concentrations of Mathura refinery wastewater (20 µl, 25 µl, 30 µl, 35 µl). All data were significantly different at $p < 0.05$. b. Fluorescence emission spectra of intercalated ethidium bromide incubated with calf thymus DNA by increasing concentrations of Mathura refinery wastewater at 37 °C.

aberration was noticed along with the formation of vagrant chromosomes. The genotoxicity of MRWW was also found to be positive by the formation of micronuclei and binucleated cells in *A. cepa*. Leme and Marin-Morales [29] have also reported the induction of chromosomal aberration and micronuclei in *A. cepa* cells exposed to the river water contaminated by petroleum waste.

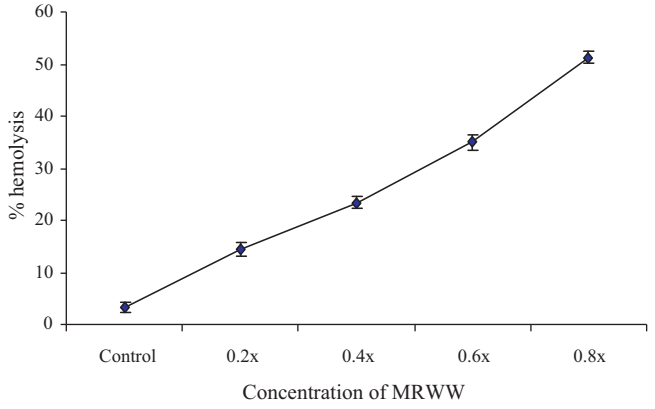

Fig. 4. % Hemolysis with increasing concentrations (0.2×, 0.4×, 0.6×, 0.8×) of Mathura refinery wastewater. All the data were significantly different at $p < 0.05$.

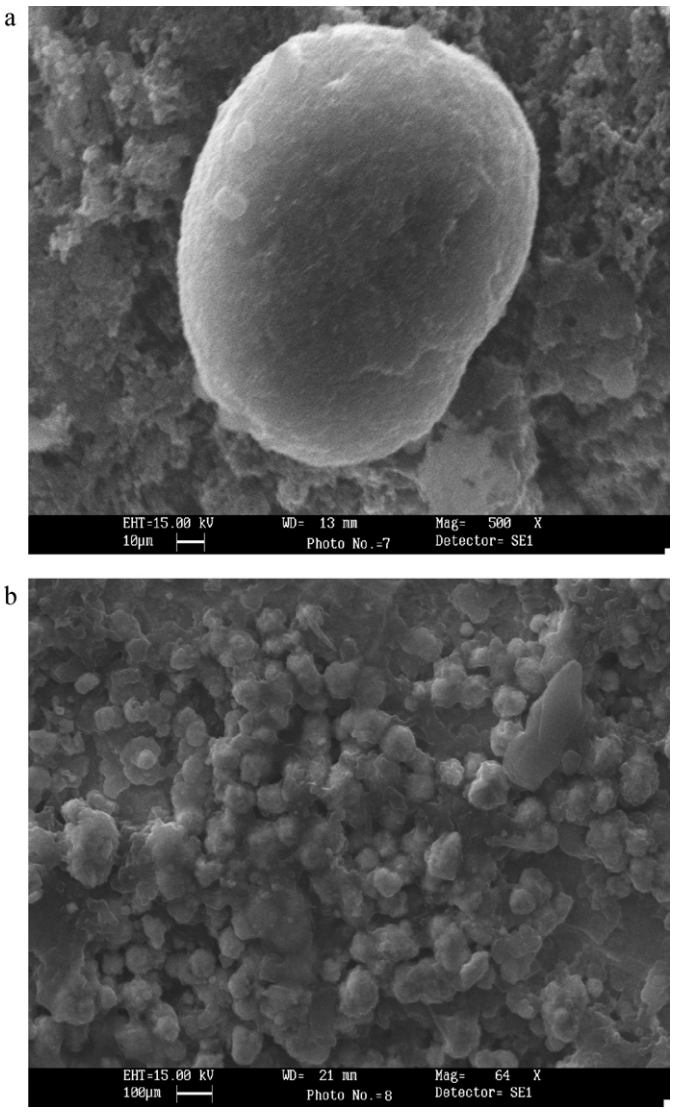

Fig. 5. a. SEM image of control RBCs. b. SEM image of RBCs after treatment with Mathura refinery wastewater.

According to Fenech [30], micronuclei result from acentric fragments or whole chromosomes that are not incorporated to the main nucleus during the cell division cycle. Chromosomal fragments can be derived from chromosomal breakages caused by clastogenic effects induced by chemicals or from chromosomal aberration, such as chromosomal bridges, which break up and originate acentric fragments [31]. Earlier studies have revealed the efficacy of *Allium* test to evaluate the genotoxicity of refinery wastewaters [32–34]. Moreover, Cavusoglu et al. [20] observed a significant increase in the MN formation in *V. faba* L. seeds exposed to refinery wastewater. An induction in chromosomal aberration and micronuclei formation in *A. cepa* cells, exposed to Atibaia River water, being contaminated by petroleum refinery waste was reported by Hoshina and Marin-Morales [33]. In organism wherever aberration occurred, there was always certain growth restriction. Most of this aberration is lethal and also can cause genetic defects in the exposed plants [35].

Fluorescence titration of solutions containing the DNA and ethidium bromide (EtBr) with MRWW was conducted. It is known that the fluorescence intensity of EtBr enhances when it goes from a polar to a nonpolar medium because of the decrease in the intersystem crossing lifetimes [36]. The displacement of

DNA-intercalated-EtBr by groove binding molecules has been used as a standard technique to assay DNA binding agents. The molecular fluorophore EtBr, a phenenthridine fluorescence dye, forms soluble complexes with nucleic acids and emits intense fluorescence in the presence of DNA due to the intercalation of the planar phenenthridinium ring between adjacent base pairs on the double-helix EtBr. Fig. 3a and b shows a significant reduction in the fluorescence intensity at different concentrations of MRWW under the experimental conditions. This decrease in EtBr fluorescence (up to 30% of the initial EtBr–DNA fluorescence intensity) is suggestive of the competition of MRWW components with EtBr in binding to DNA [37].

Bacterial tests employing DNA repair defective mutants have been used extensively for the study of genotoxicity of wastewater samples [8,38]. Besides the chromosomal abnormalities in onion cells, a significant decrease in the survival of the DNA repair defective mutants of *E. coli* K12 was also indicative of genotoxicity of MRWW (Table 3). The double mutant, *recAuvrA*, was the most sensitive strain to the killing effect of the test sample suggesting the generation of lesions reparable by both pathways. Among the single mutants, *recA*, was found to be the most sensitive strain against the damage brought about by the sample, thereby suggesting the predominant role of recA mediated pathway in the test water induced lesions. The sensitivity of single mutants was: recA > uvrA > lexA > polA. These lesions might have been generated by the free radical species due to the presence of heavy metals in MRWW. Other wastewaters have also shown the same trends of sensitivity due to the presence of heavy metals [8,26].

Besides the normal environmental and ecological shocks of routine oil activities, oil operations can exert certain pathological effects in the cells of exposed species including man [39]. We also found an increase in hemolysis (Fig. 4) that might be originated from enhanced production of free radicals as a result of MRWW exposure. The release of reactive species occurs notably in response to the copper present in human blood [40]. Boge and Roche [10] used red blood cells of marine fish to study the (eco)toxicity of industrial effluents by hemolysis assay and demonstrated an increasing degree of hemolysis with the increase in concentrations of the effluent. The exposure of cockerels to crude oil also caused a dose dependent reduction in RBC counts, concomitant with hemolysis [39].

6. Conclusion

Petroleum refinery wastewaters contain various toxicants/genotoxicants. To evaluate the genotoxicity of Mathura refinery wastewater (MRWW), we performed various tests namely chromosomal aberration and micronucleus assay in *A. cepa* cells, *E. coli* survival assay and DNA–EtBr fluorescence measurements. Human RBCs hemolysis assay was conducted to assess its toxicity only. The treatment of MRWW with *A. cepa* resulted in various types of chromosomal and nuclear abnormalities. Decrease in *E. coli* survival and fluorescence intensity whereas increase in hemolysis is suggestive of remarkable toxicity of MRWW. Moreover, study invariably demonstrated the genotoxic nature of MRWW also. Since, all the tests employed in this investigation are quite specific and sensitive, they can be used in monitoring the pollution hazard caused by refinery waste.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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