Heliyon 7 (2021) e08116

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Cyto-genotoxic potential of petroleum refinery wastewater mixed with domestic sewage used for irrigation of food crops in the vicinity of an oil refinery

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ARTICLE INFO

Keywords:
Petroleum refinery wastewater
ICP-OES and GC-MS analysis
Ames *salmonella* mutagenicity test
DNA repair defective mutants
Allium cepa chromosomal aberrations assay

ABSTRACT

Petroleum refinery wastewater combined with domestic sewage were collected from the open channel in the vicinity of Mathura oil refinery, UP (India) and analysed by inductively coupled plasma optical emission spectrometry (ICP-OES) and gas chromatography-mass spectrometry (GC-MS) for elemental analysis and organic pollutants, respectively. Several potentially toxic and non-toxic elements were found to be present in the wastewater samples. GC-MS analysis revealed the presence of several organic contaminants including pesticides. Wastewater samples were extracted using amberlite XAD4/8 resins and liquid-liquid extraction procedures using different organic solvents. The extracts were tested for their cyto-genotoxic potential using bacterial (*Salmonella* mutagenicity test, *E. coli* K-12 DNA repair defective mutants, Bacteriophage λ assay) and plant (*Vigna mungo* phytotoxicity test, *Allium cepa* chromosomal aberration assay) systems. A significant increase was observed in the number of revertants of TA97a, TA98 and TA100 strains with the test samples and XAD concentrated samples were found to be more mutagenic than liquid-liquid extracts. Colony forming units (CFUs) of DNA repair defective mutants of *E. coli* K-12 *recA*, *lexA* and *polA* declined significantly as compared to their isogenic wild-type counterparts with the test samples. Significant reduction in plaque forming units (PFUs) of bacteriophage λ was also found on treatment with the solvent extracts. Presence of several toxic pollutants in the wastewater apply prohibitive action on the seed germination process. Germination rate of *Vigna mungo* seeds as well as radicle and plumule lengths were found to be affected when treated with different concentration of wastewater as compared to control. Present study also indicated concentration dependent reduction in mitotic index of *A. cepa* i.e., 16.38% at 5% and 9.74% at 100% wastewater and percentage of aberrant cells were highest at 100% effluent. Present findings indicated that mutagenicity/genotoxicity of wastewater is due to the mixture of genotoxins; poses serious hazards to the receiving waterbodies which require continuous monitoring and remedial measures for their improvement.

1. Introduction

With rapid industrialization and fast urbanization, the problem of pollution in some countries has accentuated so much that survival of living creatures and damage to the ecosystem has reached to an alarming level due to the industrial effluent discharge into natural water bodies which results in deterioration of water quality (Iqbal et al., 2019) as well as accumulation of xenobiotic compounds and heavy metals in the food chain (Chou et al., 2014; Richardson and Kimura 2017). Petroleum refineries are one of the sources which have been reported to contaminate water reservoirs (Gupta and Ahmad 2012). In order to overcome the scarcity of water, wastewater irrigation is commonly practiced in developing countries (Jiménez and Asano 2008; Uzen 2016) which have been reported to have both beneficial and harmful effects (Singh and Kumar 2006; Khalid et al., 2018). Refinery wastewater consists of oil and greases, phenols, sulphides, ammonia, cyanides, heterocyclic aromatic amines, nitrogenous compounds, heavy metals and hydrocarbons which tend to be toxic and more persistent in the environment (Gupta and Ahmad 2012; Tian et al., 2019). Some of which such as polycyclic aromatic hydrocarbons, heterocyclic aromatic amines and nitrogenous compounds are regarded as toxic chemicals due to their genotoxic and carcinogenic nature (Ohe et al., 2004; Alghamdi et al., 2015) and their impact on environmental and public health is of global concern (Schwarzenbach et al., 2006). Although the detection techniques in the

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https://doi.org/10.1016/j.heliyon.2021.e08116
Received 16 July 2020; Received in revised form 2 December 2020; Accepted 29 September 2021

field of environmental chemistry such as HPLC, GC-MS, ICP-OES, ICP-MS have been advanced but the tracking of potential pollutants present in wastewater is still not feasible due to the limited analytical procedure, cost and time. Sometimes, we fail to identify the environmental contaminants due to the trace concentration, unknown structure or lack of sample preparation and detection techniques (Jia et al., 2015). Therefore, the effluent of refinery wastewater should be monitored meticulously using integrative approach which involves both chemical and biological methods. Biological assays (prokaryotic and eukaryotic test systems) have been proven to be more reliable and accurate than chemical tests for the evaluation of water quality. They are able to detect DNA damage from point mutations to chromosomal alterations (Cabrera and Rodriguez 1999; Mazzeo et al., 2015) and differs in their principles as well as biological endpoints, indicating that these test models are complementary and can conveniently be combined in battery of short-term assays for eco-toxicological assessment (Aleem and Malik 2003; Anjum and Malik 2013; Ghosh et al., 2017). Ames test involves *his*$^-$ auxotrophs of *Salmonella typhimurium* strains which are commonly used to detect the mutagenicity of complex mixtures in environmental samples (Tejs, 2008; Iqbal and Nisar, 2015; Aiman and Malik, 2017; Balabanič et al., 2017). The induction of SOS DNA repair system in *E. coli* K-12 mutants on exposure of DNA damaging agents is another assay for genotoxicity testing (Ferná ndez de Henestrosa et al., 2000; Maslowska et al., 2015). Several plant systems such as *Allium cepa*, *Vigna radiata*, *Vicia faba*, *Tradescantia*, *Zea mays* and *Nicotiana tabacum* have been used as an indicator of toxicity of industrial wastewater (Han et al., 2011; Iqbal and Nisar 2015; Haq et al., 2016; Iqbal 2016; Placencia et al., 2019). Out of these, *Allium cepa* and *Vigna radiata* are commonly used in monitoring of genotoxic potential due to their ability to detect different endpoints e.g. phytotoxicity (related to root length and germination index), cytotoxicity (mitotic index), genotoxicity (chromosomal aberrations) and mutagenicity (micronuclei) (Leme and Marin-Morales, 2009; Mazzeo et al., 2018; Iqbal et al., 2019; Khan et al., 2019).

Present study focuses on physicochemical characteristics of petroleum refinery wastewater mixed with domestic sewage used for irrigation of food crops for more than three decades and characterized by ICP-OES for potentially toxic (PTEs) and non-toxic elements while organic pollutants were identified by GC-MS. Further, we also determined the cyto-genotoxic potential of the wastewater employing Ames *Salmonella*/mammalian microsomal assay, *E.coli* DNA repair defective mutants, bacteriophage λ system, *V. mungo* seed germination and *A. cepa* chromosomal aberration assays.

2. Materials and methods

2.1. Sampling

Mathura Oil Refinery, a constituent of the Indian Oil Corporation Ltd. is situated close to the Agra-Delhi National highway at the latitude 27.28°N and longitude 77.41°E in the outskirts of Mathura City (Uttar Pradesh). The refinery discharges its physically, chemically and biologically treated wastewater into the River Yamuna at downstream of Mathura City through an open channel which is used to irrigate agricultural lands by the native farmers. A total of 12 composited wastewater samples were collected from March 2015 to March 2018 (usually at three months' interval) and carried to the laboratory according to standard methods (APHA 2005).

2.2. Physicochemical analysis

pH, electrical conductivity (EC), salinity and resistivity (ORP), Total dissolved solids (TDS) of wastewater samples were determined by using METTLER TOLEDO S47 SevenMulti™ dual meter pH/conductivity meter. Chemical Oxygen Demand (COD) of water sample was calculated using HACH Spectrophotometer (DR/2010, HUCH, UK) with O_2 concentration range 0–1000 mgL^{-1} according to the method described by Li et al. (2009).

Water samples were also analysed for anions using an 850 Professional IC AnCat MCS-Gradient ion chromatography system (Metrohm, UK). Separation was achieved using Metrosep A Supp 5–150/4.0 analytical column at constant temperature 24 °C. A mobile phase composed of 1mM sodium bicarbonate and 3.2 mM sodium carbonate delivered at a flow rate of 0.7 ml/min. Instrument control and data collection were accomplished using MagIC NetTM software. Calibration standards of 0.1, 1, 10 and 25 mgL^{-1} was prepared from 1000 mgL^{-1} stock standard solution (Thermo Fisher Scientific, UK) using laboratory ultrapure de-ionized water (18.2 MΩ cm).

2.3. Digestion of wastewater samples

Digestion of wastewater was achieved using reverse aqua regia (1:4 solution of Hydrochloric Acid: Nitric Acid) and a Microwave Accelerated Reaction System (MARS 5, CEM, USA). 6 mL of water samples were predigested (overnight) with 10 ml of 1:4 reverse aqua regia into digestion tubes (55 mL PFA liner Xpress vessels). Following pre-digestion, the sample tubes were capped and placed on the microwave carousel within Teflon sleeves, at specific points as directed by the MARS5 manual. The microwave operating parameters were same for soil and water samples which was programmed as follows:

1. Ramping of heat to 160 °C for 20 min and holding at 160 °C for another 20 min.
2. Ramping of heat from 160 to 180 °C within 20 min and holding at 180 °C for a final 20 min.

Each digested sample was filtered through hardened ash-less filters to remove any precipitate and silica particles into 50 ml volumetric flasks. Volume was adjusted with ultra-pure deionised water (resistivity of 18.2 MΩ cm, Direct Q3 Millipore Water Purifier, Millipore, USA) and filtered by 0.45 μm syringe filter prior ICP-OES analysis.

2.4. Elemental analysis of wastewater by ICP-OES

Dissolved and pseudo-total concentrations of 27 elements were determined by an ICP-OES (Thermo Scientific iCAP 6200 Duo View ICP Spectrometer, Thermo Fisher Scientific, Cambridge, UK). The standard sample introduction system consisted of a Mira Mist nebulizer and glass cyclonic mixing chamber. Thermo Scientific iTEVA 6300 software version was used for instrument operations. The spectral lines for the analysis were chosen in order to obtain maximum sensitivity and minimum interference: Al (396.1 nm), As (193.7 nm), Ba (455.4 nm), Ca (317.9 nm), Cd (226.5 nm), Co (228.8 nm), Cr (267.7 nm), Cu (324.7 nm), Fe (238.2 nm), Hg (194.2 nm), K (766.4 nm), Li (670.7 nm), Mg (279.5 nm), Mn (257.6 nm), Mo (202.0 nm), Na (589.5 nm), Ni (231.6 nm), Pb (177.4 nm), Pb (220.3 nm), S (182.0 nm), Sb (206.8 nm), Se (203.9 nm), Si (212.4 nm), Sr (421.5 nm), Ti (323.4 nm), V (292.4 nm) and Zn (213.8 nm).

Multi-elemental calibration standards were prepared from single element BDH Spectosol and stock standards solutions (Fisher Scientific) of 1000 mgL^{-1} concentrations. Calibration standards of 0.1, 1 and 10 mgL^{-1} concentrations were prepared in 5% HNO_3 (Analytical grade, Thermo Fisher Scientific, Cambridge, UK) and 15% HCl (Analytical grade, Thermo Fisher Scientific, Cambridge, UK). Deionised water was used for preparation of calibration solutions and blanks. The 1 mgL^{-1} quality control (QC) solution was repeatedly analysed after each 10 samples to check recovery and drift during the run. Yttrium solution (5 mgL^{-1}) in 0.5% HNO_3 was used as an internal standard.

2.5. GC-MS analysis of wastewater extracts

The organic compounds were identified by GC-MS (VARIAN GC-MS-4000), a VARIAN CP-8410 auto sampler and ion trap mass spectrometer.

The system was controlled by a 0 varian star MS work station v6.9.1. The chromatographic column was a Zebron ZB-1701 (30 m 0.25 mm i.d; 0.15 mm film thickness). The head pressure of the helium carrier gas was 8.7 psi. 1 μL sample was injected in split mode (split ratio 10) using a bruker glass liner. The compounds were identified on the basis of mass spectra using NIST (National Institute of Standards and Technology) MS search library v2.0.

2.6. XAD-concentration of wastewater

1 L of wastewater sample filtered through Whatman's filter papers No. 1 and 0.45 μm membrane filter (Millipore, India) was used to concentrate the organic constituents. Equal ratio of XAD-4 and XAD-8 was used to prepare column adsorbent (Santos et al., 2001). Adsorption of organic substances present in wastewater on the XAD resins was performed as described by Wilcox and Williamson (1986). The adsorbed organic substances were then eluted with acetone (20 mL, HPLC grade). The eluate was subjected to evaporation at room temperature until dryness under reduced pressure and re-dissolved in dimethyl sulfoxide (DMSO, HPLC grade, SRL, India) and sterilized through 0.22 μm syringe filter and stored at -30 °C till genotoxicity testing is completed.

2.7. Liquid-liquid extraction

1 L of wastewater filtered through Whatman filter paper No.1 and 0.45 μm membrane filter (Millipore, India) was extracted with two different organic solvents (separately) i.e., dichloromethane (DCM) and n-hexane (HPLC grades) as described in Standard Methods (APHA 2005) by shaking vigorously with 20 ml of extraction solvent using a separatory funnel and allowed to separate the aqueous and organic layers. The extraction procedure was repeated three times, using same amount of fresh extraction solvent each time. Organic layer was collected in a beaker (100 mL), evaporated to 5 mL at room temperature under reduced pressure and sterilized using syringe filter of 0.22 μm size and stored at -30 °C till further testing (Masood and Malik 2013).

2.8. Ames Salmonella mammalian microsomal mutagenicity assay

The Ames *Salmonella* mammalian microsomal mutagenicity assay was performed by pre-incubation procedure according to Maron and Ames (1983) with slight modifications (Aleem and Malik 2003). Five different doses of each wastewater extract i.e., 5.0, 10, 20, 40 and 80 μL per plate (equivalent to 1.0, 2.0, 4.0, 8.0 and 16 mL of wastewater, respectively) were separately incubated at 37 °C for 30 min with 100 μL of overnight grown Ames strains. Each experiment was carried out in triplicate. Following incubation, 2 mL of top agar with traces of histidine and biotin was uniformly mixed and poured on to minimal glucose agar plates and incubated at 37 °C for 48–72 h. Negative and positive controls were also included in each set of experiment; bacteria treated with DMSO used as a negative control while MMS/sodium azide as a positive control. All the extracts were also assayed in the presence of rat liver microsomal fraction (S9 mix). 20 μL of S9 mix per plate was added to determine the presence of any pro-mutagen in the test samples. The samples were considered mutagenic if the number of revertants doubled as compared to the spontaneous reversion frequency (Vargas et al. 1993, 1995).

Mutagenic index, Induction factor (Mi) and Mutagenic potential (m) was calculated according to Ansari and Malik (2009):

$$\textit{mutagenic index} = \frac{\textit{Number of his} + \textit{revertants induced in sample}}{\textit{Number of his} + \textit{revertants induced in the negative control}}$$

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

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

2.9. Survival of DNA repair defective E. coli K-12 mutants

The survival of *E. coli* K-12 DNA repair defective mutants was carried out as described by Rehana et al. (1995). Both DNA repair defective *recA*, *lexA* and *polA* mutants of *E. coli* K-12 and their isogenic wild-type counterparts were cultured in nutrient broth at 37 °C and exponentially growing cultures (1–3x 10^8 viable cells/mL) were centrifuged and the obtained pellets were re-suspended in 0.01M $MgSO_4$ solution and treated with 40 μL of each wastewater extracts separately. The samples were taken at regular time gaps (0, 2, 4 and 6 h), suitably diluted and spread on nutrient agar plates; incubated overnight at 37 °C to assay the colony forming units. Solvent control was also tested concurrently.

2.10. Extracellular treatment of bacteriophage λ with the test samples

Purified bacteriophage λ (10^{10} PFUs/mL) was treated with 40 μL of the wastewater extracts at 37 °C. 100 μL of the treated bacteriophage was withdrawn at regular intervals (0, 2, 4 and 6 h); suitably diluted in 0.01M MgSO4 solution (pH 8.0) and allowed to adsorb on exponentially growing DNA repair defective *recA* and *lexA* as well as wild type *E. coli* K-12 strains at 37 °C for 20 min and plated on hard nutrient agar plates with 2 mL of molten soft agar by double layer method as described by Aleem and Malik (2003). Plates were incubated at 37 °C overnight to assay plaques forming units (PFUs).

2.11. Vigna mungo phytotoxicity

Seed germination assay was performed by the method of Haq et al. (2016) with some modifications. Black gram seeds (*Vigna mungo* L.) were sterilized using 70% ethanol followed by 3% sodium hypochlorite solution (each for 3 min) and then washed with sterile double distilled water. The wastewater was sterilized using 0.22 μm syringe filter while distilled water was autoclaved. The sterilized seeds were then soaked overnight with different concentrations (5, 10, 25, 50 and 100%) of sterile wastewater and distilled water and seeded in petriplates in which filter paper layered on sterile cotton soaked with 10 ml of respective concentration of wastewater were poured. Petriplates with sterile ddH_2O were served as control. All the plates were incubated at room temperature in the dark. Seed germination was recorded after 24 h. Radicle and plumule lengths were recorded after 3 days by randomly selecting the seedlings from each petriplate. Percent seed germination was calculated as follows:

$$\textit{Percent seed germination} = \frac{\textit{Seeds grown}}{\textit{Total no. of seeds treated}} \times 100$$

To estimate the biomass, 10 seedlings from each petriplate were wrapped in aluminium foil and weighed fresh then they were oven dried at 70 °C for 24 h and dry weight was noted. Biomass was calculated using the formula:

$$\textit{Biomass} = \textit{fresh weight} - \textit{dry weight}$$

The germination index was calculated as under (Oktiawan and Zaman 2018):

$$\textit{Germination Index } (GI\%) = \frac{Gt \times Lt}{Go \times Lo} \times 100$$

Where G_t is the number of seeds germinated in the test plate, G_0 is the number of seeds germinated in the control plate. Where L_t is the average of radicle length in the test plate and L_0 is the average length of radicle in the control plate.

Seedling vigour index I and vigour index II were calculated according to Kumar et al. (2012) as follows:

$$Seedling\ Vigour\ Index\ I = (Mean\ root\ length + Mean\ shoot\ length) \times \%\ Germination$$

$$Seedling\ Vigour\ Index\ II = Mean\ seedling\ drymass \times \%\ Germination$$

The percent phytotoxicity for the treatments was calculated by using the formula (Revathi and Subhashree 2014):

$$Percentage\ Phytotoxicity = \frac{Radicle\ length\ of\ control - Radicle\ length\ of\ test}{Radicle\ length\ of\ control} \times 100$$

The % of relative seed germination (RSG), relative root growth (RRG) and the germination index (GI) were calculated according to Walter et al. (2006). RSG is the ratio of the no. of seeds germinated in the test sample to the number of seeds germinated in the control while RRG is the ratio of mean root length in the test sample to mean root length in the control. Stress tolerance index was calculated using the formulae:

$$Root\ length\ stress\ Tolerance\ index = \frac{Mean\ length\ of\ longest\ root\ in\ treatment}{Mean\ length\ of\ longest\ root\ in\ control} \times 100$$

$$Shoot\ length\ stress\ tolerance\ index = \frac{Mean\ length\ of\ shoot\ in\ treatment}{Mean\ length\ of\ shoot\ in\ control} \times 100$$

2.12. *Allium cepa* anaphase-telophase chromosomal aberration assay

The cyto-genotoxicity of refinery wastewater was assessed with the actively dividing cells of *Allium cepa* root tips as described by Fiskesjö (1985). Small (~1.5–2.0 cm diameter) and healthy onion bulbs (2n = 16) were used in this assay. The outermost dead scales and dried root tips of the bulb were cut off carefully without disturbing the root primordia. Before treatment, onion bulbs were placed in beaker, having deionised water, with their basal ends dipped in water and left to germinate at room temperature (37±2 °C). The root-tips of newly germinated roots (~2.0 cm in length) were then treated with different wastewater concentration i.e., 5, 10, 25, 50 and 100% for 72 h. Deionised water and methyl methane sulfonate (10 mgL^{-1}) was used as a negative and positive control respectively in each assay. Following treatment, randomly selected root tips were fixed in freshly prepared 3:1 ethanol: glacial acetic acid (v/v) at 4 °C overnight. After rinsing with water these fixed root tips can be stored for longer time in 70% alcohol at 4 °C or they can be microscopically examined immediately. Squash technique as described by (Fiskesjo 1985) was used to prepare the root tip chromosomal slides. The water rinsed root tips were heated in 1N HCl for 2–3 min at 60 °C in order to dissolve the cell wall, then washed with distilled water and stained with acetocarmine for ~10 min and slides were examined under light microscope (Olympus BX60).

Mitotic index (MI) was determined by surveying about 3000 cells (1000 cells per slide) as follows:

$$Mitotic\ index = \frac{Total\ number\ of\ dividing\ cells}{Total\ number\ of\ cells\ counted} \times 100$$

Chromosomal aberrations (CA) were counted as described by Asita and Matobole (2010) with some modification by observing about 300 dividing cells (100 cells per slide) as follows:

$$\%\ CA = \frac{Total\ no.\ of\ Aberrant\ cells}{Total\ cells\ in\ division} \times 100$$

Cells with different kinds of the aberrations were distinguished and documented.

Each value is presented as mean ± standard deviation (SD). Duncan multiple range test (DMRT) was used to find out level of significance in different treatment groups and control values.

3. Results and discussion

Petroleum refineries involve several techniques i.e., drilling, exploration, cracking, crude desalting, fractional distillation, polymerization, isomerization, alkylation, catalytic reforming, hydro-treating and transportation etc (Cholakov, 2009). In each process, huge amount of water is used and significant amounts of toxic substances are discharged with wastewater which lead to an adverse impact on the environment (Pedrozo et al., 2002). Although most of the contaminants are broken down or recovered at the refinery; but significant amounts of toxic substances and compounds may remain in the wastewater. The physicochemical characteristics of the treated refinery wastewater, presented in Table 1, showed the pH of 7.82 ± 0.32 and yellow in color, which may indicate the presence of soluble organics and insoluble alkalis. The mean values of salinity and resistivity in the wastewater were found to be 2.01 ± 0.24 PSU and 2.62 ± 0.14 × 10^2 Ω-cm respectively. The high salinity of refinery wastewater is due to the oil desalination steps which is mainly attributed to the chlorides (Nacheva 2011). The electrical conductivity was recorded as 3460 ± 216 μS/cm which may directly related to the salinity, total salt content and dissolved ions of the wastewater as chemicals used in refining of petroleum products are directly discharged into it. It is essential to control the water pollution level as high EC in water used for irrigation might results in reduced crop production (Edmund 1998; Heinen et al., 2001). The COD value of the wastewater samples was recorded as 310 ± 32.1 mgL^{-1} which is indicative of the level of toxicity of the wastewater as well as the presence of biologically inert organic compounds (Dutta 1999). Petroleum wastewater contains several inorganic cations and anions such as Ca^{2+}, Mg^{2+}, S^{2-}, Cl^{-}, PO_4^{3-}, Fe^{2+}, Fe^{3+}, SO_4^{2-} (Mansouri et al., 2014). In our study, bicarbonates, carbonates and chloride were found to be 14.6 ± 1.63 mgL^{-1}, 79 ± 7.0 mgL^{-1} and 173.67 ± 11.59 mgL^{-1} respectively. The most common toxicity is from chloride in the irrigation water as it not adsorbed by soil and moves quickly with soil-water, absorbed by crops, passes in the transpiration stream and ultimately accumulated in the leaves (Pescod, 1992). The dissolved anions concentrations of fluoride, nitrite, bromide, nitrate, phosphate and sulphate were found to be 3.59 ± 0.46, 1.00 ± 0.03, 2.05 ± 0.06, 2.88 ± 0.005, 4.76 ± 0.21 and 41 ± 5.79 mgL^{-1} respectively.

Heavy metal ions, being highly toxic, non-destructible and tend to bioaccumulate and biomagnify in food chain are the most significant

Table 1. Physicochemical characteristics of refinery wastewater.

Parameters	Wastewater
pH	7.82 ± 0.32
Colour	Yellowish
EC (uS/cm)	3460 ± 216
Salinity (PSU)	2.01 ± 0.24
Resistivity (Om cm)	2.62 ± 0.14 E+02
TDS (mgL^{-1})	1910 ± 54.5
COD (mgL^{-1})	310 ± 32.1
Carbonate (mgL^{-1})	79 ± 7.0
Bicarbonate (mgL^{-1})	14.6 ± 1.63
Chloride (mgL^{-1})	173.67 ± 11.59
Fluoride (F^{-}) (mgL^{-1})	3.59 ± 0.46
Nitrite (NO^{2-}) (mgL^{-1})	1.00 ± 0.03
Bromide (Br^{-}) (mgL^{-1})	2.05 ± 0.06
Nitrate (NO^{3-}) (mgL^{-1})	2.88 ± 0.005
Phosphate (PO_4^{3-}) (mgL^{-1})	4.76 ± 0.21
Sulphate (SO_4^{2-}) (mgL^{-1})	41 ± 5.79

contributors of water pollution (Ayangbenro and Babalola, 2017; Ali and Khan, 2018; Kahlon et al., 2018). They sufficiently affect living systems in aquatic ecosystems directly or indirectly. Several techniques such as Flame atomic absorption spectrometry (FAAS), graphite furnace absorption spectrometry (GFAAS), Inductively-coupled plasma optical emission spectrometry (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS) are being used to quantify the trace elements in environmental samples (Bettinelli et al., 2000; Tokalioğ lu et al., 2000; Liang and Sang 2008; Islam et al., 2017). The average concentrations of dissolved as well as pseudo-total metals are shown in Table 2; some elements such as As, Cd, Co, Cr, Hg, Mo,Pb, Sb, Se, Ti and V were found to be less than the detectable limits. Water samples were also found to be contaminated with various potentially toxic elements (PTEs) as analysed by ICP-OES (Table 2). Out of 11 analysed PTEs, six (Cu, Pb, Zn, Cr, Ni and V) were detected in all the samples tested while five (Cd, As, Mo, Hg and Sb) were found below the detection limits. The five non-PTE metals i.e., Al, Co, Fe, Mn and Ti, seven cations i.e., Ca, K Mg, Na, Ba, Li and Sr and some non-metals like S, P, Si, and Se were also found. PTEs play remarkable functions in the survival of living organisms. Several PTEs are physiologically required at the optimum levels for the biota but they might induce adverse health impacts if their concentrations exceeds than they are required (Antoniadis et al., 2019a; b), such as dysfunction of different organs of the body such as bone, kidney and liver. Hence, the excessive levels of PTEs can endanger ecosystem endurance and human health in the environment. The concentration of heavy metals in the test wastewater samples were in the following order Fe > Al > Zn > Mn > Ti > Pb > Ni and were found to be 5.14 ± 0.17, 2.73 ± 0.06, 0.75 ± 0.03, 0.58 ± 0.01, 0.37 ± 0.23, 0.04 ± 0.01, 0.02 ± 0.003 mgL^{-1} respectively. Heavy metals present in refinery wastewater give rise to a serious threat to the environment and humans depending upon their concentrations, toxicity level and exposure time (Ray et al., 2014). Rasheed and Saleh (2016) examined the physico-chemical characteristics of well water, wastewater and soil in the vicinity of Bazian petroleum oil refinery (Iraq) and reported the presence of several heavy metals like Cr, Pb, Cd, Cu, Zn and Ni. They found Cr in the range of 1.27–1.34 mgL^{-1}, mean Pb concentration was 1.64 mgL^{-1} and Cd from 0.71 to 0.88 mgL^{-1} in wastewater, while concentration of Cu, Ni and Zn were 0.188, 0.76 and 1.44 mgL^{-1} respectively. Wokoma and Edori (2017) reported Zn, Fe and Pb in the range of 0.206–0.330 mgL^{-1}, 0.231–0.275 mgL^{-1} and 0.018–0.135 mgL^{-1} respectively, while studying the heavy metal contents of oily wastewater discharged from an oil firm in Port Harcourt, Nigeria and showed the order of contamination in the following manner: Zn > Fe > Pb > Cd. Our results validated the earlier findings, where excessive concentrations of metals were found in wastewater mixed with industrial discharges (Rasheed and Saleh 2016; Olayebi and Adebayo 2017; Oyetibo et al., 2017).

Table 2. Elemental analysis of petroleum refinery wastewater.

			Pseudo-total Metal (mgL^{-1})	Dissolved Metal (mgL^{-1})
Cations		Potassium	28.46 ± 1.88	28.65 ± 1.27
		Barium	0.16 ± 0.00	0.0722 ± 0.01
		Sodium	495.64 ± 4.90	532.3 ± 26.78
		Lithium	0.03 ± 0.00	0.021 ± 0.003
		Calcium	104.08 ± 0.84	94 ± 1.31
		Strontium	1.24 ± 0.01	1.09 ± 0.04
		Magnesium	55.19 ± 2.5	47.6 ± 6.94
Metals	Non-PTEs	Aluminium	2.73 ± 0.06	0.0119 ± 0.002
		Cobalt	<0.01	<0.001
		Iron	5.14 ± 0.17	0.09 ± 0.004
		Manganese	0.58 ± 0.01	0.469 ± 0.012
		Titanium	0.37 ± 0.13	<0.001
	PTEs	Copper	<0.01	0.003 ± 0.001
		Lead	0.04 ± 0.01	<0.003
		Cadmium	<0.02	<0.0002
		Chromium	<0.01	<0.001
		Zinc	0.75 ± 0.03	0.102 ± 0.07
		Nickel	0.02 ± 0.003	0.008 ± 0.001
		Arsenic	<0.4	<0.004
		Molybdenum	<0.08	<0.0008
		Vanadium	<0.03	<0.004
		Mercury	<0.15	<0.002
		Antimony	<0.5	<0.005
Non-metals		Sulphur	140.50 ± 18.96	156.7 ± 13.17
		Phosphorous	5.28 ± 0.08	1.59 ± 0.003
		Silicon	12.79 ± 0.39	8.34 ± 0.23
		Selenium	<0.7	<0.007

Gas chromatography and mass spectrometry (GC-MS) is a robust analytical device to identify organic pollutants in the environmental samples (Haleyur et al., 2016; Manamsa et al., 2016; Chandra et al., 2017). In gas chromatography, the separation of different components present in the samples are separated on the basis of retention times which results several peaks in the chromatogram. GC–MS can detect traditional volatile and semi-volatile pollutants, thus have an essential role in the analysis of persistent organic pollutants. However, GC–MS cannot determine the compounds that are neither vaporizable nor esterified (Zhang et al., 2020). The mass spectra of the major peaks in the gas chromatograms of different wastewater extracts at particular retention time were compared with the NIST library which reveals the presence of various aliphatic and aromatic organic compounds and broadly categorised as pthalates, phthallic acid derivatives, alkanes and their derivatives, PAHs derivatives, esters of acids, cholestane derivatives and some pesticides such as chlorpyrifos etc (Table 3; Figure 1). Organic compounds found in the test samples under the condition tested were Tetrapentacontane, 1,54-dibromo-, Tetradecane, 2,6,10-trimethyl-, 17-Pentatriacontene, 9-(2′,2′-Dimethyl-propanoilhydrazono)-3,6-dichloro-2,7-bis-[2-(diethylamino)-ethoxy] fluorine, Octadecenal, 3-acetoxy-7,8-Epoxylanostan-11-ol, and Chlorpyrifos. Occurrence of organophosphorous such as chlorpyrifos compounds in rivers and wastewaters is associated with strong acetylcholinesterase inhibitory activity (Castillo and Barcelo′, 2001). Saien and Shahrezaei (2012) found different concentrations of aliphatic and aromatic petroleum hydrocarbons viz. methyl-tetrabutyl ether, phenol, 2,3,5,6-tetramethylphenol, naphthalene, xylene, tetradecane, 4-chloro-3-methylphenol and 3-tert-butylphenol in the pre-treated refinery wastewater from a biological treatment unit in Kermanshah refinery plant (Iran) by GC-MS. Boczkaj et al. (2016) found 2, 3-Dihydropyran, 2-Butanol, Ethyl acrylate, 2-Pentanone, 2-Hexanone, 1-Hexanol, Cyclohexanol, Cyclohexanone, 3-Methylcyclohexanone, Furfural in raw refinery wastewater, while *o*-cresol, Phenol and *m*-Cresol were detected in their treated petroleum refinery effluents. Al-Saleh et al. (2017) identified bisphenol and six phthalate esters, namely dimethyl phthalate, diethyl phthalate, dibutyl phthalate, butyl benzyl phthalate, bis (2-ethylhexyl) phthalate and dioctyl phthalate in secondary- and tertiary-treated wastewater collected from water treatment plants using headspace SPME followed by GC-MS. Dai et al. (2016) reported eight major categories of organic compounds (organic acids, esters, alcohol, heterocyclic compounds, alkanes, aromatic hydrocarbons, aldehydes, ketones and phenols) in which organic acids were most prominent and accountable to 50.94% of total organic compounds.

The mutagenicity, and cyto-genotoxicity of refinery wastewater was assessed by bacterial and plant systems as they are genetic models for the screening and monitoring of environmental pollutants. In order to carry out the genotoxic potential of wastewater, the samples need to be concentrated or fractionated as the compounds may be present in small concentrations (Mortelmans and Zeiger 2000). Adsorption on Amberlite XAD resins (nonpolar copolymers of styrene–divinylbenzene) is the common method for concentrating organic constituents from water and wastewater samples; followed by elution with organic solvents which efficiently extracts all the polar and non-polar toxic chemicals and mutagens/genotoxins (Junk et al., 1974; Pan et al., 2009). Various authors have reported that XAD concentrates of wastewater gave positive results in the

Table 3. Compounds identified in refinery wastewater using Gas-chromatography-Mass Spectrometry.

Extraction Solvent	Identified Compounds in refinery wastewater
hexane	Tetrapentacontane, 1,54-dibromo-
	7,8-Epoxylanostan-11-ol, 3-acetoxy-
	Tetradecane, 2,6,10-trimethyl-
	17.alfa.,21á-28,30-Bisnorhopane
	Rhodopin
	D-Homo-24-nor-17-oxachola-20,22-diene-3,16-dione, 7-(acetyloxy)-1,2:14,15:21,23-triepoxy-4,4,8-trimethyl-, (5à,7à,13à,14á,15á,17aà)-
	17-Pentatriacontene
	Oleic acid, eicosyl ester
	Chlorpyrifos
Dichloromethane (Acidic fraction)	[1,1′-Bicyclopropyl]-2-octanoic acid, 2′-hexyl-, methyl ester
	Cholestan-3-ol, 2-methylene-, (3á,5à)-
	Tetradecane, 2,6,10-trimethyl-
	4-Octadecenal
	Hexadecane, 1,1-bis(dodecyloxy)-
	Octadecane, 3-ethyl-5-(2-ethylbutyl)-
	Dasycarpidan-1-methanol, acetate (ester)
	17-Pentatriacontene
	9-(2′,2′-Dimethylpropanoilhydrazono)-3,6-dichloro-2,7-bis-[2-(diethylamino)-ethoxy]fluorine
	Oleic acid, eicosyl ester
	Cholestan-3-one, cyclic 1,2-ethanediyl aetal, (5á)-
	18,19-Secoyohimban-19-oic acid, 16,17,20,21-tetradehydro-16-(hydroxymethyl)-, methyl ester, (15á,16E)-
	9-Hexadecenoic acid, 9-octadecenyl ester, (Z,Z)-
	3-[3-(1,5-Dimethylhexyl)-7-(2-hydroxy-1-methylethyl)-3a,6,9b-trimethyl-2,3,3a,4,5,6,7,8,9,9b-decahydro-1Hcyclopenta [a] naphthalen-6-yl]propanoic acid, methyl ester
Dichloromethane (Basic fraction)	1,16-Cyclocorynan-17-oic acid, 19,20-didehydro-, methyl ester, (16S,19E)-
	4-Piperidineacetic acid, 1-acetyl-5-ethyl-2-[3-(2-hydroxyethyl)-1H-indol-2-yl]-à-methyl-, methyl ester
	8-Octadecenal
	Propanoic acid, 2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl)-
	Phen-1,4-diol, 2,3-dimethyl-5-trifluoromethyl-
	8,14-Seco-3,19-epoxyandrostane-8,14-dione, 17-acetoxy-3á-methoxy-4,4-dimethyl-
	5-Octadecenal
	9-Octadecene, 1-[2-(octadecyloxy)ethoxy]-
	9-(2′,2′-Dimethylpropanoilhydrazono)-3,6-dichloro-2,7-bis-[2-(diethylamino)-ethoxy]fluorene

bacterial mutagenicity assays (Masood and Malik 2013; Liu et al., 2015; Jiang et al., 2015; Guan et al., 2017). Industrial wastewater containing different chemicals extracted with liquid-liquid extraction procedure using solvents such as dichloromethane and hexane are helpful in extraction of several organic compounds such as herbicides, pesticides, chlorophenols and poly aromatic hydrocarbons also have been proved to be genotoxic and induced different levels of DNA/chromosomal damage in tested samples (Haleyur et al., 2016; Aiman and Malik 2017).

The mutagenic potential of XAD-concentrated and liquid-liquid extracted (LLE) refinery wastewater samples was assessed using Ames *Salmonella*/mammalian microsomal assay both in the absence and presence of S9 fraction. The reversion of *Salmonella typhimurium* tester strains with XAD-concentrated and LLE water samples are outlined in Tables 4, 5, 6, and 7. XAD concentrated sample showed significant increase in the number of revertants for all the tester strains up to the dose of 40 μl/plate and reduced at the dose of 80 μl/plate (Table 4). TA98 showed highest number of revertants (mutagenic index of 10.43 without S9 fraction) which also exhibited maximum response both in terms of induction factor (Mi 2.24 without S9). On the basis of the mutagenic index and induction factor both in the absence and presence of S9 fraction, the tester strains showed the response in the following order for XAD concentrated sample:

TA98 > TA97a > TA100 > TA102 > TA104

However, on the basis of mutagenic potential/slope, Ames tester strains showed response in the following order:

TA97 > TA100 > TA198 > TA102 > TA104

For acidic fraction of DCM extracts, the number of revertant colonies increased with increasing concentration up to 40 μL/plate and declined at the dose of 80 μL/plate (Table 5). TA98 was found to be the most responsive in terms of mutagenic index.

Reversion of the tester strains treated with n-hexane extracted wastewater samples are presented in Table 6, displaying maximum response of 9.19 (-S9) in terms of mutagenic index; 2.10 (-S9) in terms of induction factor with TA98; and 5.3 (-S9) and 5.5 (with S9 fraction) in terms of mutagenic potential for TA97 strain.

In all the tester strains, the mutagenic index for XAD concentrated and LLE wastewater samples were considerably higher when compared with control, suggesting dose-dependent mutagenicity. The mutagenic activity was slightly increased in the presence of S9 fraction when treated with XAD-concentrated wastewater samples as indicated by the highest values of mutagenic index, induction factor and mutagenic potential observed at the dose level of 40 μl/plate. Wastewater extracts can be arranged as follows on the basis of toxicity:

XAD concentrated sample > DCM (Acidic fraction) > Hexane extract > DCM (Basic fraction).

A dose dependent increase in number of revertants was found when significance of the number of *his*$^{+}$ revertants in the sample was compared to the negative control using one way analysis of variance (ANOVA) at $P \leq 0.05$.

Ames *Salmonella* strains have different types of mutations i.e. frameshift mutations (TA97a, TA98), base-pair substitution/missense mutations (TA100) and transitions/transversions (TA102, TA104) and thus have potential to detect the specific kind of mutagens (Maron and Ames 1983; Tejs 2008). Our results clearly indicated that the wastewater (Refinery wastewater mixed with domestic sewage) consisted of huge amount of frameshift and missense mutagens as indicated by the significant increase in the number of revertants of TA97a, TA98 and TA100 when treated with the different extracts (Tables 4, 5, 6, and 7). Present study also indicated the presence of pro-mutagens in the test samples as mutants showed higher mutagenic response in the presence of S9 fractions. Moreover, it was also observed that XAD concentrated samples considered to be more mutagenic in comparison to LLE which might be due to the ability of XAD resins to effectively concentrate broad category of mutagenic compounds such as polycyclic aromatic hydrocarbons, aryl amines, nitro compounds, quinolines, anthraquinones etc (Junk et al., 1974; Aleem and Malik 2005; Guan et al., 2017). Iqbal et al. (2017) tested mutagenicity of photo-catalytically treated petroleum refinery wastewater using Ames *Salmonella* test and samples were considered to be mutagenic with TA98 and TA100 tester strains and decreased when treated with UV and gamma radiation. Khan et al. (2019) used battery of short-term assays in order to evaluate the potential toxicity of textile wastewater and also reported significant mutagenic activity with XAD concentrated sample than LLEs, TA98 was the most responsive strain and displayed mutagenic index of 15.42 and 15.60 without and with S9 fraction.

DNA repair defective mutants of *E. coli* K-12 have extensively been used for genotoxicity testing of environmental samples (Siddiqui et al., 2011; Gupta and Ahmad 2012; Khan et al., 2019). DNA repair defective *recA*, *lexA*$^{-}$ and *polA*$^{-}$ mutants of *E. coli* K-12 and wild-types were treated

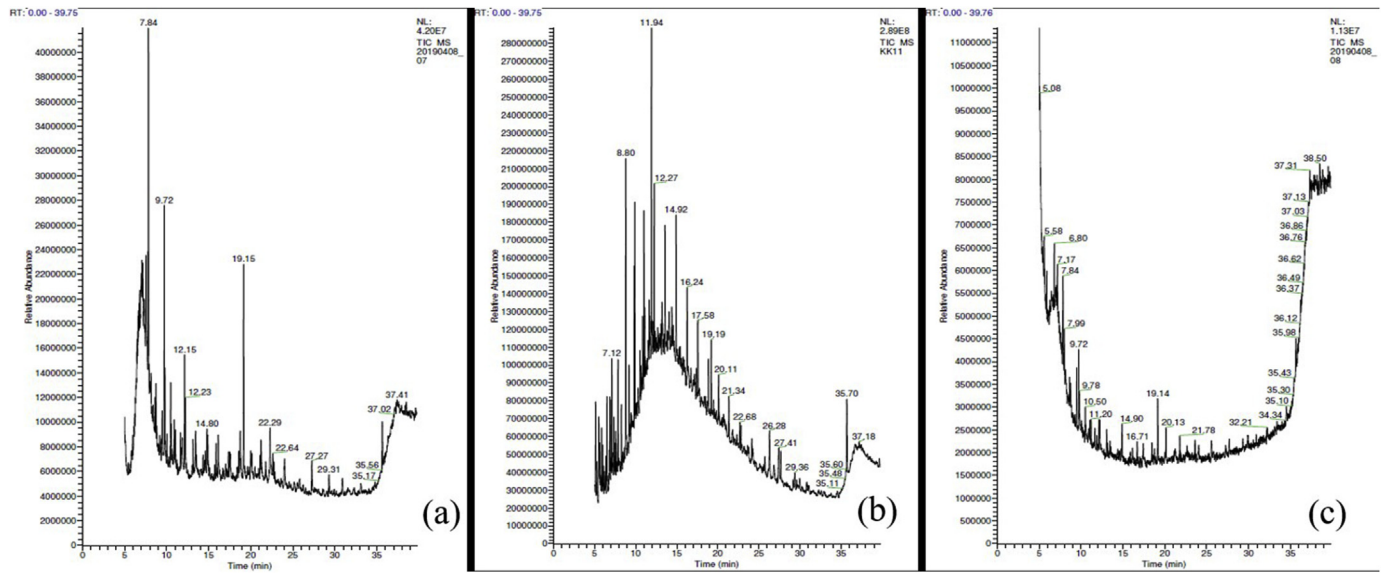

Figure 1. Gas chromatogram of refinery wastewater extracts: DCM acidic (a); n-hexane (b); and DCM basic fraction (c).

Table 4. Reversion of *Salmonella* tester strains in the presence of XAD-concentrated wastewater samples.

Wastewater extract (µl/plate)										
Strain	S9	Control	5	10	20	40	80	Mi	m	LSD P ≤ 0.05
TA97a	-	94±8^{a}	182±7^{b} (1.94)	230±9^{c} (2.45)	308±6^{d} (3.28)	411±9^{f} (4.37)	337±8^{e} (3.59)	1.22	5.4938	1.81927
	+	97 ± 11^{a}	198 ± 11^{b} (2.04)	262±8c (2.70)	328 ± 10^{d} (3.38)	427 ± 11^{f} (4.40)	400±9^{e} (4.12)	1.22	6.6463	2.57283
TA98	-	32±6^{a}	179 ± 10^{b} (5.59)	218±1^{c} (6.81)	235±8^{d} (7.34)	334 ± 12^{f} (10.43)	287 ± 11^{e} (8.97)	2.24	4.7856	4.06801
	+	36±8^{a}	202 ± 13^{b} (5.61)	246±9^{c} (6.83)	268 ± 10^{d} (7.44)	376±7^{f} (10.44)	323 ± 18^{e} (8.97)	2.25	5.4087	17.2591
TA100	-	121±9^{a}	209±8^{b} (1.72)	243±9^{c} (2.01)	327±7^{d} (2.70)	402±9^{f} (3.32)	361±8^{e} (2.98)	0.84	5.3150	1.81927
	+	129 ± 12^{a}	227±8^{b} (1.76)	269±5^{c} (2.09)	349 ± 10^{d} (2.71)	428 ± 12^{f} (3.32)	385 ± 10^{e} (2.98)	0.84	5.5569	16.1700
TA102	-	241 ± 10^{a}	348±7^{b} (1.44)	392±6^{c} (1.62)	427 ± 10^{d} (1.77)	509 ± 12^{f} (2.11)	472±5^{e} (1.95)	0.11	4.6912	4.81333
	+	250±7^{a}	360±9^{b} (1.44)	406 ± 11^{c} (1.62)	442±8^{d} (1.77)	529±7^{f} (2.12)	491 ± 10^{e} (1.96)	0.11	4.9217	3.15107
TA104	-	320±8^{a}	412 ± 12^{b} (1.29)	469±8^{c} (1.47)	513±8^{d} (1.60)	602±9^{e} (1.88)	528 ± 10^{d} (1.65)	-0.13	4.3620	12.6043
	+	327±5^{a}	423±8^{b} (1.30)	487±9^{c} (1.49)	525±9^{d} (1.61)	617 ± 11^{f} (1.89)	542±8^{e} (1.66)	-0.12	4.4531	3.63854

Values in parentheses are mutagenic index; Mi = induction factor; m = mutagenic potential; LSD = least significant difference.
Mean values followed by different letters are significantly different at p ≤ 0.05 (Duncan multiple range test).

Table 5. Reversion of *Salmonella* tester strains in the presence of Acidic fraction of DCM extracted wastewater samples.

Wastewater extract (µl/plate)										
Strain	S9	Control	5	10	20	40	80	Mi	m	LSD P ≤ 0.05
TA97a	-	93±5^{a}	169±7^{b} (1.82)	224±9^{c} (2.41)	295 ± 10^{d} (3.17)	377±8^{f} (4.05)	339 ± 14^{e} (3.64)	1.11	5.5355	5.45781
	+	95±7^{a}	173 ± 10^{b} (1.82)	243 ± 11^{c} (2.56)	310 ± 13^{d} (3.26)	385 ± 11^{f} (4.05)	347 ± 13^{e} (3.65)	1.12	5.5458	4.06801
TA98	-	33±5^{a}	151±4^{b} (4.57)	198±8^{c} (6.00)	238±7^{d} (7.21)	312±9^{f} (9.45)	289 ± 12^{e} (8.76)	2.13	5.1108	12.0677
	+	37±9^{a}	170±7^{b} (4.59)	225±6^{c} (6.08)	269±9^{d} (7.27)	351 ± 11^{f} (9.48)	325±8^{e} (8.78)	2.14	5.7279	3.15107
TA100	-	122±9^{a}	200±5^{b} (1.64)	224 ± 10^{c} (1.84)	309 ± 13^{d} (2.53)	372 ± 14^{f} (3.05)	350±9^{e} (2.87)	0.72	5.1145	5.75303
	+	130±5^{a}	215±7^{b} (1.65)	240±9^{c} (1.85)	329 ± 12^{d} (2.53)	398 ± 11^{f} (3.06)	373±7^{e} (2.87)	0.72	5.4352	4.81333
TA102	-	254 ± 11^{a}	345 ± 16^{b} (1.36)	376 ± 13^{c} (1.48)	402 ± 11^{cd} (1.58)	481±9^{e} (1.89)	440 ± 10de (1.73)	-0.11	3.8046	42.0405
	+	261 ± 07^{a}	359 ± 12^{b} (1.38)	388 ± 14^{c} (1.49)	423 ± 15^{cd} (1.62)	507 ± 13^{e} (1.94)	457±8de (1.75)	-0.06	4.0276	43.5486
TA104	-	318 ± 11^{a}	404±9^{b} (1.27)	438±7^{c} (1.34)	479 ± 16^{d} (1.51)	551 ± 12^{f} (1.73)	500±8^{e} (1.57)	-0.31	3.7729	19.1672
	+	325 ± 13^{a}	416 ± 10^{b} (1.28)	450 ± 11^{c} (1.38)	495 ± 13^{d} (1.52)	575 ± 11^{e} (1.77)	511 ± 12^{d} (1.57)	-0.26	3.8710	1.81927

Values in parentheses are mutagenic index; Mi = induction factor; m = mutagenic potential; LSD = least significant difference.
Mean values followed by different letters are significantly different at p ≤ 0.05 (Duncan multiple range test).

with wastewater extracts to detect the percent of DNA damage in terms of survival (Table 8; Figure 2). *polA*$^{-}$ was the most sensitive to XAD concentrates (22.70%) after 6 h of treatment. *lexA*$^{-}$ and *recA*$^{-}$ also exhibited sensitivity with survival of 33.80% and 38.12% respectively under similar experimental conditions as compared to their isogenic wild-type

Table 6. Reversion of *Salmonella* tester strains in the presence of n-hexane extracted wastewater samples.

Wastewater extract (µl/plate)										
Strain	S9	Control	5	10	20	40	80	Mi	m	LSD P ≤ 0.05
TA97a	-	91±8^{a}	159 ± 10^{b} (1.74)	214 ± 11^{c} (2.35)	285 ± 14^{d} (3.13)	342 ± 12^{e} (3.75)	330 ± 10^{e} (3.62)	1.01	5.3113	3.63854
	+	93±6^{a}	164±8^{b} (1.76)	221 ± 13^{c} (2.38)	294 ± 11^{d} (3.16)	358 ± 16^{e} (3.84)	340 ± 07^{e} (3.66)	1.05	5.5100	7.04600
TA98	-	32±7^{a}	137±9^{b} (4.28)	178 ± 11^{c} (5.56)	228 ± 12^{d} (6.51)	294±8^{f} (9.19)	271 ± 10^{e} (8.47)	2.10	4.8730	3.63854
	+	38±8^{a}	163±7^{b} (4.29)	214 ± 12^{c} (5.63)	249 ± 15^{d} (6.55)	350 ± 12^{f} (9.21)	323 ± 11^{e} (8.50)	2.11	5.8555	5.45781
TA100	-	123±8^{a}	198 ± 11^{b} (1.61)	224±9^{c} (1.82)	288 ± 13^{d} (2.32)	381 ± 15^{f} (3.10)	328 ± 10^{e} (2.67)	0.74	4.7049	4.81333
	+	127±7^{a}	210±9^{b} (1.65)	232±7^{c} (1.82)	299 ± 12^{d} (2.35)	395 ± 12^{f} (3.11)	349 ± 13^{e} (2.74)	0.75	5.0551	4.81333
TA102	-	255±7^{a}	314±9^{b} (1.23)	351 ± 11^{c} (1.37)	397 ± 11^{d} (1.55)	457 ± 14^{f} (1.79)	428 ± 13^{e} (1.68)	-0.23	3.8289	4.81333
	+	257±6^{a}	333 ± 10^{b} (1.30)	369 ± 15^{c} (1.44)	400 ± 13^{d} (1.57)	468 ± 10^{f} (1.82)	440 ± 15^{e} (1.71)	-0.20	3.8536	6.30213
TA104	-	313±5^{a}	383±9^{b} (1.22)	409 ± 10^{c} (1.31)	441 ± 13^{d} (1.41)	503±7^{f} (1.61)	473 ± 15^{e} (1.51)	-0.50	3.3770	7.71850
	+	324±7^{a}	394±6^{b} (1.22)	424±9^{c} (1.31)	458±8^{d} (1.41)	521 ± 11^{f} (1.61)	494 ± 11^{e} (1.52)	-0.50	3.6170	14.5541

Values in parentheses are mutagenic index; Mi = induction factor; m = mutagenic potential; LSD = least significant difference.
Mean values followed by different letters are significantly different at p ≤ 0.05 (Duncan multiple range test).

counterparts. The sensitivity of mutants and their wild type was in the following order:

$polA^{-} > lexA^{-} > recA^{-} > polA^{+} > recA^{+}\ lexA^{+}$

Similar declining trend was also observed by the all the mutants with n-hexane and DCM extracted (acidic and basic fraction) wastewater. Gupta and Ahmad (2012) investigated the genotoxicity of refinery effluent employing *E. coli* survival assay and found that AB2480 (*recA uvrA*) double mutant was the most sensitive to damage by the test sample with the percent survival decreased to zero after exposure of 6 h, followed by AB2463 (*recA*) and AB2494 (*lexA*) with the survival of 3% and 19% respectively. In our study, the *polA* mutant was considered the most sensitive as indicated by the maximum damage induced by wastewater extracts, thereby indicating the role of *polA* mediated pathway in the test water induced lesions which may be due to the free radicals produced by heavy metals present in refinery wastewater. Several authors have also demonstrated the declining trends in the sensitivity of DNA repair defective *E. coli* K-12 mutants with wastewaters (Siddiqui et al., 2011; Tabrez and Ahmad 2011; Khan et al., 2019). In the present findings, maximum damage was noticed in DNA repair defective mutants on treatment with XAD concentrated samples followed by acidic fraction of DCM, n-hexane extracted and basic fraction of DCM extracts and thus the results are in support of Ames *Salmonella* mutagenicity assay. Our findings showed the importance of *recA*, *lexA* and *polA* genes in DNA repair and thereby helping the bacteria to tolerate pollution stress (Walker 1985; Masood and Malik 2013).

Extracellular treatment of bacteriophage λ with the test samples also showed considerable reduction in PFUs at the dose of 40 µl (test samples)/ml (Table 9). The *lexA* mutant appeared to be most sensitive strain in the presence of test samples compared to *recA*. Survival was 33.80% in *lexA* mutant in the presence of XAD concentrated sample after 6 h of treatment, while it was 36.29%, 42.98% and 45.01% when phage was treated with acidic fraction of DCM, n-hexane and basic fraction of DCM extracts, respectively (Table 9). Anjum and Malik (2013) also found *lexA* mutant as highly sensitive strain when treated with different industrial wastewater extracts.

Germination, being a complicated biological activity involves various factors to run concomitantly for the emergence of a seedling. Water uptake is required to activate the hydrolytic enzymes responsible for metabolizing the stored foods of the seeds into simple molecules, required for cell division as well as cell differentiation. Presence of several toxic pollutants in the wastewater apply prohibitive action on the process of seed germination (Table 10; Khan and Malik, 2018). Seeds of *V. mungo* were germinated in various concentrations of wastewater and did not inhibit germination at 5–50% diluted wastewater, while 100% wastewater showed 13.33% reduction (Table 10). The lengths of radicle and plumule were reduced when seeds were treated with different concentrations of wastewater compared to distilled water treated seeds. Significant reduction in radicle length (75.37%) and plumule length (83.6%) was observed at 100% (v/v) wastewater and biomass of 25.49, 53.69, 61.90, 69.01, and 74.41% at 5, 10, 25, 50, and 100% (v/v), respectively as compared to control (Table 10). Haq et al. (2016) found that diluted effluents (12.5–50%) had not affected *V. radiata* seed

Table 7. Reversion of *Salmonella* tester strains in the presence of Basic fraction of DCM extracted wastewater samples.

Wastewater extract (µl/plate)										
Strain	S9	Control	5	10	20	40	80	Mi	m	LSD P ≤ 0.05
TA97a	-	92±9^{a}	126±7^{b} (1.37)	159±8^{c} (1.73)	213±9^{d} (2.32)	297 ± 14^{f} (3.23)	276±6^{e} (3.00)	0.80	4.5991	5.14567
	+	97±7^{a}	138±9^{b} (1.42)	172 ± 12^{c} (1.77)	242±8^{d} (2.49)	319 ± 13^{f} (3.29)	291±8^{e} (3.00)	0.83	4.7628	7.93001
TA98	-	38 ± 11^{a}	156 ± 10^{b} (4.11)	188 ± 10^{c} (4.95)	223 ± 10^{d} (5.87)	315±8^{f} (8.29)	290±9^{e} (7.63)	1.99	5.1591	14.6674
	+	42 ± 10^{a}	175 ± 12^{b} (4.17)	210±7^{c} (5.00)	249 ± 10^{d} (5.93)	361±5^{f} (8.60)	321 ± 11^{e} (7.64)	2.03	5.8042	4.81333
TA100	-	121±8^{a}	185 ± 11^{b} (1.53)	221±8^{c} (1.83)	269±7^{d} (2.22)	373 ± 11^{f} (3.08)	301±9^{e} (2.49)	0.73	4.2205	3.15107
	+	126±6^{a}	199±9^{b} (1.58)	232 ± 13^{c} (1.84)	283 ± 11^{d} (2.25)	389±9^{f} (3.09)	316 ± 13^{e} (2.51)	0.74	4.3808	4.81333
TA102	-	247 ± 12^{a}	293±8^{b} (1.19)	327 ± 12^{c} (1.32)	376 ± 13^{bd} (1.52)	428±8^{f} (1.73)	399 ± 10^{e} (1.61)	-0.31	3.4595	75.8223
	+	251 ± 10^{a}	303 ± 12^{b} (1.21)	336±9^{c} (1.34)	382 ± 12^{d} (1.52)	449 ± 10^{f} (1.79)	408 ± 10^{e} (1.63)	-0.24	3.5904	2.57283
TA104	-	312±7^{a}	361±7^{b} (1.16)	394 ± 11^{c} (1.26)	427±9^{d} (1.37)	495±7^{e} (1.59)	441±8^{d} (1.41)	-0.53	2.9154	13.8909
	+	320±6^{a}	383 ± 13^{b} (1.20)	403±9^{c} (1.26)	441±8^{d} (1.38)	509±9^{f} (1.59)	467±7^{e} (1.46)	-0.53	3.2328	4.41899

Values in parentheses are mutagenic index; Mi = induction factor; m = mutagenic potential; LSD = least significant difference.
Mean values followed by different letters are significantly different at p ≤ 0.05 (Duncan multiple range test).

Table 8. Survival pattern of *E. coli* K12 strains exposed to different extract of wastewater.

Test Sample	*E.coli* K12 strain	0 h	2 h		4 h		6h	
		No. of colonies	No. of colonies	% mean Survival	No. of colonies	% mean Survival	No. of colonies	% mean Survival
XAD concentrated extract	Wild type	479 ± 23.71	468 ± 25.94	97.61 ± 0.61	449 ± 25.24	93.65 ± 0.65	434 ± 24.42	90.60 ± 0.64
	pol A$^+$	435 ± 21.03	400 ± 19.08	92.03 ± 0.17	376 ± 16.09	86.52 ± 0.52	354 ± 14.11	81.46 ± 0.73
	rec A$^-$	479 ± 28.02	382 ± 24.70	80.08 ± 9.15	259 ± 15.13	54.05 ± 1.47	183 ± 10.69	38.12 ± 1.25
	lex A $^-$	357 ± 26.08	221 ± 15.82	62.35 ± 7.48	146 ± 10.97	40.96 ± 4.91	120 ± 9.29	33.80 ± 3.64
	pol A$^-$	378 ± 17.24	235 ± 17.16	62.32 ± 6.00	138 ± 17.35	36.57 ± 6.06	86 ± 10.15	22.70 ± 1.99
DCM (Acidic fraction) extract	Wild type	550 ± 4.00	536 ± 08.8	97.46 ± 1.89	513 ± 9.16	93.28 ± 2.00	506 ± 7.02	92.06 ± 1.05
	pol A$^+$	504 ± 11.06	462 ± 9.54	91.65 ± 3.35	446 ± 9.71	88.54 ± 3.47	424 ± 14.19	84.05 ± 3.95
	rec A$^-$	674 ± 12.53	543 ± 8.88	80.60 ± 2.77	325 ± 8.62	48.30 ± 2.17	272 ± 14.57	40.44 ± 2.89
	lex A $^-$	505 ± 11.59	330 ± 13.01	65.37 ± 3.60	216 ± 8.18	42.84 ± 2.59	183 ± 12.53	36.29 ± 2.99
	pol A$^-$	660 ± 19.31	424 ± 12.53	64.31 ± 3.73	245 ± 9.71	37.12 ± 2.54	154 ± 9.17	23.33 ± 1.22
n-hexane extract	Wild type	726 ± 7.76	709 ± 7	97.71 ± 1.23	693 ± 10.54	95.50 ± 0.48	689 ± 12.50	94.89 ± 0.77
	pol A$^+$	573 ± 11.01	547 ± 16.01	95.58 ± 2.45	522 ± 24.02	91.14 ± 5.23	498 ± 32.23	86.97 ± 5.64
	rec A$^-$	674 ± 18.77	582 ± 8.74	86.28 ± 1.12	425 ± 20.30	63.05 ± 3.60	336 ± 18.61	49.88 ± 2.46
	lex A $^-$	659 ± 34.93	540 ± 36.50	82.10 ± 6.91	362 ± 4.58	54.98 ± 2.42	285 ± 61.49	42.98 ± 7.73
	pol A$^-$	779 ± 27.22	518 ± 28.29	66.43 ± 1.50	384 ± 23.63	49.23 ± 1.91	246 ± 18.01	31.67 ± 2.90
DCM (basic fraction)	Wild type	665 ± 18.00	648 ± 10.07	97.51 ± 1.16	638 ± 17.58	95.94 ± 0.92	633 ± 17.47	95.24 ± 0.61
	pol A$^+$	418 ± 25.42	405 ± 24.79	96.62 ± 2.07	389 ± 23.35	92.97 ± 3.94	372 ± 22.37	89.16 ± 9.01
	rec A$^-$	612 ± 14.01	545 ± 12.50	89 ± 3.59	410 ± 10.50	66.95 ± 3.25	324 ± 7.51	53 ± 2.44
	lex A $^-$	603 ± 18.34	518 ± 15.53	86.08 ± 4.43	331 ± 10.82	54.97 ± 2.94	271 ± 8.89	45.01 ± 2.45
	pol A$^-$	346 ± 17.52	249 ± 19.08	71.90 ± 1.94	176 ± 18.08	50.77 ± 2.69	119 ± 17.04	34.38 ± 3.19

germination, but at 75 and 100% concentrated effluent, germination was inhibited by 10 and 30%, respectively. Radicle and plumule lengths as well as biomass of seedlings were reduced significantly at 100% effluent that may be corresponded with the combined effect of excessive organic and inorganic contaminants present in the wastewater. Baruah and Das (1997) reported a delayed germination in the presence of higher metal and salt concentrations, while Yasmin et al. (2011) obtained reduction in root and shoot lengths with the higher concentration of total dissolved solids which may be related to the fact that some of the nutrients present in the wastewater are essential but toxic which become hazardous at higher concentrations. Powel et al. (1996) obtained reducing trend in fresh weights of seedlings, while no change was observed in dry weights

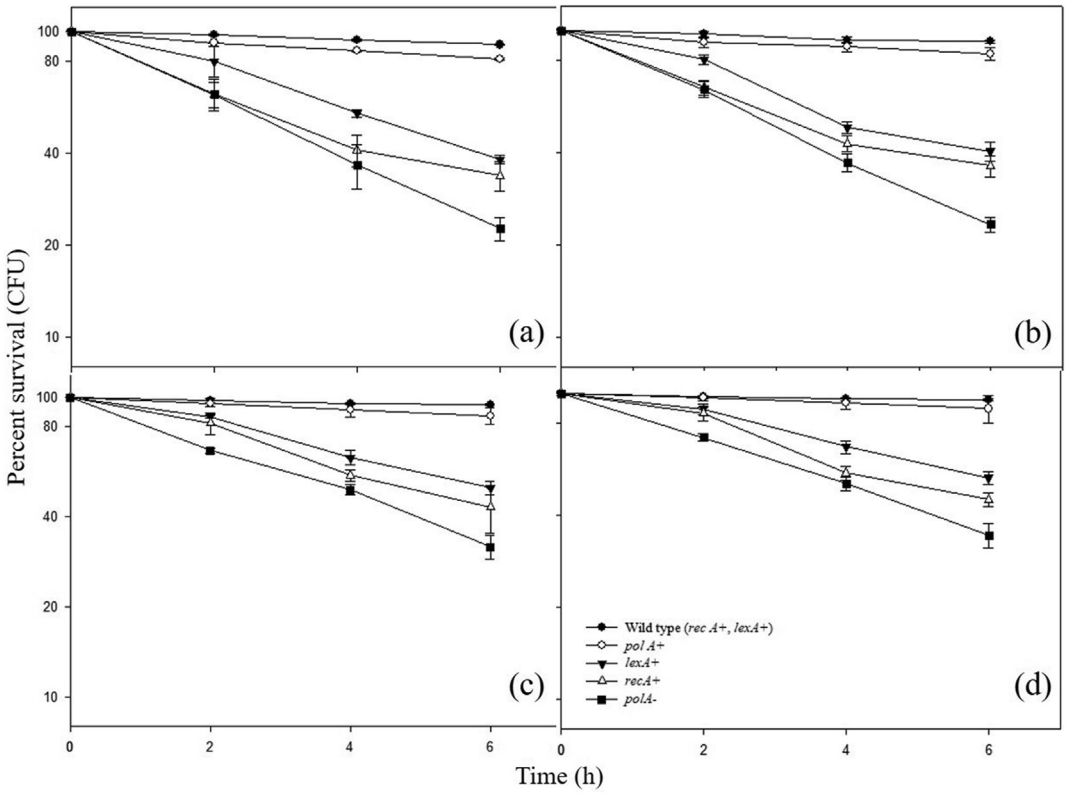

Figure 2. Survival of *E.coli* K-12 strains treated with XAD-concentrated wastewater (a), acidic fraction of DCM extract (b), n-hexane extracted wastewater (c) and basic fraction of DCM extract (d).

Table 9. Survival of extracellularly treated bacteriophage λ exposed to different extract of wastewater.

	E.coli K12 strain	0 h	2 h		4 h		6h	
		No. of plaques	No. of plaques	% mean survival	No. of plaques	% mean survival	No. of plaques	% mean survival
XAD concentrated extract	*recA*$^+$*lexA*$^+$	203.33 ± 4.26	187.67 ± 4.60	92.29 ± 0.34	175 ± 2.12	86.13 ± 2.59	159.67 ± 5.67	78.53 ± 2.47
	recA$^-$	357.67 ± 18.90	301 ± 5.13	84.19 ± 3.93	212.67 ± 2.52	59.59 ± 3.64	157.33 ± 4.73	44.07 ± 2.58
	lexA$^-$	372 ± 14.44	249.33 ± 5.72	67.09 ± 1.43	167 ± 5.34	44.91 ± 0.44	103.33 ± 4.81	27.78 ± 0.72
DCM (Acidic fraction) extract	*recA*$^+$*lexA*$^+$	211 ± 1.41	193.67 ± 5.67	91.77 ± 2.07	174.33 ± 8.73	82.59 ± 3.59	169 ± 6.75	80.11 ± 3.44
	recA$^-$	343.67 ± 9.45	301 ± 3.51	87.74 ± 3.33	194.33 ± 21.39	56.46 ± 4.73	157.33 ± 4.73	45.82 ± 2.52
	lexA$^-$	375.67 ± 24.42	261 ± 10.98	69.61 ± 1.61	168.33 ± 9.91	44.84 ± 0.73	115 ± 4.42	30.81 ± 2.62
n-hexane extract	*recA*$^+$*lexA*$^+$	222.67 ± 1.47	206.33 ± 2.86	92.67 ± 1.48	191 ± 3.54	85.79 ± 1.82	182.67 ± 4.60	82.05 ± 2.49
	recA$^-$	334 ± 16.70	267 ± 13.32	80.07 ± 2.92	206.67 ± 4.51	61.99 ± 3.76	164 ± 7.00	49.18 ± 3.16
	lexA$^-$	363 ± 32.18	263 ± 21.92	72.62 ± 3.31	187.67 ± 6.38	52.06 ± 3.16	113 ± 3.24	31.47 ± 3.03
DCM (Basic fraction) extract	*recA*$^+$*lexA*$^+$	208.67 ± 3.67	204 ± 3.94	97.76 ± 0.22	189.67 ± 4.81	90.88 ± 0.75	183.33 ± 4.26	87.85 ± 0.56
	recA$^-$	323.67 ± 23.76	267 ± 7.21	82.71 ± 4.79	222.67 ± 4.51	68.98 ± 3.74	193 ± 9.00	53.62 ± 4.53
	lexA$^-$	349.67 ± 34.77	275.33 ± 18.33	79.23 ± 4.87	227.33 ± 25.54	64.94 ± 1.66	144.33 ± 6.57	41.58 ± 2.32

under the pollution stress. Both increase and decrease in the weight parameters were reported by Nawaz et al. (2006) and Yousaf et al. (2010). Seedling vigour index determines the degree of activity and performance of the seeds during germination and seedling emergence. Seedling vigour index and stress tolerance index was gradually decreased with increasing wastewater concentration from 5-100% (Table 11). The average seedling vigour index reduced from 916.67 to 222.45 in wastewater compared to 1286.67 in control. The stress tolerance indices i.e., RLSTI and SLSTI were highest at 5% i.e., 74.69 and 68.44% respectively, while 24.69 and 18.17% at 100%, respectively. Kabir et al. (2008) used seedling vigour index as a phytotoxicity index to evaluate the impact of heavy metals on seedling growth and found that seedlings vigour index of *Thespesia populnea* gradually reduced with the increase in concentrations of lead and cadmium. Seed germination, seedling growth and seedling dry weights were also significantly ($p < 0.05$) affected by lead and cadmium concentrations. Many of the low molecular weight hydrocarbons such as benzene, toluene, ethylbenzene and xylene also exhibited phytotoxicity for food crops (de Oliveira et al., 2012).

Allium cepa chromosomal aberration assay is considered as one of the best biological method for monitoring the hazardous consequences of persistent pollutants present in the environmental samples. MI and CA in meristematic root-tips cells of *Allium cepa* is thought to be an excellent method of choice in the assessment of the genotoxicity of chemical agents, sewage and industrial wastewaters (Lutterbeck et al., 2015; Martins et al., 2016; Haq et al., 2017). The cyto- and genotoxicity of wastewater was also tested using *Allium cepa* chromosomal aberration assay and the frequency of mitotic phases, mitotic indices and chromosomal aberrations were also determined (Table 12). The frequency of mitotic phases was altered by the treatments, as the percentage of prophage declined slowly with increasing wastewater concentrations up to 100%, whereas no uniform pattern was observed in metaphase as well as anaphase-telophase stages. Moreover, when the bulbs were placed in different wastewater concentrations, a dose-dependent reduction of MI was found (MI of 16.38% at 5% and 9.74% at 100% wastewater concentrations). Negative control (ddH_2O) displayed the highest MI (24.855), while positive control (MMS) exhibited the lowest MI (6.10%). Iqbal et al. (2017) found a remarkable decline in cytotoxicity of refinery wastewater by *A. cepa* when raw wastewater was treated with $UV/H_2O_2/TiO_2$ and gamma radiation/H_2O_2.

A high level of chromosomal aberrations (18.95%) was observed at 100% wastewater compared to diluted wastewater and distilled water (Table 13). Moreover, root tip cells treated with wastewater also showed various kinds of anomalies such as stickiness, chromosomal loss, anaphasic bridge, c-mitosis, tripolar anaphase, vagrant chromosome and telophasic bridge (Figure 3) in which c-mitosis, stickiness, vagrant chromosomes, and bridges were the most common aberrations found in each concentration of effluents. Sticky chromosomes were the most frequent kind of aberration induced by refinery effluent. Abnormal chromosome orientation and movement such as multipolar anaphase and binucleus were also found. The observed aberrations can be correlated with the several toxic chemicals present in the refinery wastewater. Percentage of aberration increased with increasing concentrations of wastewater and recorded highest at 100% wastewater. Binucleated cells were frequently seen in our test samples indicated that the effluent had suppressed the cell plate formation which is responsible for binucleated cells (Somashekar and Gowda, 1984). The effluent had caused nuclear

Table 10. Effect of different concentrations of refinery wastewater on radicle and plumule growth of *Vigna mungo L.* seeds.

Test Sample		G (%)	% Reduction in germination	Radicle length (cm)	% radicle reduction	Plumule length (cm)	% plumule reduction	Mean seedling length	Fresh weight	Dry weight	Biomass (gm)	% Biomass reduction
C	ddH_2O	100 ± 0.00		5.4 ± 1.06^{a}	-	7.5 ± 0.38^{a}	-	12.87 ± 1.42^{a}	2.52 ± 0.18	0.335 ± 0.03	2.181 ± 0.15	-
Wastewater	5%	100 ± 0.00	0	4.03 ± 0.57^{ab}	25.37	5.13 ± 0.72^{b}	31.6	9.17 ± 1.29^{b}	1.94 ± 0.10	0.313 ± 0.01	1.625 ± 0.09	25.49
	10%	100 ± 0.00	0	3.53 ± 1.45^{bc}	34.63	3.1 ± 0.52^{c}	58.67	6.63 ± 1.55^{c}	1.32 ± 0.07	0.288 ± 0.04	1.01 ± 0.09	53.69
	25%	100 ± 0.00	0	3.43 ± 0.40^{bc}	36.48	2.53 ± 0.35^{cd}	66.27	5.97 ± 0.31^{c}	1.29 ± 0.15	0.455 ± 0.11	0.831 ± 0.05	61.90
	50%	100 ± 0.00	0	2.00 ± 0.50^{cd}	62.96	2.1 ± 0.53^{d}	72	4.10 ± 0.17^{d}	1.06 ± 0.33	0.388 ± 0.25	0.676 ± 0.05	69.01
	100%	86.67 ± 15.3	11.37	1.33 ± 0.45^{d}	75.37	1.23 ± 0.25^{e}	83.6	2.57 ± 0.59^{d}	0.87 ± 0.02	0.310 ± 0.02	0.558 ± 0.04	74.41

Table 11. Effect of different concentration of Refinery wastewater on germination and growth of *Vigna mungo L.*

Test Sample	Seedling Vigour Index I	Seedling Vigour Index II	Relative seed germination rate (RSG)	Relative root growth (RRG)	Percentage phytotoxicity	G.Index (%)	Stress Tolerance Index %	
							RLSTI*	SLSTI**
Control	1286.67 ± 142.24^{a}	33.53 ± 2.93^{bc}	1.00	1.00	0.00^{c}	100	100^{a}	100^{a}
5%	916.67 ± 128.58^{b}	31.30 ± 1.45^{bc}	1.00	0.75	25.31 ± 10.52^{b}	74.63	74.69 ± 10.53^{b}	68.44 ± 9.64^{b}
10%	663.33 ± 155.03^{c}	28.83 ± 4.43^{c}	1.00	0.65	34.57 ± 26.85^{b}	65.37	65.43 ± 15.50^{b}	41.33 ± 6.92^{c}
25%	596.67 ± 30.55^{c}	45.47 ± 10.72^{a}	1.00	0.63	36.42 ± 7.48^{b}	63.51	63.58 ± 7.48^{b}	33.78 ± 4.69 ^{cd}
50%	410.00 ± 17.32^{d}	38.83 ± 2.55^{ab}	1.00	0.37	62.96 ± 9.26^{a}	37.04	37.04 ± 9.26^{c}	28.00 ± 7.05^{de}
100%	222.45 ± 50.79^{e}	26.90 ± 2.04^{c}	0.867 ± 0.15	0.25	75.31 ± 8.35^{a}	24.63	24.69 ± 8.35^{c}	18.17 ± 2.02^{e}

*RSG = Relative shoot growth; **RRG = Relative root growth; *** RLSTI = Root length stress tolerance index; ****Shoot length stress tolerance index.

Table 12. Effect of different concentrations of wastewater extract on mitotic index and mitotic phase of *Allium cepa* root meristem cells.

	NC	PC	Wastewater				
	ddH_2O	MMS	5%	10%	25%	50%	100%
Total cells counted	3135	3218	2180	2361	3702	2283	2722
Interphase	2356	3022	1805	2000	3199	2020	2442
In division	779	196	375	361	503	263	280
Prophase	467	128	217	203	271	125	117
% Prophase	59.94	65.31	57.86	56.23	53.87	47.53	41.78
Metaphase	172	48	81	78	102	55	77
% Metaphase	22.08	24.49	21.60	21.61	20.28	20.91	27.5
A + T	140	20	77	80	130	83	86
% A + T	17.97	10.20	20.53	22.16	25.84	31.56	30.71
%MI	24.85 ± 2.04^{a}	06.10 ± 1.68^{f}	16.38 ± 1.23^{b}	14.45 ± 0.81^{bc}	13.03 ± 0.89^{cd}	10.87 ± 1.52^{de}	09.74 ± 1.24^{e}
%CA	2.54 ± 0.49^{e}	28.79 ± 2.60^{a}	4.84 ± 2.6^{e}	9.67 ± 2.06^{d}	14.20 ± 0.34^{c}	15.75 ± 1.99^{bc}	18.95 ± 1.45^{b}

%P= Percent Prophase; %M = Percent Metaphase; A + T = Anaphase and Telophase; %MI = Percent Mitotic Index; %CA = Percent chromosomal aberration. Mean values followed by different letters are significantly different at $p \leq 0.05$ (Duncan multiple range test).

Table 13. Chromosome aberration in the root meristem cells of *Allium cepa* exposed to different concentrations of wastewater for 72 h.

Conc. (%)	Major types of aberrations (in approx. 300 cells i.e. ~100 per slide)											Total aberrant cells (%)
	B	CM	CS	CB	L	BN	SN	VC	DAT	DM	Others	
5	4	3	3	-	-	1	-	-	-	4	-	4.84 ± 2.6^{e}
10	-	4	9	3	-	2	3	2	3	2	3	9.67 ± 2.06^{d}
25	8	5	9	-	-	-	13	7	1	-	2	14.20 ± 0.34^{c}
50	4	4	11	6	3	3	8	3	4	6	-	15.75 ± 1.99^{bc}
100	3	7	4	13	-	4	10	3	3	9	3	18.95 ± 1.45^{b}
PC	9	8	12	7	8	8	9	5	10	9	3	28.79 ± 2.60^{a}
NC	1	1	2	-	-	-	-	1	2	1	-	2.54 ± 0.49^{e}

B=Bridges; CM = C-mitosis; CS = Chromosomal stickiness; L = Laggards; BN = Binucleated cells; SN = Strap nucleus; VC = Vagrant chromosomes; DAT = Disturbed Anaphase/Telophase; DM = Disturbed metaphase; PC = Positive control; NC = Negative control.

irregularities associated with chromosomal aberrations and abnormal nuclear shape resulted in strap nucleus. Positive control (MMS) had highest no. of aberrations, whereas cells treated with distilled water displayed minimum aberrations. Wastewater treatment had caused significant ($P < 0.05$) effect on MI and %CA to that of both the controls (i.e., positive and negative) using DMRT. Several industrial effluents and heavy metals were found to induce c-metaphase, chromosomal breakage and fragmentation as well as micronucleus formation in root tip cells of *A. cepa* (Olusegun et al., 2010; Shrivastava 2015; Gupta et al., 2018). Yadav et al. (2019) reported genotoxic nature of tannery wastewater by *A. cepa* chromosomal aberration assay where they found several abnormalities like stickiness, chromosome loss, C-mitosis, vagrant chromosome, micronucleated and binucleated cells. Hemachandra and Pathiratne (2016) also reported various kinds of physiological and clastogenic effects in *Allium* root cells exposed to industrial effluents with harmful chemicals which is used for the irrigation and ultimately reaching to a Kelani river, Sri Lanka. Hara and Marin-Morales (2017) investigated the genotoxic potential of river water which was under the influence of a petroleum refinery (Sao Paulo State, Brazil) using *A. cepa* test and found high frequencies of chromosomal aberrations and micronuclei in dividing cells with the physico-chemically treated samples, but none was observed with the samples after biological treatment.

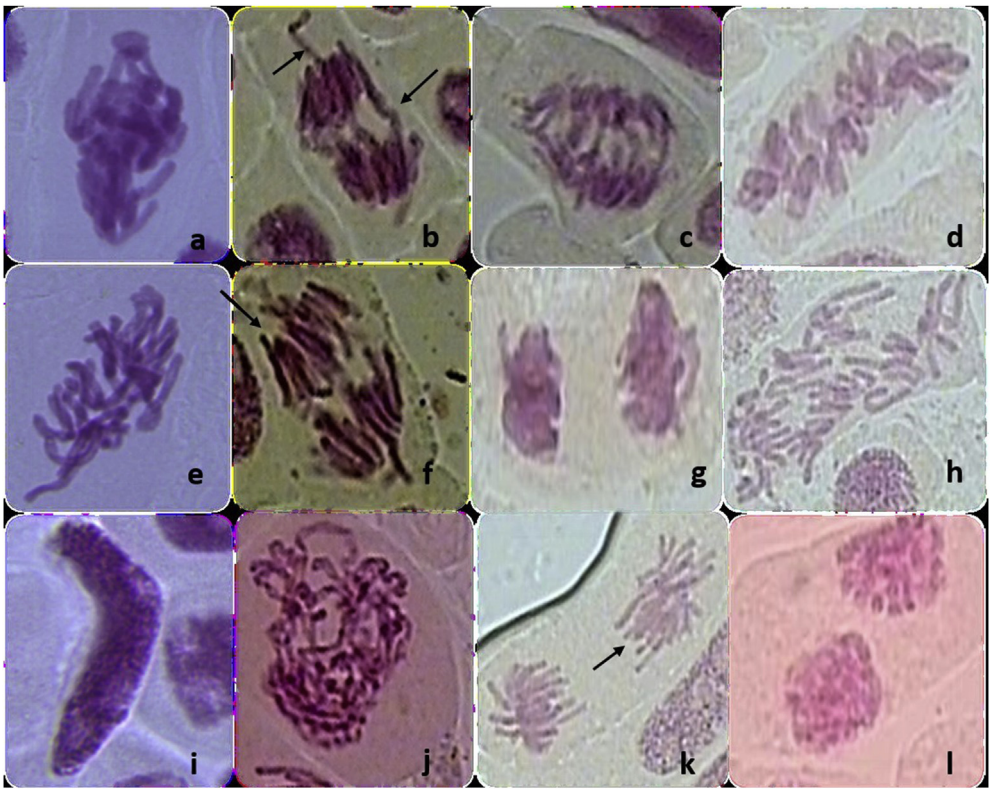

Figure 3. Different kinds of chromosomal aberrations induced by wastewater in *Allium cepa* root tips: Stickiness (a), vagrant chromosome with bridges (b), Anaphase bridge (c), C-mitosis (d), spindle disturbance in anaphase (e), tripolar anaphase (f), sticky telophase (g), chromosomal breakage (h), strap nucleus (i), disturbed metaphase (j), star anaphase (k), Binucleated cell(l).

4. Conclusion

Present study demonstrated that petroleum refinery wastewater mixed with domestic sewage found to have higher EC, COD, salts, nitrites, nitrates, bromides and sulphates etc. and contains various potentially toxic (PTEs) and non-toxic elements as determined by ICP-OES; and wide variety of toxic organic pollutants viz. phthalates, phthallic acid derivatives, alkanes and their derivatives, PAHs derivatives, esters of acids, cholestane derivatives and pesticides etc as identified by GC-MS. The complex mixtures of chemicals in the wastewater exhibited cyto-genotoxic hazards in terms of mutagenicity and genotoxicity as observed in Ames *Salmonella* reverse mutation assay. Present findings showed that wastewater consisted of several kinds of genotoxins due to which number of revertant colonies increased and SOS response in *E.coli* K-12 was triggered, causing mutation in the bacterial DNA. The wastewater also showed the adverse effect on germination of *V. mungo* seeds as well as reduction in radicle and plumule lengths and biomass. Moreover, *A. cepa* root tips chromosomal aberration assay also confirmed the presence of cyto-genotoxic compounds in the test samples as decreased in MI and induction of several chromosomal aberrations in the plant root meristem. Present findings suggested that cyto-genotoxic effect triggered by wastewater to the receiving waterbodies require continuous monitoring to provide serious attention and remedial measures for its improvement as it is continuously used to irrigate the food crops where pollutants might be accumulated into the food chain and are hazardouss to humans and animals health.

Declarations

Author contribution statement

Khalida Khatoon: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Abdul Malik: Contributed reagents, materials, analysis tools or data.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

Acknowledgements

Authors are thankful to DST-UKIERI joint project (DST/INT/UK/P-152/2017) for their visit to UK. Department of Civil and Environmental Engineering, University of Strathclyde, Glasgow, UK is acknowledged for Ion chromatography, ICP-OES and GC-MS analysis. Authors are thankful to Dr. Charles W. Knapp, Dr. Tatyana Peshkur and Mara Knapp of Civil and Environmental Engineering, University of Strathclyde for inorganic and organic analysis.

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