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Research article

Genotoxic hazard and oxidative stress induced by wastewater irrigated soil with special reference to pesticides and heavy metal pollution

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HIGHLIGHTS

- Genotoxicity evaluation of WWI soil applying bacterial and plant-based assays was carried out.
- Ames tester strains and eukaryotic plants systems revealed the toxicity of WWI soil.
- The findings endorse the significant cyto-genotoxic potential of polluted soil.
- Genotoxicity and mutagenicity were found on dose dependent manner of soil extracts.

GRAPHICAL ABSTRACT

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ABSTRACT

Due to enhancement of industrial growth and urbanization, soil contamination is increasing prominently. Therefore, it is important to examine possible adverse effects of industrial waste. Soil samples were might to be polluted with several heavy-metals and pesticides. Gas chromatographic results showed occurrence of high-level of organochlorine and organophosphate pesticides in studied soil samples. Genotoxicity of soil extracts was assessed using environmental-risk assessment models. Soil samples were extracted in hexane and dichloromethane solvents and were evaluated for genotoxic potential by prokaryotic (Ames test, plasmid nicking assay and *E. coli* K-12 DNA repair defective mutants) and eukaryotic (*Allium cepa* root chromosomal aberration and *Vigna radiata* seed-germination test) bioassays. Strain TA98 was found the most susceptible among soil extracts. The mutagenicity of hexane soil extract from wastewater irrigation was found to be higher than that of DCM samples in terms of mutagenic index, mutagenic potential, and induction factor for Ames strains. The damage in DNA repair defective mutants of hexane extracts were found higher compared to DCM extracts at dose of 20 µl/ml of culture. Survival in *polA*, *lexA* and *recA* mutants were 39%, 47% and 55% while treated with hexane extract. *Allium cepa* test, mitotic index was decreased in dose-dependent way and various kinds of chromosomal aberrations were found. *Vigna radiata* seeds germination and other parameters were also affected when treated with wastewater irrigated (WWI) soil. Oxidative stress in *V. radiata* roots were also showed under CLS microscope. Genotoxicity of WWI soil extract was also confirmed by plasmid nicking test. Our study provides possible explanation for the assessment of potential health and environmental hazards of the industrial region.

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1. Introduction

In developing nations, it is a common practice of using wastewater for irrigation of agricultural lands (Amerasinghe et al., 2013; Haroon et al., 2019). Many industries discharge untreated wastewater into adjoining agricultural fields due to a lack of access to sufficient sewage treatment amenities and organisation, which may result in the deposition of various organic and inorganic contaminants (Ahmed et al., 2018; Gupta et al., 2020) which alter physico-chemical properties of the soil. Due to high level of dissolved solids (TDS), sulfate, phosphate, chloride, biochemical oxygen demand (BOD), chemical oxygen demand (COD), heavy metals, and organic pollutants in the industrial wastewater, it is unfit for crop irrigation. The irregular and indiscriminate usage of wastewater for the irrigation of agricultural lands may eventually leads to the deposition of these hazardous pollutants in soil (Barakat et al., 2020; da Silva Júnior and Vargas, 2009; Men et al., 2018), ultimately taken up by plants and gets accumulated in different organs of human body by consuming that plants (Bonanno et al., 2018; Qiu et al., 2016).

Chemical pesticides and heavy metals played a vital role in sustaining crops and livestock yield for several decades and have negative influence on environment (Duke, 2018; Onwona-Kwakye et al., 2020). Irrigation of agricultural soils with wastewater have high risk of accumulation of persistent organic and inorganic pollutants in soil. The presence of heavy metals and pesticide residues in soil has an influence on agricultural crops, posing a major threat to public health (Al-Nasir et al., 2020; Herrero-Hernández et al., 2020; Wang et al., 2012).

Among various industries that contribute to the overall pollution of water bodies, pesticide industry is of particular importance (Bachmann Pinto et al., 2018; Srilatha et al., 2019). The discharged contaminants from industries are complex mixture (organic and inorganic pollutants) and can lead to unpredictable genotoxicity responses. These hazardous wastes have the potential to bind to DNA and produce negative alterations in biological processes such as cytotoxicity, cancer, altered metabolism, and reproductive failure (Du et al., 2020; Singh and Chandra, 2019; Zhao et al., 2019). According to Zhang et al. soil exposed with organochlorine pesticides has an endocrine disrupting potential (Zhang et al., 2018). The lack of information on the fate and potential toxicity of such pollutants make it difficult for regulatory authorities to manage these contaminants.

Many researchers focused on detecting and identifying pollutants in industrial wastewater that pose possible health risks, rather than agricultural land (Amerasinghe et al., 2013; Mandal and Kaur, 2019; Ying et al., 2021). The biological approach has some benefits as it represents the overall toxicity of a vast number of chemicals found in any environmental sample without specific knowledge on their physical and chemical composition. The direct effect of toxic contaminants can be identified by *in vivo* testing using whole living organisms (such as Zebrafish, Polychaete etc) (Luan et al., 2020). However, due to their high cost, time consuming and ethical issues, these tests are not feasible. Therefore, a combination of *in vitro* experiments involving microorganisms and plants have been used to provide more practical view of the genotoxicity of the mutagens (Gupta et al., 2020; Khan et al., 2019). Genotoxicity of wastewater irrigated soil have been evaluated by many researchers using plants such as *Allium cepa* (Gallego and Olivero-Verbel, 2021) *Vicia faba* (Gupta et al., 2020) *Tradescantia* (Šiukšta et al., 2019) and bacterial assay likewise Ames test and SOS DNA repair (Ansari and Malik, 2009). The aim of current study was genotoxicity assessment of soil irrigated with the wastewater employing Ames *Salmonella*/mammalian microsome test, plasmid nicking test, DNA repair defective *E. coli* K-12 mutants, *Allium cepa* root chromosomal aberration test and oxidative damage in the roots of *Vigna radiata* plant.

2. Materials and methods

2.1. Sample collection

Ghaziabad is hub of industries, housing a large number of industries such as steel, automobile, agrochemicals, pharmaceuticals. Soil samples were taken from agricultural fields to a depth of ±15 cm using the sterilized spatula as described by Aleem and Malik (2003). Two different soil sites were selected: (i) wastewater irrigated (WWI) soil (near industrial area), and (ii) Ground water irrigated (GWI) soil, which is located at ±15 km away from the Industrial site. From every field, 1 kg soil samples were taken from four distinct spots within the same field and 4 kg composite soil samples were prepared.

2.2. Physicochemical and heavy metal analysis of soil samples

The samples were subjected to physicochemical examination in accordance with the procedure given by Gupta (2007). Samples were dried at 40 °C and finely ground (<0.1 mm), then burned to ashes in crucible. In a conical flask, 1 g of each soils ashes moistened with 1 ml of double-distilled (DD) water. After that, aqua regia (concentrated hydrochloric acid and nitric acid in 3:1 ratio) were added. The flask was put on hot plate and digested until the clear solution obtained above the soil ash and volume was reduced to 1 ml (Alef and Nannipieri, 1995). After digestion, soil samples were diluted, and make up the volume to 100 ml with double distilled water and filtered through Whatman filter paper with pore size 11 μm. The digested samples were analysed for the presence of heavy metals (Ni, Cu, Cr, Pb, Cd, Zn, Fe and Mn) by atomic absorption spectrophotometer (Model: GBC 932 Plus, Australia).

2.3. Quantification of pesticides in soil samples

Pesticides in soil were determined using the method described by Gan et al. (1999). Ten-gram soil samples were vigorously shaken with 25 ml of methanol and water in 4:1 ratio (v/v) for one hour and decanted the supernatant then the mixture was centrifuged for 15 min at 10,000 rpm. In a rotatory evaporator, this step was repeated twice to reduce the extract volume to 15 ml.

2.4. Gas chromatographic analysis of pesticides

The gas chromatographic (GC) analysis was carried out using a SHIMADZU GC-2010 gas chromatograph containing an electron capture detector (Zeyad et al., 2019). Peaks of samples were identified by matching the retention time of multi-standard CRM-47426 of 20 organochlorines and 48391 organophosphorus pesticide mixtures (Sigma-Aldrich). The concentration of standards of CRM-47426 (organochlorines) and 48391 (organophosphorus) were 2000 μg/ml of each pesticide.

2.5. Extraction of soil samples with different solvents

Soil samples were extracted using hexane and dichloromethane solvents (HPLC grade, SRL, India) as described by the method of Knize (Knize et al., 1987). 10 g of soil were extracted with 10 ml of solvent. The extracted samples were dried using rotary vacuum evaporator (Hahnshin S and T Co. Ltd, Korea) and redissolved in 1 ml of DMSO (SRL, India). The dissolved soil extracts were centrifuged at 8000 rpm for 20 min and filtered by 0.22 μm syringe filter (Axiva).

Aqueous extracts of soil was prepared following the method of Cotelle et al. (1999). 100 g soil was mixed with 1000 ml of deionized water and placed overnight at room temperature. The aqueous extract of soil was used for *A. cepa* root chromosomal aberration test and mung bean seed germination assay.

2.6. Ames Salmonella/microsome mutagenicity assay

The *Salmonella* mutagenicity assay was done according to standard Ames protocol with few modifications (Maron and Ames, 1983). Briefly, 10 μl bacterial culture was incubated with varying concentrations of soil extracts ranging from 2.5 to 40 μl/plate (equivalent to 10–400 mg soil per plate) incubated for 30 min at 37 °C. Two ml of top agar comprising trace amount of biotin and histidine were added and the mixture was poured on the glucose agar plates (minimal). The plates were incubated at 37 °C for 48–72 h. Both negative and positive controls were included in each experiment. MMS was used as positive control and DMSO was taken for negative control. The extracts of soil were also evaluated for the detection of pro-mutagens in presence of S9 microsomal fraction.

Mutagenic potential (m), induction factor (Mi) and mutagenic Index, were recorded as described by Ansari and Malik (2009).

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

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

where, n = number of colonies of revertant; c = number of control colonies of revertants.

The initial linear portion of the curve of dose-response with each tester strains of Ames was used to calculate the mutagenic potential of samples. The slope was calculated using least square regression of initial linear component of the curve.

2.7. Effect soil extracts on E. coli K-12 strains

E. coli K-12 DNA repair defective mutants (*recA*, *lexA* and *polA*) and isogenic wild type strains were cultivated separately in nutrient broth at 37 °C to obtain exponentially growing bacterial cells (Masood and Malik, 2013). The pellets were reconstituted in $MgSO_4$ solution (10 mM) and then treated with soil extracts (20 μl). To observe variations in colony forming units (CFUs), an aliquot from each treatment was taken at different time intervals, diluted, and plated onto the nutrient agar plates. The plates were then incubated at 37 °C for overnight. Solvent (negative) control was also included in each experiment.

2.8. Allium cepa chromosomal aberration assay

To assess the negative impact of WWI soil samples, *A. cepa* root chromosomal aberration assay were used by the method of Fiskesjo (1985). Onion bulbs of similar size (diameter) (1.5–2.0 cm) were received from a recognised retailer. Before beginning the experiment, the outer dead scales of onion bulbs were removed without harming the root primordia. The basal part onion bulbs were dipped into small beakers containing DD water. These bulbs were allowed to grow for 48–72 h at room temperature. Freshly developed roots up to 2 cm in length were employed in the test, and they were treated with a series of aqueous soil extracts (5, 10, 25, 50, and 100%) up to 3 days. In each test, MMS and DD water were also included as positive and negative controls respectively. After three days of treatment, root tips were chosen randomly. The root tip cells were fixed in a 3:1 ratio (v/v) of ethanol and glacial acetic acid and kept in 70% ethanol at 4 °C until examination. For slide preparation, the fixed root tips were washed with DD water and hydrolysed using HCl (1N) followed by washing with DD water. Acetocarmine were used to stain the hydrolysed cells and observed under microscope. Mitotic index (MI) was determined by viewing approximately 6000 dividing cells (2000 cells per slide) as follows:

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

Approximately 300 cells (100 cells from each slide) were examined for chromosomal aberrations.

2.9. Effect of soil extracts on germination and growth of Vigna radiata seed under in vitro condition

Phytotoxicity of soil extracts on mung bean seeds were performed as defined by Bharagava and Chandra (Bharagava and Chandra, 2010; Silambarasan et al., 2019). Seeds of mung bean (*Vigna radiata* L.) were bought from a nearby certified store. Fresh seeds were washed using sterile DD water and sterilized by ethanol (70%) for 1 min, followed by sodium hypochlorite (3% solution) for 3 min. The seeds were again washed with water (DD) several times. These seeds were soaked for overnight in a series of concentration of soil extracts and put on 0.7% agar plate, followed by incubation at 25 °C (with 75% humidity). Seed germination, plumule and radicle length, and dry biomass were measured after 5–7 days of incubation.

$$\% \text{ Germination} = \frac{\text{number of seeds germinated}}{\text{total number of seeds}}$$

The seedling vigour index (SVI) was calculated based on the percentage of seed germination.

$$\text{SVI} = (\text{root} + \text{shoot length}) \times \% \text{ germination of seed}$$

2.10. Oxidative damage induced by WWI soil under CLS microscopy

Oxidative damage in the root cells caused by WWI soil extracts were determined by CLS microscopy. *Vigna radiata* roots were germinated on 0.7% agar plates with varying concentrations of soil extract (10%, 25%, 50% and 100%) for seven days (Yadav et al., 2019). The roots were thoroughly rinsed using phosphate buffer saline (PBS) after treatment and stained with propidium iodide (PI). The roots were then fixed on slide and examined under an LSM-780 CLS Microscope (Zeiss, Germany).

2.11. Plasmid nicking assay

Damage in the plasmid DNA (pBR322) was assessed following the method of Siddiqui et al. with some modifications (Siddiqui et al., 2011). Plasmid DNA (500 ng) was treated with a series of concentration of soil extracts (both WWI and GWI soil) containing a final volume of 25 μl for 3 h at 25 °C. After incubation, agarose gel electrophoresis was done at 50 mA for 90 min. The damaged DNA bands of plasmid were visualized under Gel Doc (BIO RAD) and photographs were captured.

2.12. Statistical analysis

The one-way ANOVA (analysis of variance) with $p \leq 0.05$ was used for the comparison of total number of his^{+} revertant colonies to the control. The findings of the *A. cepa* anaphase-telophase test are presented as a % mitotic index and a percentage of aberrant cells. For seed germination assays, percentage of seed germination and radicle-plumule length are shown as average with standard deviation. DMRT (Duncan Multiple Range Test) was used to assess significance in treatment groups, positive and negative control data (SPSS software).

3. Results

3.1. Physicochemical and heavy metal analysis of soil samples

Physiochemical analysis of agricultural soils is presented in Table 1. The pH of WWI soil was found to slightly alkaline (pH 8.36), while it was 7.97 for GWI soil. Carbonate and bicarbonate were found to be 147.8 and 129.13 mg/kg in WWI soil, while 154 and 114.4 mg/kg were detected in

Table 1. Physico-chemical and heavy metal analysis of soil.

Parameters	WWI soil	GWI soil
pH	8.36 ± 0.12	7.97 ± 0.12
EC (dS/m)	1.2 ± 0.21	1.3 ± 0.25
Texture	Loamy	Loamy
Carbonate (mg/kg)	147.8 ± 10.16	154 ± 6.84
Bicarbonate	129.13 ± 7.02	114.4 ± 15.2
Organic carbon (%)	1.13 ± 0.12	0.15 ± 0.01
Chloride (mg/kg)	42.7 ± 1.03	24.7 ± 3.13
Phosphorus (mg/kg)	20.55 ± 1.4	18.71 ± 1.1
Potassium (mg/kg)	174.87 ± 15.24	95.8 ± 11.6
Sulphur (mg/kg)	12.64 ± 0.98	10.8 ± 1.1
Ni (mg/kg)	12.57 ± 0.98	1.41 ± 0.12
Cd (mg/kg)	3.33 ± 1.24	0.16 ± 0.02
Pb (mg/kg)	22.95 ± 2.67	1.29 ± 0.14
Cu (mg/kg)	17.6 ± 0.78	0.98 ± 0.19
Cr (mg/kg)	33.35 ± 1.98	2.78 ± 0.35
Zn (mg/kg)	11.86 ± 0.61	1.64 ± 0.22
Fe (mg/kg)	14.06 ± 2.95	9.12 ± 0.88
Mn (mg/kg)	15.13 ± 1.76	4.24 ± 0.55

GWI soil. The electrical conductivity (EC) of WWI and GWI soils were recorded as 1.2 and 1.3 dS/m with loamy soil texture. Total organic carbon was 1.13% in WWI soil, whereas, it was 0.15% in GWI soil. The amount of chloride (Cl^{-1}), phosphorous (P), and sulphur (S) was found to be 42.7, 20.55 and 12.64 mg/kg respectively while potassium (K) was 174.81 mg/kg in WWI soil. On the other hand, the concentration of Cl^{-1}, P, S and K in GWI soil were found to be 24.7 mg/kg, 18.71 mg/kg, 10.8 mg/kg, and 96.07 mg/kg respectively (Table 1). The soil samples were WWI with some heavy metal ions including Ni, Cd, Pb, Cu, Zn, Cr, Fe, and Mn as confirmed by AAS (Table 1).

3.2. Quantitative determination of pesticides in soil samples

Organochlorine and organophosphate pesticides were found in different concentrations in soil samples analysed by gas chromatography (GC). WWI soil contained high levels of both organochlorine and organophosphate pesticide groups. The organochlorine pesticides like α-BHC, β-BHC, lindane, heptachlor, aldrin, α-endosulfan, 4-4″ DDE, dieldrin, β-endosulfan, endrin aldehyde, endosulfan sulfate and endrin ketone in WWI soil were 31.44, 6.21, 44.26, 16.75, 9.42, 30.7, 29.45, 114.18, 60.61, 12.22, 18.59 and 14.83 μg/g respectively (Table 2), whereas dichlorvos, disulfoton, parathion-methyl, chlorpyrifos, prothiofos, and azinphos-methyl (organophosphate pesticides) were found to be 4.45, 43.53, 14.39, 4.69, 41.31 and 14.93 μg/g detected respectively.

3.3. Reversion of Ames Salmonella tester strains

Salmonella tester strains (TA97a, TA98, TA100, TA102, and TA104) were also used to evaluate the mutagenicity of soil samples. Hexane extract of WWI soil sample was found to be high mutagenic compared to DCM extract of soil samples in the terms of mutagenic parameters (mutagenic index, induction factor, and mutagenic potential) (Tables 3, 4, 5, and 6). The number of reversion colonies of *Salmonella* strains were increased with rising doses up to 20 μl/plate then decreased at 40 μl/plate. Both the solvent extracts (hexane and DCM) of WWI soil showed the significant mutagenicity with tester strains (such as TA98, TA100 and TA102) in the absence as well as in presence of S9 fraction. In hexane extracts of WWI soil, strain TA98 was most sensitive concerning of mutagenic index (13.41 and 13.46 without and with S9 fraction), induction factor (2.52 and 2.53 without and with S9 fraction) and mutagenic potential (6.37 and 7.72 without and with S9 fraction) (Table 3). The responsiveness order (based on mutagenic index and induction factor) is as follow:

(TA98 > TA97a > TA100 > TA102 > TA104)

Table 2. Concentration of pesticides in WWI and GWI soil samples determined by Gas Chromatography.

Organochlorine (OC)	Concentration in WWI soil (μg/g)	Concentration in GWI soil (μg/g)	Organophosphate (OP)	Concentration (μg/g) WWI soil (μg/g)	Concentration in GWI soil (μg/g)
4-4″ DDD	ND	0.32 ± 0.06	Azinphos-methyl	14.93 ± 1.61	3.95 ± 0.3
4-4″ DDE	29.45 ± 5.51	ND	Chlorpyrifos	4.69 ± 1.11	ND
4-4″ DDT	ND	ND	Dichlorvos	4.45 ± 0.81	1.51 ± 0.25
Aldrin	9.42 ± 1.95	ND	Disulfoton	43.53 ± 1.5	1.24 ± 0.07
Dieldrin	114.18 ± 12.08	20.59 ± 3.6	Ethoprophos	ND	ND
Endosulfan sulfate	18.59 ± 1.76	0.49 ± 0.05	Fenchlorphos	ND	ND
Endrin	ND	ND	Malathion	ND	ND
Endrin aldehyde	12.22	ND	Parathion-methyl	14.39 ± 4.48	ND
Endrin Ketone	14.83 ± 2.90	ND	Prothiofos	41.31 ± 9.89	7.55 ± 1.05
Heptachlor	16.75 ± 3.26	0.22 ± 0.05			
Heptachlor epoxide	ND	ND			
Lindane	44.26 ± 6.56	1.3 ± 0.2			
Methoxychlor	ND	ND			
α-BHC	31.44 ± 6.80	0.55 ± 0.1			
α-Chlordane	ND	ND			
α-Endosulfan	30.7 ± 7.20	0.18 ± 0.02			
β-BHC	6.21 ± 1.01	0.34 ± 0.05			
β-Endosulfan	60.61 ± 13.06	0.35 ± 0.04			
γ-Chlordane	ND	2.2 ± 0.3			
σ-BHC	ND	ND			

ND = Not detected.

Table 3. Reversion of *Salmonella* tester strains in the presence of hexane extract of WWI soil.

Soil extract (µl/plate)											F value
Strain	S9	Control	2.5	5	10	20	40	Mi	m	LSD ($p \leq 0.05$)	
TA97a	–	87 ± 9	113 ± 11 (1.3)	162 ± 15 (1.86)	254 ± 18 (2.93)	359 ± 15 (4.15)	263 ± 20 (3.03)	1.14	4.73	26.3	90.5
	+	93 ± 7	129 ± 10 (1.39)	174 ± 14 (1.87)	277 ± 16 (2.97)	388 ± 22 (4.17)	288 ± 17 (3.10)	1.15	5.15	11.3	110.4
TA98	–	32 ± 5	129 ± 14 (4.03)	225 ± 19 (7.02)	346 ± 18 (10.80)	429 ± 23 (13.41)	321 ± 19 (10.04)	2.52	6.37	24.3	145.9
	+	37 ± 7	154 ± 20 (4.16)	267 ± 23 (7.21)	403 ± 21 (10.90)	498 ± 17 (13.46)	390 ± 14 (10.54)	2.53	7.72	13.3	184.9
TA100	–	133 ± 19	234 ± 16 (1.75)	370 ± 22 (2.78)	441 ± 20 (3.31)	502 ± 24 (3.77)	404 ± 26 (3.03)	1.02	5.40	33.6	83.3
	+	147 ± 17	261 ± 19 (1.78)	411 ± 21 (2.79)	489 ± 22 (3.32)	556 ± 23 (3.78)	451 ± 20 (3.07)	1.02	6.07	37.6	113.4
TA102	–	213 ± 13	319 ± 16 (1.49)	443 ± 20 (2.07)	507 ± 17 (2.38)	569 ± 22 (2.67)	468 ± 21 (2.19)	0.51	5.01	25.6	101.1
	+	227 ± 19	341 ± 20 (1.50)	472 ± 23 (2.07)	543 ± 26 (2.39)	611 ± 25 (2.69)	507 ± 27 (2.23)	0.52	5.50	35.3	71.3
TA104	–	301 ± 14	348 ± 18 (1.16)	402 ± 20 (1.33)	498 ± 23 (1.65)	577 ± 20 (1.97)	501 ± 21 (1.66)	−0.08	4.86	2.67	57.6
	+	316 ± 18	379 ± 20 (1.20)	452 ± 18 (1.43)	528 ± 16 (1.67)	610 ± 18 (1.93)	544 ± 21 (1.72)	−0.07	5.20	15.7	69.4

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

Table 4. Reversion of *Salmonella* tester strains in the presence of hexane extract of GWI soil.

Soil extract (µl/plate)											F value
Strain	S9	Control	2.5	5	10	20	40	Mi	m	LSD ($p \leq 0.05$)	
TA97a	–	91 ± 8	108 ± 13 (1.19)	125 ± 19 (1.38)	152 ± 21 (1.38)	183 ± 23 (2.01)	170 ± 20 (1.87)	0.02	1.92	17.3	8.34
	+	96 ± 10	124 ± 15 (1.28)	135 ± 16 (1.41)	161 ± 13 (1.68)	200 ± 18 (2.08)	185 ± 15 (1.93)	0.08	2.10	11.6	14.1
TA98	–	32 ± 7	46 ± 12 (1.44)	56 ± 11 (1.77)	71 ± 15 (2.25)	96 ± 15 (3.03)	80 ± 14 (2.53)	0.71	1.16	9.0	7.18
	+	39 ± 8	59 ± 10 (1.52)	72 ± 16 (1.84)	98 ± 11 (2.51)	120 ± 14 (3.07)	103 ± 13 (2.63)	0.73	1.46	4.7	12.3
TA100	–	122 ± 11	142 ± 14 (1.16)	171 ± 16 (1.40)	202 ± 19 (1.65)	234 ± 20 (1.92)	190 ± 25 (1.56)	−0.08	1.60	12.0	10.3
	+	137 ± 15	174 ± 16 (1.27)	214 ± 13 (1.56)	244 ± 19 (1.78)	265 ± 24 (1.93)	231 ± 19 (1.68)	−0.07	1.90	12.7	13.8
TA102	–	215 ± 16	240 ± 19 (1.12)	260 ± 15 (1.21)	279 ± 19 (1.29)	307 ± 13 (1.42)	291 ± 20 (1.35)	−0.86	1.67	12.0	7.87
	+	228 ± 17	255 ± 16 (1.12)	285 ± 15 (1.25)	309 ± 19 (1.36)	342 ± 14 (1.49)	319 ± 18 (1.40)	−0.69	2.03	9.7	13.1
TA104	–	300 ± 16	321 ± 21 (1.06)	347 ± 20 (1.55)	369 ± 14 (1.23)	419 ± 14 (1.39)	401 ± 19 (1.33)	−0.93	2.46	18.0	13.8
	+	317 ± 16	341 ± 16 (1.07)	368 ± 20 (1.16)	395 ± 19 (1.24)	444 ± 18 (1.40)	423 ± 22 (1.33)	−0.92	2.54	21.0	15.6

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

Table 5. Reversion of *Salmonella* tester strains in the presence of DCM extract of WWI soil.

Soil extract (µl/plate)											F value
Strain	S9	Control	2.5	5	10	20	40	Mi	m	LSD ($p \leq 0.05$)	
TA97a	–	90 ± 11	102 ± 16 (1.13)	152 ± 19 (1.68)	243 ± 17 (2.70)	338 ± 15 (3.75)	247 ± 24 (2.74)	1.01	4.36	3.3	59.6
	+	97 ± 15	112 ± 18 (1.15)	167 ± 17 (1.72)	263 ± 17 (2.71)	365 ± 21 (3.76)	270 ± 18 (2.78)	1.02	4.76	6.7	68.8
TA98	–	32 ± 6	101 ± 14 (3.15)	189 ± 19 (5.91)	269 ± 17 (8.41)	353 ± 19 (11.03)	248 ± 20 (7.76)	2.30	4.84	20.7	98.8
	+	37 ± 6	118 ± 16 (3.18)	219 ± 16 (5.93)	311 ± 21 (8.48)	411 ± 23 (11.11)	288 ± 19 (7.77)	2.31	5.62	23.6	118.8
TA100	–	133 ± 13	236 ± 17 (1.77)	345 ± 14 (2.58)	402 ± 19 (3.01)	500 ± 23 (3.75)	408 ± 22 (3.05)	1.01	5.74	6.0	104.6
	+	146 ± 16	259 ± 20 (1.77)	378 ± 23 (2.59)	441 ± 23 (3.02)	549 ± 26 (3.76)	447 ± 24 (3.06)	1.01	6.30	5.7	86.5
TA102	–	221 ± 18	303 ± 17 (1.37)	369 ± 19 (1.67)	450 ± 24 (2.03)	523 ± 26 (2.37)	427 ± 17 (1.93)	0.31	4.44	22.3	56.6
	+	244 ± 15	337 ± 19 (1.38)	414 ± 23 (1.70)	513 ± 20 (2.10)	579 ± 24 (2.37)	493 ± 22 (2.02)	0.32	5.32	20.0	72.0
TA104	–	297 ± 16	343 ± 19 (1.16)	410 ± 17 (1.38)	505 ± 27 (1.70)	564 ± 24 (1.90)	486 ± 25 (1.63)	−0.11	4.45	19.0	44.1
	+	316 ± 16	371 ± 20 (1.17)	440 ± 23 (1.39)	539 ± 24 (1.71)	601 ± 25 (1.90)	518 ± 27 (1.64)	−0.10	4.68	21.0	45.1

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

Whereas following trends was found in terms of mutagenic potential/slope:

TA98 > TA100 > TA102 > TA97a > TA104

DCM extract of WWI soil, strain TA98 was also found maximum response with mutagenic index (11.03 and 11.11 without and with S9 fraction), induction factor (2.30 and 2.31 without and with S9 fraction), whereas TA100 displayed highest mutagenic potential (5.74 without and 6.30 with S9 fraction) (Table 5). The responsiveness order (on the basis of mutagenic index along with induction factor) are as follow:

(TA98 > TA97a > TA100 > TA102 > TA104)

However, different trends were found in terms of mutagenic potential/slope:

TA100 > TA98 > TA102 > TA97a > TA104

Table 6. Reversion of *Salmonella* tester strains in the presence of DCM extract of GWI soil.

Soil extract (µl/plate)											F value
Strain	S9	Control	2.5	5	10	20	40	Mi	m	LSD ($p \leq 0.05$)	
TA97a	–	91 ± 12	107 ± 14 (1.17)	139 ± 12 (1.53)	148 ± 13 (1.62)	169 ± 18 (1.86)	154 ± 16 (1.70)	−0.14	1.38	6.7	8.8
	+	98 ± 9	126 ± 12 (1.28)	151 ± 18 (1.54)	170 ± 12 (1.73)	210 ± 18 (2.14)	175 ± 14 (1.78)	0.13	1.74	5.7	14.8
TA98	–	31 ± 4	42 ± 16 (1.35)	54 ± 8 (1.71)	66 ± 9 (2.09)	102 ± 11 (3.24)	81 ± 7 (2.57)	0.81	1.27	11.3	21.9
	+	37 ± 8	55 ± 11 (1.47)	69 ± 8 (1.86)	93 ± 10 (2.50)	125 ± 15 (3.39)	101 ± 13 (2.72)	0.87	1.55	8.0	17.1
TA100	–	126 ± 11	138 ± 16 (1.10)	152 ± 18 (1.21)	177 ± 15 (1.41)	217 ± 18 (1.72)	196 ± 17 (1.56)	−0.32	1.83	12.0	9.6
	+	139 ± 18	161 ± 16 (1.15)	177 ± 15 (1.27)	211 ± 19 (1.51)	242 ± 22 (1.73)	224 ± 14 (1.61)	−0.30	2.03	10.1	15.3
TA102	–	231 ± 16	257 ± 20 (1.11)	284 ± 15 (1.22)	311 ± 21 (1.34)	332 ± 23 (1.43)	315 ± 16 (1.36)	−0.83	1.83	4.3	8.4
	+	254 ± 20	285 ± 23 (1.12)	313 ± 24 (1.23)	344 ± 17 (1.35)	365 ± 26 (1.43)	347 ± 19 (1.37)	−0.83	1.99	3.6	7.5
TA104	–	326 ± 18	345 ± 20 (1.05)	368 ± 22 (1.12)	382 ± 24 (1.17)	415 ± 22 (1.27)	390 ± 16 (1.20)	−1.29	1.49	8.0	4.9
	+	347 ± 18	369 ± 21 (1.06)	392 ± 19 (1.13)	427 ± 22 (1.23)	448 ± 25 (1.29)	419 ± 26 (1.21)	−1.23	1.63	8.3	6.0

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

The response of all *Salmonella* strains was significantly greater when treated with WWI soil extracts in comparison to GWI soil extracts (Tables 4 and 6). As expected, the GWI soil extracts of hexane (GWI) displayed low mutagenic response with TA98 strains. The mutagenic index were 3.07 with S9 and 3.03 without S9 fraction and induction factor were found to be 0.73 and 0.71 (with and without S9 fraction) (Table 4). Whereas the mutagenic potential was found to be 1.46 and 1.16 (with S9 and without S9) (Table 4).

Salmonella tester strains exhibited modest levels of mutagenicity when treated with a DCM extract of GWI soil (Table 6). Strain TA98 showed maximum responsive regarding mutagenic index (3.24 and 3.39 without as well as with S9 fraction) and induction factor (0.81 without and 0.87 with S9 fraction), while strain TA100 was most sensitive concerning mutagenic potential (1.83 and 2.03 without and with S9 fraction) (Table 6).

3.4. Survival of *E. coli* K-12 strains treated with test samples

E. coli K-12 DNA repair defective mutants (*recA*, *lexA* and *polA*) and isogenic wild type counterparts is shown in Figure 1 when treated with soil extracts (at dose of 20 µl/ml of culture). The tester strains displayed reduction in CFUs after treatment with hexane extract of WWI soil and survival percentage was 39% in *polA*, 47% in *lexA* and 55% in *recA E. coli* K-12 mutants after 6 h (Figure 1a). DCM extract of WWI soil exhibited survival of 25% in *polA*, 39% in *lexA* and 46% in *recA* mutants after 6 h of treatment (at dose of 20 µl/ml of culture) under similar experimental condition (Figure 1c). Moreover, the mutant strains were also treated with GWI soil (both DCM and hexane) and the survival was observed to be 56%, 64% and 78% in *polA*, *lexA* and *recA* mutants respectively with hexane extract (Fig. 1b), and 51% in *polA*, 59% in *lexA* and 77% in *recA* mutants with DCM extract under similar conditions (Figure 1d).

3.5. Allium cepa chromosomal aberration test

The effect of aqueous soil extracts on the mitotic index (MI) of root meristematic cells of *A. cepa* was more affected in dose dependent manner (5–100%). With increasing concentration of soil extracts, MI was significantly reduced, and MI was more in WWI soil than GWI. MI was observed to be 9.1% at 100% concentration for WWI soil, whereas it was 22.6% at 100% concentration for GWI soil. Mitotic index of GWI soil

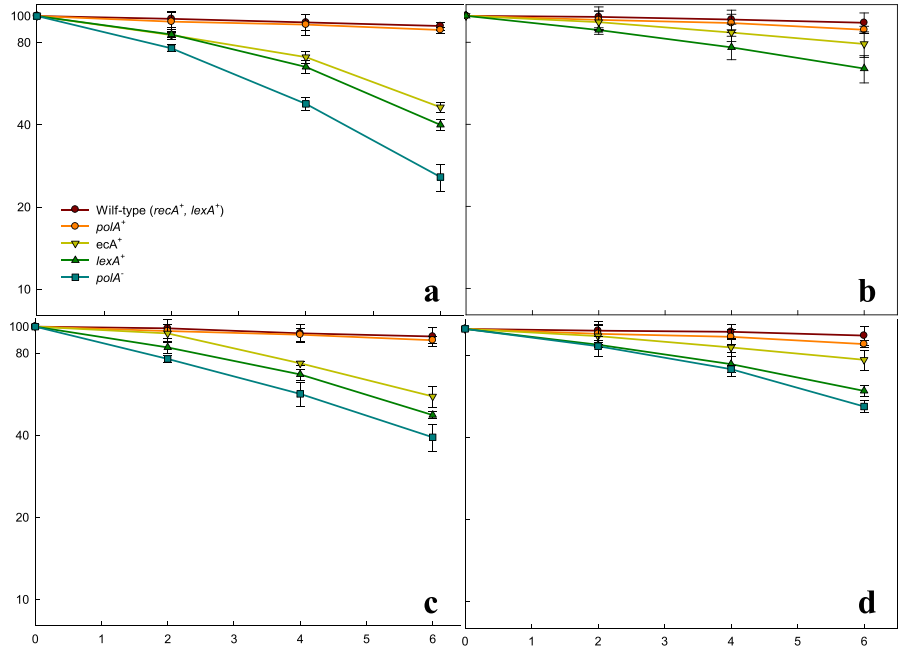

Figure 1. Survival of *E. coli* K-12 strains treated with hexane extracted WWI soil extract (a); hexane extracted GWI soil extract (b); DCM extracted WWI soil extract (c), DCM extracted GWI soil extract (d).

Table 7. Effect of different concentrations of soil extract on mitotic index and mitotic phase of *Allium cepa* root meristematic cells.

Samples	Concentration (% v/v)	Mitotic Phases (%)			Mitotic index (%±SD)
		Prophase	Metaphase	Anaphase-Telophase	
WWI soil	5	49.66	19.95	30.39	22.60 ± 0.89^{cd}
	10	53.3	19.18	27.52	19.45 ± 1.14^{de}
	25	54.87	19.71	25.42	16.53 ± 0.9^{ef}
	50	58.56	17.14	24.3	10.7 ± 0.32^{g}
	100	60.58	19.97	19.45	9.1 ± 0.72^{g}
GWI soil	5	42.97	23.84	33.19	31.9 ± 2.14^{a}
	10	43.29	25.60	29.23	29.23 ± 0.52^{ab}
	25	43.45	26.41	30.14	26.4 ± 3.15^{bc}
	50	50.12	23.12	26.76	21.63 ± 3.44^{cd}
	100	58.84	17.50	23.66	14.93 ± 0.61^{f}
Positive control		61.44	18.53	20.03	9.1 ± 0.73^{g}
Negative control		48.81	20.22	30.97	31.47 ± 2.61^{a}

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

extract (31.9% at 5% soil extract) was comparable to that MI of negative control (31.47%) (Table 7).

Statistically significant ($p < 0.05$) frequencies of chromosomal abnormalities and % aberrant cells were observed with soil extracts of WWI soil compared to GWI soil (Table 8). Chromosomal aberrations and % aberrant cells were increased from 8.44% to 39.95% with increasing extract concentrations of WWI soil (5–100%). The soil samples caused different forms of aberrations in the root tips, such as breakage, anaphase spindle disturbance, disturbance at metaphase, C-mitosis, stickiness, multipolar anaphase with chromosomal break, anaphase bridge with vagrant chromosome and uneven proportions of chromosomes at anaphase stage (Figure 2). Therefore, the WWI soil sample displayed greater frequency of aberrations (39.95%) as compared to GWI soil (17.81%) at 100% concentration for WWI and GWI soil extracts (Table 8).

3.6. *In vitro* toxicity of soil extracts to *Vigna radiata*

Toxicity of WWI soil and GWI soil were also assessed by *V. radiata* seed germination test. The germination rate of mung bean seeds and other plant parameters were found to be influenced and reduced when treated with different doses of WWI soil extract (Figure 3). At 100% dose of WWI soil extract, % seed germination, seedling vigour index (SVI), plumule length (PL), radicle length (RL), dry biomass of plumule (DBP) and dry biomass of radicle (DBR) were found to be 52.22%, 698 SVI, 7.8 cm, 5.53 cm, 0.19 g and 0.09 g, respectively (Figure 4). The damage to root tip cells caused by pollutants in soil extracts were observed and easily visible under a fluorescent microscope using propidium iodide to produce red fluorescence. The fluorescence intensity increased as the concentration of WWI soil extract increased (Figure 5).

3.7. Plasmid nicking assay

DNA band pattern of plasmid nicking test with different doses of WWI soil extract is shown in Figure 6. Different concentration (5, 10, 15 and 20 µl) of WWI soil extract were used to analyse the effect in partial transformation of pBR322 plasmid DNA from supercoiled state to the open circular (Figure 6, lane b-e). The test samples also caused the conversion of supercoiled pBR322 DNA into linear (Figure 6, lane b-e). Highest loss of pBR322 plasmid (supercoiled form) was observed in 20 µl of soil extract. The band intensity of pBR322 DNA (open circular form) was increased and supercoiled form was decreased in dose dependent manner. Positive control (MMS) caused in the compete loss of supercoiled pBR322 plasmid DNA (Figure 6; lane f).

4. Discussion

Environmental pollution caused by growing urbanisation and industrialisation is a big problem across the world (Peña et al., 2020; Shahid and Khan, 2019). Toxic pollutants in industrial effluents, as a result, have a detrimental effect on the surrounding soil and water bodies (Bielen et al., 2017; Libutti et al., 2018). Among all sources, the agrochemical sector is a major contributor to environmental pollution, owing to its large consumption of freshwater and discharge of wastewater at the end of an industrial operation (Khan et al., 2022; Rasmussen et al., 2015; Woodrow et al., 2018). In agricultural soils, pesticides and heavy

Table 8. Chromosomal aberrations in the root meristematic cells of *Allium cepa* exposed to different concentrations of soil extract for 72 h.

Sample	Concentration (% v/v)	Types of aberrations									Total aberrant cells (%±SD)
		VC	CM	LC	MA	DM	SC	BN	AB	DAT	
WWI soil	5	–	1	2	–	–	2	–	1	2	8.44 ± 1.3^{f}
	10	3	4	2	1	3	2	–	–	1	12.82 ± 1.55^{e}
	25	3	5	5	1	4	2	–	3	4	21.51 ± 2.74^{d}
	50	8	4	7	4	4	2	2	3	5	34.73 ± 3.27^{bc}
	100	12	9	5	7	8	2	3	9	8	39.95 ± 5.34^{b}
GWI soil	5	2	1	2	–	1	–	–	–	1	4.99 ± 0.36^{h}
	10	1	2	2	–	2	–	–	–	2	6.83 ± 0.72^{fg}
	25	2	3	1	1	2	1	–	1	3	10.45 ± 1.2^{ef}
	50	2	1	2	1	3	1	–	2	4	13.91 ± 2.27^{e}
	100	4	4	3	2	2	2	–	2	3	17.81 ± 2.02^{de}
Positive control		14	9	11	6	7	13	5	10	17	44.45 ± 4.49^{a}
Negative control		1	1	1	–	2	–	–	1	1	4.72 ± 0.87^{h}

VC: vagrant chromosome CM: C-mitosis, LC: laggard chromosome, MA: multipolar anaphase, DM: disturbed metaphase, SC: sticky chromosome, BN: bi-nucleated cell, AB: anaphase bridge, DAT: disturbed anaphase-telophase,; Means with the same letters do not significantly differ at 0.05 level (Duncan multiple range test); ±: Standard deviation.

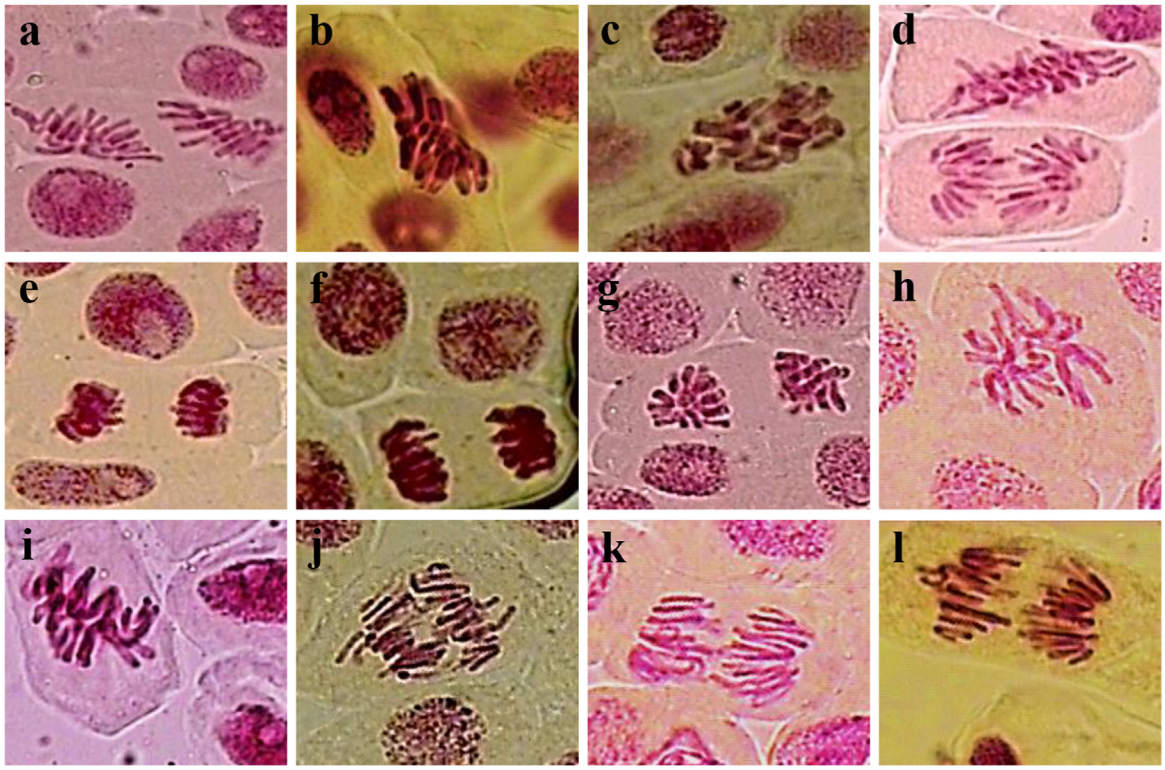

Figure 2. Different types of chromosomal aberrations induced by the soil extracts in root tips of *Allium cepa*: Anaphase spindle disturbance (a); C-Mitosis (b, c); Multipolar anaphase with chromosomal break (d); Stickiness in telophase (e, f); Anaphase with break and star shape chromosome (g); Disturbance at metaphase (h, i); Anaphase bridge with vagrant chromosome (j, k); Multipolar anaphase with chromosomal break (l).

elements are serious contaminants. Various studies believe that this combination poses a toxicological risk to both the environment and human health (Gallego and Olivero-Verbel, 2021).

In the present study, the concentration of Ni, Cd, Pb, Cu, Cr, Zn, Fe and Mn in WWI soil were 12.57, 3.33, 22.95, 17.6, 33.35, 11.86, 14.06 and 15.13 mg/kg (Table 1). One of the primary problems with food security may be high levels of trace elements in soils. Several authors have also reported significantly higher concentration of heavy metal ions in WWI soil (Barakat et al., 2020; Chaoua et al., 2019; Shao et al., 2015; Woldetsadik et al., 2017). Chaoua et al. reported high concentration of Zn, Cu, Pb, and Cd as 112.71 mg/kg, 17.70 mg/kg, 57.36 mg/kg and 11.22 mg/kg in WWI soils in Marrakech region (Morocco) (Chaoua et al., 2019). Trace elements in agricultural soils poses major safety concerns for long-term environmental sustainability and has an impact on the human health (Jolly et al., 2013; Sharma and Nagpal, 2020). However, if heavy metals are present even below the appropriate limits, there is always a high possibility that the metal concentration will be bioaccumulated and biomagnified over the course of time, eventually producing significant health consequences. Moreover, irrigation of soils with wastewater contributes to the deposition of toxic metals in agriculturally important and other crops, which eventually causes toxicity to humans and grazing animals via food chain (Rezapour et al., 2019a; 2019b).

Organochlorine and organophosphorus group of pesticides are extensively used to combat crop pests and vector-borne diseases. Therefore, these two groups have been considered a key environmental concern due to its persistency and subsequent harmfulness (Samare et al., 2020; Zhou et al., 2021). Due to their deleterious effect on the environment and members of the food chain or food web, many pesticides have been prohibited or banned to use in many country including India (Lan et al., 2019; Sah et al., 2020). The concentration of organochlorine and organophosphate pesticides of WWI soils are shown in Table 2. Anjum and Krakat reported that soil irrigated with pesticide industry wastewater comprises a high level of organochlorine pesticides such as lindane, α-endosulfan and β-endosulfan and were found to be 547, 422 and 421 ng/g, respectively and also found high level of toxic metals (like Cr, Zn, Ni, Fe, Cu and Cd were 36.2, 42.5, 241, 13.2 and 11.2 μg/g of soil respectively) (Anjum and Krakat, 2015). Fang et al. reported α and β isomers endosulfan, and endosulfan sulfate from the soil at endosulfan manufacturing site, Jiangsu, China (Fang et al., 2016). They found in the range of 0.01 mg/kg to 114 mg/kg of soil at production site whereas in the surrounding area of manufacturing site, the concentration of endosulfan were detected in the range of 1.37–415 ng/g of soil. We also found α-endosulfan (0.18 μg/g), β-endosulfan (0.35 μg/g) and endosulfan sulfate (0.49 μg/g) in contaminated soil (Table 2). A mixture of pesticides using GC-MS/MS and trace elements via ICP-MS (Cr, Cu, Ni and Se) were also detected in the soil used for conventional and organic crops of *Allium cepa* (Gallego and Olivero-Verbel, 2021). Many other authors reported different pesticides in various environmental samples (Devault and Karolak, 2020; Kathavarayan et al., 2019; Marsin et al., 2020; Muckoya et al., 2020).

Numerous pesticides and metal ions were found in the test soils during analysis. Since a polluted site may include a high number of dangerous substances that might interfere with efficient chemical analysis, it is challenging to determine the toxicity of different soil samples (Haleyur et al., 2016). Moreover, bioassay-directed chemical tests can consequently be used to identify the putative mutagens by integrating the effects of all components of the mixture, whether or not they are known and identified (Kirkland et al., 2008, 2016; Man et al., 2013). In the current study, WWI soil showed considerable mutagenicity with increase in revertant colonies as dose increased. The results indicated that strain TA98 was the most sensitive, followed by TA97a and TA100 with test soil extracts (Tables 3, 4, 5, and 6), thus demonstrating the presence of frame shift (TA98 and TA97a) and base pair substitution (TA100) mutagens in test soil. However, all tester strains displayed high response when treated with WWI soil in comparison to GWI soil, thus indicating accumulation of

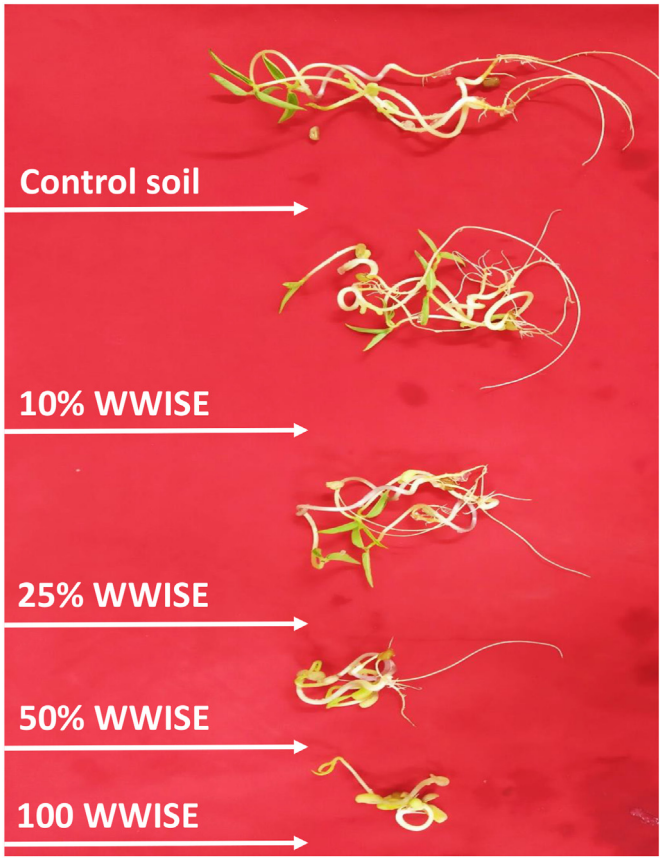

Figure 3. Effect of different concentration of wastewater irrigated soil extract (WWISE) on radicle and plumule length of mung bean seeds germination in soft agar plates (0.7%).

toxic pollutants in the WWI soil. Many researchers have also used the *Salmonella* mutagenicity test to assess the genotoxicity of WWI soil (de Souza Pohren et al., 2019; Lah et al., 2008; Man et al., 2013; Okunola et al., 2016). Monarca et al. demonstrated genotoxicity of contaminated soil collected from industrial area in the Lombardy region (Northern Italy) using Ames mutagenicity and *Tradescantia*/micronucleus test (Monarca et al., 2002). They observed that the polluted soil showed mutagenic activity with the TA98 and TA100 strains and clastogenicity with the *Tradescantia*/micronucleus assay. Watanabe et al. taken soil samples from 12 different sites at Kyoto district (Japan) and tested their soil extracts using Ames *Salmonella* and found that 90% samples showed mutagenicity in strain TA98 (without and with S9 fraction) (Watanabe et al., 2008). As a result, we can state that TA98 is the most sensitive Ames tester strain.

Katnoria et al. revealed mutagenic activity of industrial waste contaminated soil by TA100 and reported dose-dependent increase of revertant colonies per plate (Katnoria et al., 2011). In the present study, maximum mutagenic index and mutagenic potential was found to be 13.46 and 7.72 with S9 mix, respectively. Man et al. demonstrated significant soil mutagenicity of 12 different lands conducted by *S. typhimurium* strains and found mutagenic potential of 13.8 and 7.43 in the TA98 strain (Man et al., 2013). Masood and Malik reported mutagenic activity in *S. typhimurium* strains TA98, TA97a and TA100 and found to be the most responsive while treated with different organic solvent extracts of tannery WWI soil, Kanpur (India) (Masood and Malik, 2013). Okunola et al. also tested mutagenicity of soil contaminated with automobile industrial waste and demonstrated an increase revertant colonies in TA98 and TA100 strains (Okunola et al., 2016).

In current study, DNA repair defective mutants (*recA*, *lexA* and *polA*) were highly sensitive to both hexane and DCM extracts of the WWI soil (Figure 1). The test also indicated the existence of DNA-damaging chemicals in WWI soil and demonstrated the role of genes (*recA*, *lexA* and *polA*) in combating the harmful effects of these contaminants. Many workers have reported the role of these genes in SOS repair in *E. coli* K-12 strains (Serment-Guerrero et al., 2020; Siddiqui et al., 2011; Zhang et al., 2010). Anjum and Krakat evaluated the genotoxic effect of soil irrigated with pesticide industry wastewater using *E. coli* K-12 mutants (DNA repair defective) assay and lambda bacteriophage system and reported decline in CFUs when treated with soil extracts (Anjum and Krakat, 2015). Masood and Malik reported the genotoxic potential of WWI soil by mammalian microsomal assay and found substantial decline in the survival of *E. coli* K-12 DNA repair defective mutant as compared to its

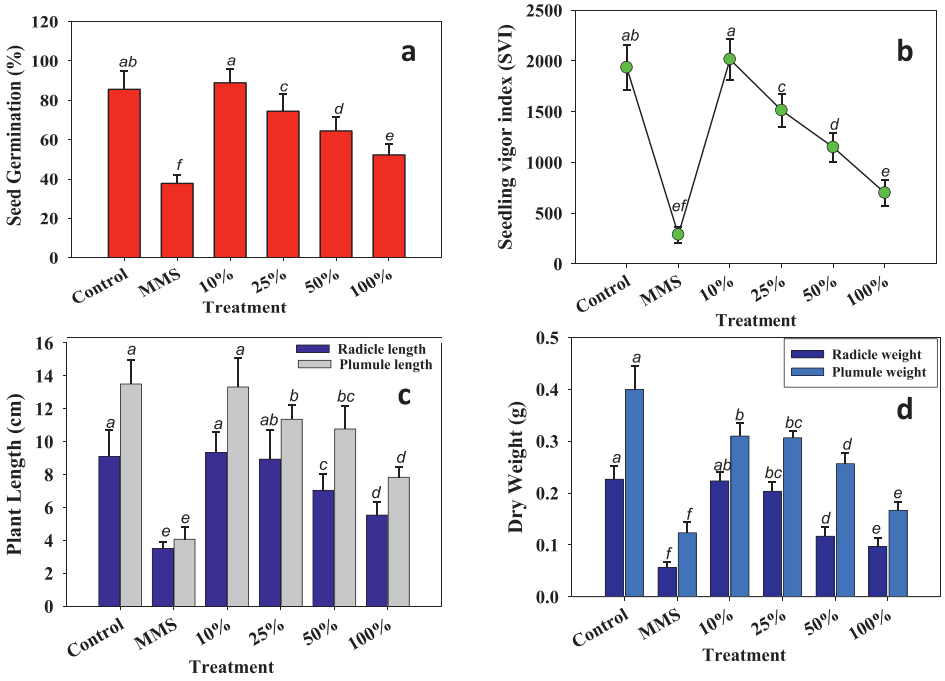

Figure 4. Plant parameters of *Vigna radiata* (mung bean) seeds germinated on soft agar plates treated with different (10, 25, 50 and 100%) concentrations of WWI soil extract; % germination (a), seedling vigor index (SVI) (b), radicle and plumule length (cm) (c) and dry biomass (d). Each value is a mean of five independent replicates (n = 5) where each replicate constituted five seeds/plates. Mean values followed by different letters are significantly different at $p \leq 0.05$ according to DMRT test. Vertical bars represent means ± SD.

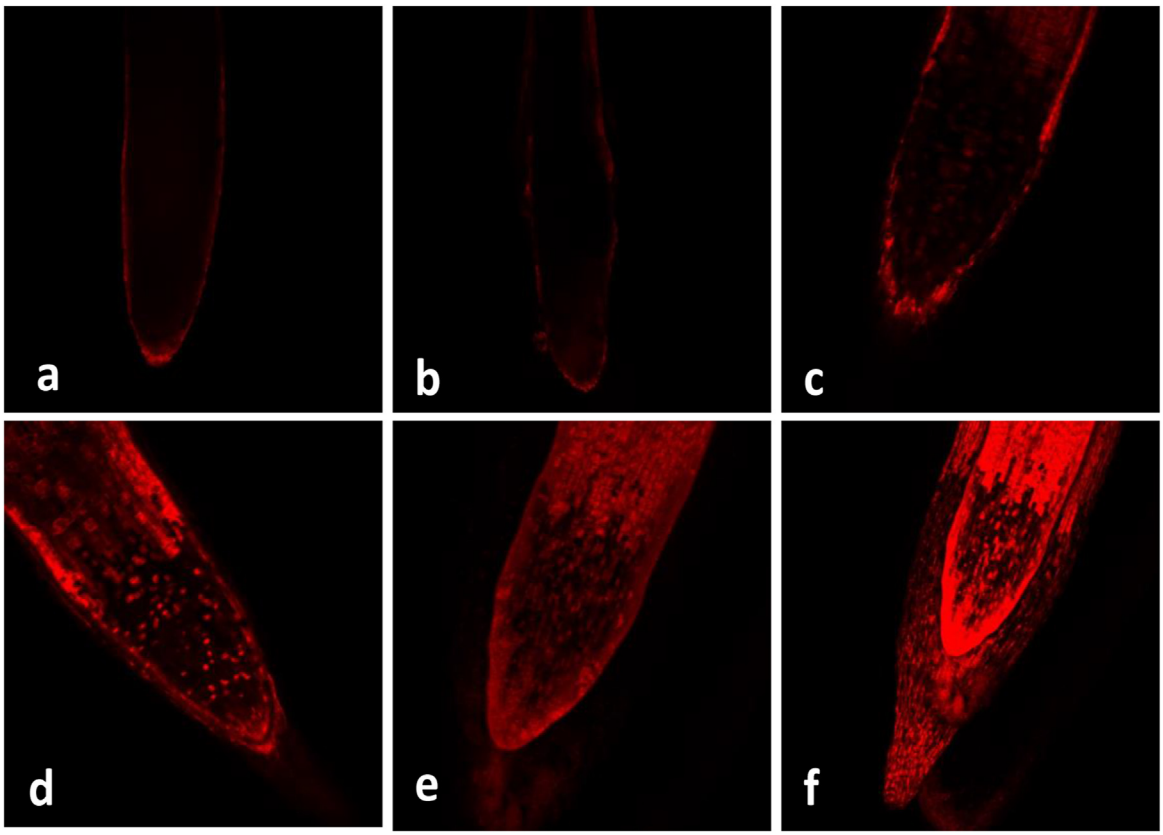

Figure 5. Confocal laser scanning microscopic (CLSM) images of *Vigna radiata* roots stained with propidium iodide (PI) and treated with GWI soil (a), and different concentration of WWI soil (b) 10%, (c) 25% (d) 50% (e) 100% and positive control (MMS) (f).

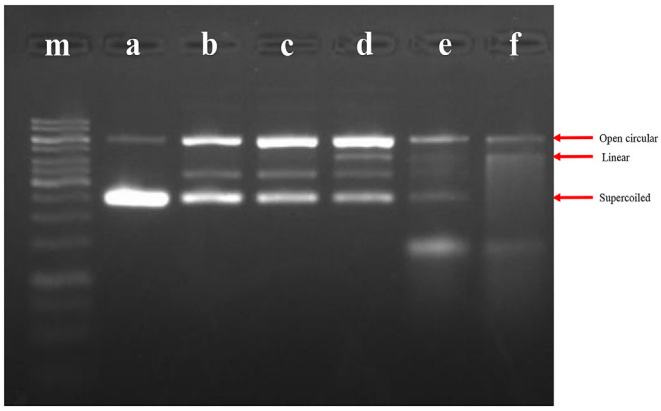

Figure 6. Effect of WWI soil extract on pBR322 DNA (Plasmid-nicking assay). Lane m: 1 kb ladder; Lane a: pBR322 DNA alone. Lane b–e: pBR322 DNA +5 μl; 10 μl; 15μl; 20μl of soil extract respectively. Lane f: pBR322 DNA + MMS.

isogenic wild-type counterparts while treating with soil extracts (Masood and Malik, 2013). Khan et al. evaluated genotoxicity of textile industry wastewater using Ames *Salmonella*/microsome test and *E. coli* K-12 DNA repair defective mutant assay and they found that TA98 strain was most responsive and maximum cell damage was observed in *pol*A mutants (Khan et al., 2019).

Allium cepa chromosomal aberration test is a popular tool for environmental monitoring and is regarded as an effective way to assess soil and water pollution (Gupta et al., 2020; Leme and Marin-Morales, 2008; Yadav et al., 2019). Root tips of *A. cepa* treated with the WWI soil showed decline of mitotic index (Table 7). This decrease might be attributed to the fact that wastewater irrigation resulted in a significant number of cytotoxic compounds being present in the test soil extract, which prevented the cell proliferation of *A. cepa* root tip cells. Nefic et al. demonstrated that the decrease in mitotic index could reflect the presence of cytotoxic chemicals in any environmental sample (Nefic et al., 2013). Dutta et al. used *A. cepa* assay for the genotoxicity and cytotoxicity of soil treated with pesticides and vermicompost and reported MI 25.4% in vermicompost treated soil and 9.7% in pesticide treated soil after 48 h (Dutta et al., 2018).

Our results of *A. cepa* chromosomal aberration assay showed different types of aberrant cells when treated with soil extracts (Table 8, Figure 2). Sabeen et al. reported chromosomal aberrations in some vegetables irrigated with industrial wastewater (Sabeen et al., 2020). In the present study, the percentage of aberrant cells increased with increasing concentration of WWI soil extract and 39.95% cells were aberrant at 100% (v/v) of soil extract (Table 8). Dutta et al. reported gradual increase in chromosomal aberrations as the dose of industrial effluents increased from 25 to 100% (v/v) after 3 days of exposure to *A. cepa* root tips (Dutta et al., 2018). These aberrations were the results of the cumulative effect of being aneugenic and clastogenic actions of the contaminants present in the wastewater. Soodan et al. have also found different types of physiological and clastogenic aberrations in *A. cepa* roots exposed to agricultural soil contaminated with hazardous pollutants (Soodan et al., 2014). Moreover, when the root tips were treated with GWI soil extract, the frequency of chromosomal abnormalities was reduced (Table 8). In a similar study, diverse forms of chromosomal aberrations viz., micronucleus, C-mitosis and anaphase bridges etc. in roots of *A. cepa* developed in soil extract obtained from the pesticide contaminated sites (Cherednichenko et al., 2020). Bianchi et al. observed that the combination of the insecticide imidacloprid with the herbicide sulfentrazone poses a danger to organisms due to the possibility for chromosomal damage and a delay in cell cycle development (Bianchi et al., 2016).

Environmental toxicity evaluation of WWI soil extracts using plant seed germination test is perhaps one of the easiest short-term test (Haq et al., 2016a). The seed germination involves sufficient amount of water for proper growth and development (Gonzaga et al., 2020). Pesticide industry wastewater contains a huge amount of organic and inorganic pollutants, resulting in the accumulation of harmful chemicals in irrigated soil (Zhang et al., 2013). In this study, seed germination rate, SVI, RL, PL, DBR and DBP was inhibited when mung bean seeds treated with WWI soil extract (Figure 4). Haq et al. reported significant decrease in seed germination, root length, shoot length and dry biomass in *V. radiata* and *A. cepa* after treated with different concentration of pulp and paper mill effluent at Saharanpur, UP (India) (Haq et al., 2016b). However, they reported that the above parameters were not affected when treated with GWI soil extract. Yadav et al. also reported reduction in seed germination and radicle-plumule length when treated with leather industry effluent at Unnao, UP (India) (Yadav et al., 2019).

Breakage in plasmid DNA is also one of the critical parameter in determining the genotoxicity of wide range of chemicals and this test is very commonly used in environmental monitoring (Gupta et al., 2015; Kaur et al., 2019; Kour et al., 2020; Rocha et al., 2009). Incidence of DNA damage is profound indicator of potential risk of malignancy in human being (Bonassi et al., 2005). In the present study, plasmid nicking assay showed transformation of supercoiled DNA (pBR322 plasmid) to open circular and linear form after treating with WWI soil extract (Figure 6). Whenever any mutagens (such as chemicals, free radicles) interfere with supercoiled form of plasmid DNA, single or double DNA strands are damaged. Gupta et al. reported the genotoxicity of oil refinery waste on plasmid pBR322 and found conversion of the supercoiled form into open circle (Gupta et al., 2015). Kaur et al. also observed conversion of supercoiled plasmid DNA (pBR322) into open circular and linear form after treating with ground water samples from industrial area of Buddha Nullah, Ludhiana of Punjab (India) (Kaur et al., 2020).

5. Conclusion

The findings clearly decipher the presence of numerous mutagens in the WWI soil. TA98 was the most responsive strain in terms of induction factor mutagenic index and the mutagenic potential representing that frameshift type of mutagens were present in test samples. A reduction in CFUs of defective *E. coli* K-12 mutants of DNA repair along with their isogenic wild type of counterpart clearly showed the presence of mutagens capable of damaging the DNA. Cyto-genotoxicity triggered by test samples is also evident from the decreased mitotic index and induction of chromosomal abnormalities in the *A. cepa* root meristematic cells. Present results also indicated that WWI soil had toxic effect on *V. radiata* seed germination where a reduced seedling development. DNA damage was also found in plasmid pBR322. Therefore, application of untreated wastewater for agricultural purposes should be strictly banned to shield the humans from the toxicological impact of the pollutants.

Declarations

Author contribution statement

Mohammad Tarique Zeyad: Conceived and designed the experiments; Performed the experiments; Wrote the manuscript.

Abdul Malik: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Sana Khan: Analyzed and interpreted the data.

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Data availability statement

Data will be made available on request.

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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