Cytotoxic and genotoxic assessment of agricultural soils from an industrial region

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Abstract Industrial effluents contain hazardous substances that can be a serious threat to the agriculture and human health. In the present study, the cytotoxic and genotoxic impacts of agricultural soil from the industrial area of Dera Bassi (Punjab, India) have been evaluated. Assays such as defects in DNA repair in K-12 mutants of *Escherichia coli* and chromosomal aberrations in *Allium cepa* were used to estimate the acute toxicity and chromosomal mutagenesis, respectively. Atomic absorption spectrometry and GC-MS analysis revealed contamination of the soil with high concentrations of heavy metals and organic compounds, respectively. Dichloromethane extract of site I soil sample caused maximum damage to 40 μL mL^{-1} DNA repair defective mutants and showed 38 and 49% survival in *lexA* and *recA* mutants, respectively, which was least among all the sites. In *A. cepa* test, an inverse relationship between soil extract concentration and the mitotic index was observed. Exposure of growing roots of *A. cepa* to soil extracts induced chromosomal abnormalities and alterations in mitotic phases in root tip cells. The study concludes that agricultural sites near the industrial area were contaminated with genotoxic and mutagenic compounds. Hence, adequate measures should be taken to reduce the toxicity of industrial effluents discharged onto the agricultural fields.

Introduction

Increased industrialization has escalated the volume and frequency of wastewater discharge into the ecosystem. Owing to inefficient treatment and improper waste disposal, several toxic compounds including heavy metals and polyaromatic hydrocarbons are released into the soil by industrial activities and modern agricultural practices (Ho et al., 2012; Anh et al., 2019). These hazardous substances pose a serious threat to soil biota, plants, and animals including humans, thereby affecting the health of the receiving ecosystem (FAO & UNEP, 2021). Exposure to heavy metals impedes proper functioning of brain, kidney, liver, and lungs causing muscular and mental degenerative diseases in humans (Reddy & Osborne, 2020). Overexposure to toxic organic compounds leads to lung problems, weakness, and damaged immune system, and it can be fatal even at low concentrations

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(Raza et al., 2019). Agricultural lands adjacent to industries have higher risk of accumulation of toxic compounds through direct and indirect ways, from where these pollutants can enter the food chain (Datta et al., 2018; Husejnovic et al., 2018). In fact, world's soil is under great pressure due to pollution (FAO & UNEP, 2021). It is thus pertinent to undertake genotoxic risk assessment of agricultural soil contaminated due to industrial pollution (Xiao et al., 2006; FAO & UNEP, 2021). The effect of pollutants in the soil varies due to synergistic and antagonist actions; it is, therefore, not advisable to undertake physicochemical methods for risk assessment of contaminated soil (Jensen & Pedersen, 2006). The toxicity of industrial waste could be evaluated using in vivo assays, but these are limited due to economical, temporal, and ethical factors. Therefore, in vitro studies employing both prokaryotic and eukaryotic systems can overcome these limitations as it detects DNA damage from point mutations to chromosomal alterations (Khan et al., 2019). Fernandez et al. (2005) opined that bioassay involving biological organisms should be used along with chemical analysis for genotoxic risk assessment of soil contaminants.

Among the various bacterial genotoxicity methods, DNA repair assays are the most widely used ones (Maslowska et al., 2019). These generally determine the level of DNA damage, thus approximating the genotoxic/mutagenic potential of a compound or test sample. Induction of SOS repair system in bacteria and exposure to DNA damaging agents is another test for genotoxicity (Simmons et al., 2008). There is a high degree of correlation in DNA repair system in bacteria with higher organisms (Ronen & Glickman, 2001). *Escherichia coli* has been employed as a model organism to study DNA repair genes in eukaryotes (Augusto-Pinto et al., 2003). SOS response system in *E. coli* involves a set of about 50 co-regulated genes (Maslowska et al., 2019), which are induced in response to the loss of genetic integrity due to DNA damaging agents and inhibition of replication. SOS response is initiated by single-stranded DNA and involves two genes, repressor *rec*A and an inducer *lex*A protein, which play a key role in repair of DNA (Maslowska et al., 2019). However, the mutant strains lacking SOS response are overly sensitive to DNA damage by mutagenic chemicals (Kuzminov, 1999).

To screen and monitor the toxicity of environmental pollutants, plant bioassays are also well acknowledged (de Souza et al., 2016). Among them, the *Allium cepa* (onion) is used widely to study the genotoxicity because of its easy handling, low cost, greater sensitivity, reliability, and high correlation with other test systems (Pasha et al., 2015). *A. cepa* bioassay has been regarded as a bioindicator of pollutants and genotoxicity (Tedesco & Laughinghouse, 2012; de Souza et al., 2016).

Dera Bassi (Mohali, Punjab, India) is an industrial town located in foothills of Shiwaliks in north India, with humid sub-tropical climate, and has an average elevation of 321 m above mean sea level (Sharma et al., 2021). It houses around 300 industries including chemical, pharmaceutical, electroplating, paper mills, textile, dyeing, brewery, paint, dairy, and meat etc. There have been reports of discharge of untreated industrial effluents directly onto the nearby drains/rivers and agricultural soils, which prompted National Green Tribunal (NGT) of India to shut down several industries (Dogra, 2018). Despite the release of harmful effluents from the industries in the region, no study has undertaken the cyto- and genotoxic risk assessment of the polluted agricultural soils. We, therefore, conducted a study to investigate the cytotoxicity and genotoxicity of contaminated agricultural soils from this region. The toxicity was evaluated using bacterial genotoxicity assays in terms of survivability of SOS defective K-12 mutants of *E. coli* and chromosomal aberrations in *A. cepa* root tips.

Materials and methods

Soil collection and analyses

The study was conducted on the soils collected from agricultural fields around the industrial hub, Dera Bassi (30.58° N 76.84° E; Punjab, India). Soil samples were collected at 0−15 cm depth from four sites (sites I, II, III, and IV). The soil samples were analyzed for physicochemical parameters and heavy metal content. Soil properties such as pH (1:2 soil/water, *v/v*), NH_4^+-N (kg ha^{-1}), organic carbon (%), phosphates (kg ha^{-1}), NO_3^--N (kg ha^{-1}), and

available K (kg ha^{-1}) were determined as per the methods given by Tandon (1993). To evaluate the metal content in collected soil, samples were dried in an oven at 40 °C, grounded, sieved (0.1 mm), and converted into ash at 450 °C. A slurry was prepared by adding 1 mL of distilled water to 1 g of ash followed by the addition of HCl and HNO$_3$ acid (3:1, *v/v*). The samples were digested by heating the flask until the solution became clear. The clear solution was concentrated to 1 mL, and volume was made up to 100 mL using double-distilled water. Then, it was filtered successively through Whatman no. 1 and no. 42 filters. The digested samples were examined through atomic absorption spectroscopy (GBC 932 Plus, Australia), and various heavy metals were quantified.

Extraction of soil samples

Solvent extraction of soil samples with dichloromethane (DCM; HPLC grade) was carried out as per Knize et al. (1987). For this, 10 mL of DCM was added to 10 g of soil, and the mixture was centrifuged (at 5000 *g*, for 10 min). The supernatant was collected and evaporated completely. The residues were dissolved in 1 mL of dimethyl sulfoxide. The solution was filtered through 0.45 μm nylon filter and stored at −20 °C for analysis.

GC-MS analysis

Soil extracts were analyzed for the presence of organic compounds using GC-MS (Thermo Trace 1300 GC with Triple Quadrupole MS, Thermo Fischer Scientific, USA) fitted with TG 5 column (30 m × 0.25 mm × 25 μm). The operating conditions were 250 °C injector temperature, sample injection at 1:15 split ratio, Helium as carrier gas at 1 mL min^{-1}, initial oven set at 60 °C for 2 min, increased to 280 °C at 10 °C min^{-1}, and held for another 10 min. The mass spectra were obtained in the range of *m/z* 50−600 amu. The fragmentation patterns of the major peaks were compared with National Institute of Standards and Technology library (NIST 2.0) to identify the compounds present in the different soil extracts.

Exposure of *E. coli* mutants

SOS response of DNA repair mechanism was studied using *rec*A and *lex*A mutants of *E. coli*. The mutants and the isogenic wild-type strains were collected from an overnight-grown culture having 100−300 × 10^6 cells mL^{-1}. The bacterial pellets were dissolved in a solution of 10 mM MgSO$_4$. Then, 40 μL of soil extract was added. Samples were obtained at 2-, 4-, and 6-h intervals. These were diluted, and their CFA (colony-forming ability) was assayed in an incubator set at 37 °C for overnight. A parallel control, with solvent only, was maintained simultaneously.

Allium cepa anaphase-telophase test

Cytotoxicity of the soil samples was tested in terms of chromosomal aberrations (CAs) and mitotic index (MI) using squash technique (Chandel et al., 2019). Onion bulbs (ϕ=1.5–2.0 cm) were procured from the local market. Prior to use, their outer scales were removed. The basal ends of the bulbs were immersed in distilled water in beakers at 25 ± 2 °C. Emerging roots (~ 1–2 cm) were harvested for determining the toxicity of soil samples. The soil extracts were mixed with distilled water (1:10, *w/v*) and shaken on a rotary shaker for 24 h at 25 °C (Cotelle et al., 1999). These were diluted to get working concentrations of 5%, 10%, 25%, 50%, and 100%. Parallel set-ups with distilled water served as negative control, whereas methyl methane sulfonate (MMS, 10 mg mL^{-1}) was used as positive control. After 72 h of treatment, root apices were removed, fixed in Clarke's solution (75 mL ethanol/25 mL acetic acid glacial) for 12 h at 4 °C. After fixation, root apices were washed in distilled water and stained with aceto-carmine.

For each soil extract, 3–4 root tips per slide were used, and three such slides were prepared as replicates. A total of 3000 cells (1000 per slide) for each treatment were analyzed under light microscope (model 66475; Getner, India) to determine MI. However, 300 dividing cells per treatment (100 per slide) were analyzed for determining CAs. According to the mechanism of action, CAs were classified as aneugenic (chromosomal breaks and bridges) resulting

from spindle disturbance or clastogenic (chromosomal adherence, losses, and *C*-metaphases).

Statistical analysis

The CAs and MI data were expressed as percent. The data were analyzed by one-way ANOVA followed by the comparison of mean values at $P \leq 0.05$ using post hoc Duncan's multiple range test. The statistical analyses were done using SPSS ver. 16.

Results

Soil samples were neutral in nature with pH ranging from 7.2 to 7.5 (Table 1). The soil organic carbon (0.6%) was found to be least for site III. Nitrate-nitrogen (NO_3-N) of the soil samples ranged from 3.0 to 9.0 kg/ha, whereas the amount of ammonium-nitrogen (NH_4-N) was highest in soil collected from site I and least in soil sample from site IV. The amount of available P and available K was in the range of 18–60 kg ha^{-1} and 90–112 kg ha^{-1}, respectively (Table 1). The amount of Cr, Cu, Cd, Ni, and Pb ranged from 19.18 to 28.23 mg per kg, 18.6 to 30.5 mg kg^{-1}, 0.1 to 0.3 mg kg^{-1}, 24.5 to 45.9 mg kg^{-1}, and 34.8 to 42.9 mg kg^{-1}, respectively (Fig. 1). Soil extracts contained various organic compounds as revealed by GC-MS (Table 2).

The percent survivability of *E. coli* mutant strains after treatment with soil extracts is presented in Fig. 2. Soil extract of site I exhibited maximum decrease in survival compared with samples from
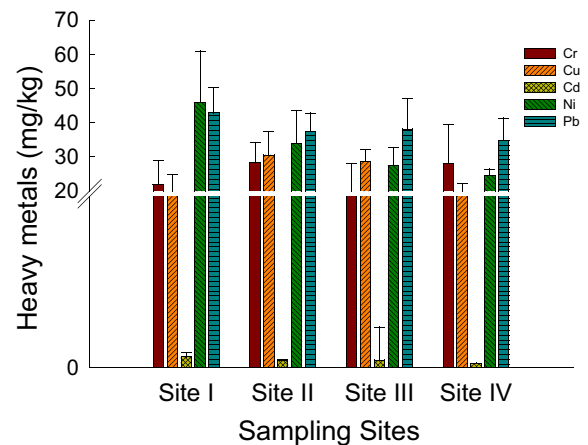

Fig. 1 Heavy metal content in soil samples collected from four sites in agricultural fields near industrial hub Dera Bassi (Punjab, India). Values are presented as mean ± SE (*n* = 3). Vertical bars represent S.E

sites II, III, and IV. After 6 h of treatment with soil extracts, the survival in *lexA* mutants was 38% for site I extract, which was least, while survival was 41, 43, and 47% in sites II, III, and IV, respectively, as compared with isogenic wild type. For *recA* mutants, the highest survival (60%) was noticed in site IV soil extract followed by 54% survival in site III extract. There was not much difference in the survivability of *recA* mutants in soil extract of site I and site II when compared with the isogenic wild type (Fig. 2).

The effect of soil extracts on the changes in MI and frequency of mitotic phases in root meristem of *A. cepa* is presented in Fig. 3 and Table 3,

Table 1 Soil properties of samples collected from four sites in agricultural fields near industrial hub Dera Bassi (Punjab, India)

Soil characteristic	Sampling site			
	I	**II**	**III**	**IV**
pH	7.3 ± 0.10	7.2 ± 0.50	7.2 ± 0.21	7.5 ± 0.70
Organic carbon (%)	1.1 ± 0.3	1.3 ± 0.1	0.6 ± 0.1	0.75 ± 0.2
Phosphate (kg/ha)	60 ± 4.1	28 ± 2.3	20 ± 1.7	18 ± 6.3
NH_3-N (kg/ha)	73 ± 5.8	70 ± 7.9	31 ± 3.3	15 ± 6.1
NO_3-N (kg/ha)	9 ± 1.3	8 ± 0.9	4 ± 0.56	3 ± 0.6
Available K (kg/ha)	112 ± 10	100 ± 15.1	90 ± 7.1	98 ± 11.3

Values are presented as mean ± SE (*n* = 3)

Table 2 Organic compounds identified in soil samples collected from agricultural fields near Dera Bassi (Punjab, India) using GC-MS

	Soil sampling site			
	Site I	Site II	Site III	Site IV
Identified organic compounds	trans-13-Octadecenoic acid 2-(2-hydroxyethoxy)ethyl ester 4-(2,6,6-trimethyl-2-cyclohexen-1-yl) Z-11-Pentadecenal cis-13-Eicosenoic acid 9-Octadecenoic acid (Z)-pentanoic acid, 2-methyl, 1,2,3-propanetriyl ester Heptadecane, 9-hexyl- 2-Nonadecanone-2,4-dinitrophenylhydrazine Benzene, 1,1'-sulfonylbis[4chloro]	Perfluorotributylamine Pyrimidin-2-one Oleic acid 4-[N-methylureido]1[4methylaminocarbony loxymethyl Octadecane, 3-ethyl-5-(2-ethylbutyl)- n-Hexadecanoic acid Pyrimidin-2-one 1-Monolinoleoylglycerol trimethylsilyl ether 1-Decanol, 10[(tetrahydro-2-Hpyran-2-yl)oxy] cis-13-Octadecenoic acid Oleic acid, eicosyl ester Cyclononasiloxane, octadecamethyl Benzene, 1,1'-sulfonylbis[4chloro] 2-Nonadecanone-2,4-dinitrophenylhydrazine Cyclodecasiloxane, eicosamethyl Pentanoic acid, 2-methyl, 1,2,3-propanetriyl ester Heptadecane, 9-hexyl- 3-Desoxo-3,16-dihydroxy-12-desoxyphorbol 8,14-Seco-3,19-epoxyandrostane-8,14-dione Phthalic acid, di(2-propylpentyl) ester Eicosane, 7-hexyl-	10,13,16,19-Tetraoxa(2,12)[26] paracyclophan-1-en Cyclohexasiloxane, dodecamethyl Perfluorotributylamine-3,4,5-Trihydroxybenzoic acid ethyl ester, tris(Otrimethylsilyl) Ethyl i isoallocholate 1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester 13-Docosenamide, (Z)- Tetracosane Tetratriacontane Stigmastan-3,5-diene 9,12,15,18,21,24-Hexaoxa(2,16)[30]metacyclophan-1-ene, 1,2-diphenyl 9-Octadecenamide, (Z)-	Cycloheptasiloxane, tetradecamethyl Heptadecane Cyclooctasiloxane, hexadecamethyl 2-Nonadecanone2,4-dinitrophenylhydrazine 2,2,4-Trimethyl-3(3,8,12,16-tetramethylheptadeca-3,7,11,15-tetraenyl)- Cyclohexanol 4-HCyclopropa[5',6']benz[1',2':7,8]azuleno[5,6-b]oxiren-4-one (5á)Pregnane-3,20á-diol 1-Monolinoleoylglycerol trimethyl-silyl ether 3-Desoxo-3,16-dihydroxy-12-desoxyphorbol 9,12,15-Octadecatrienoic acid, Triacontane Lycoxanthin Cholest-5-ene-16,22-dione, 3a,26-dihydroxy Tetratriacontane

Fig. 2 Percentage surviv-ability of *Escherichia coli* K-12 strains after treat-ment with dichloromethane extracts of soil samples collected from four sites in agricultural fields near industrial hub Dera Bassi (Punjab, India). **a** Site I, **b** site II, **c** site III, **d** site IV. Values represented as mean ± SD
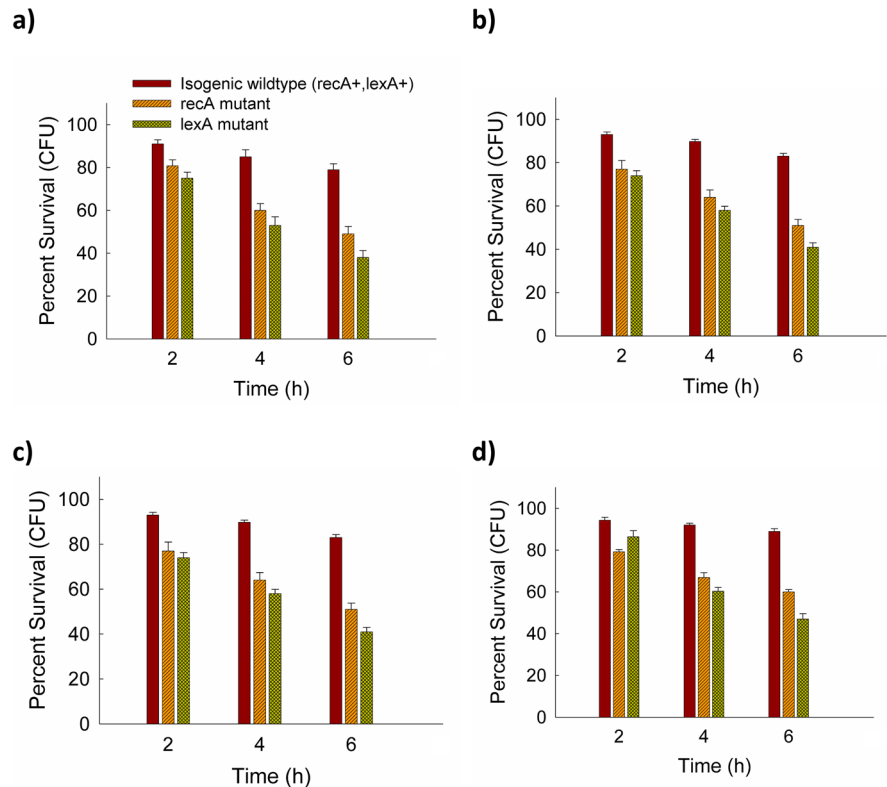
respectively. MI was the minimum in samples exposed to MMS (positive control) and exhibited the lowest average (8.0%), while the highest MI
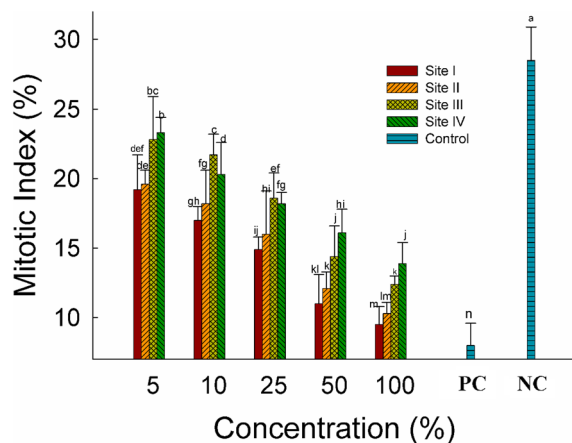

Fig. 3 Changes in mitotic index in root meristems of *Allium cepa*, measured after 72 h of treatment with extracts of soil collected from agricultural fields near industrial hub Dera Bassi (Punjab, India). PC positive control, NC negative con-trol. Values represented as mean ± standard deviation. Differ-ent lowercase letters represent significant difference at $P \leq 0.05$ applying Duncan's multiple range test

(28.5%) was observed in negative control (distilled water). MI significantly declined in a concentra-tion-dependent manner in all the test soil extracts compared with negative and positive controls (Fig. 3). For site I, the MI was 9.5% ($P \leq 0.05$) in response to 100% soil extracts, whereas, at the same concentration, sites II, III, and IV showed MI ($P \leq 0.05$) of 10.3, 12.4, and 13.9% respectively (Fig. 3). All the soil extracts affected the rate of each mitotic stage in *A. cepa* with an increase in prophase stage and a consistent decrease in the anaphase and telophase stages, thereby suggest-ing an antiproliferative activity of the compound (Table 3).

In chromosomal aberration assay, cells in ana-phase-telophase of the positive control (MMS) and the treatments showed high abnormalities ($P \leq 0.05$) as compared to the negative control (Table 4). Total CAs (%) were observed to be higher in site I than that of other sites (Fig. 4). Treatment of soil extracts induced CAs such as *C*-mitosis, sticky chromo-somes, and laggard chromosomes (Fig. 5), and the effect was concentration-dependent (Table 4).

Table 3 Change in phases of mitosis in root meristems of *Allium cepa*, measured after 72 h treatment with soil extracts

Conc. (%) ↓ Sites →	Mitotic phase (%)											
	Prophase				Metaphase				Anaphase-telophase			
	I	II	III	IV	I	II	III	IV	I	II	III	IV
5	26.56	34.18	29.8	30.0	36.5	30.1	30.7	30.9	36.9	35.7	39.47	39.05
10	31.76	42.3	24.8	36.9	32.35	26.3	34.1	28.5	35.9	31.3	41.0	34.48
25	40.3	48.1	35.48	36.8	36.24	25	31.7	30.2	23.48	26.9	32.7	32.96
50	47.5	51.4	54.8	48.4	32.67	21.9	25.6	24.8	19.8	26.6	19.4	26.7
100	54.76	59.1	56.4	53.2	28.57	31.2	27.4	25.8	16.66	9.6	16.1	20.8
PC	65.18				24.39				10.43			
NC	28.10				33.69				38.21			

PC positive control (MMS), *NC* negative control (distilled water)

Table 4 Chromosome aberrations (CA) induced in root meristems of *Allium cepa* by extracts of soil collected from agricultural fields near Dera Bassi (Punjab, India)

Conc. (%) ↓ Sites →	Chromosome aberrations (CA)																							
	CM				B				BN				S				CB				DAT			
	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV
5	3	0	1	1	1	3	1	–	0	–	–	2	4	4	1	1	–	0	0	1	3	3	–	3
10	4	3	1	4	5	3	2	3	3	0	3	1	3	6	3	1	4	1	4	3	5	5	1	1
25	5	5	3	2	8	7	5	6	5	3	4	3	4	6	7	4	7	3	5	5	6	5	3	4
50	7	7	5	6	10	8	3	8	7	6	7	7	9	8	9	10	9	7	7	7	8	8	5	3
100	10	9	13	7	12	11	12	6	8	10	10	11	15	9	12	10	13	12	5	4	14	11	6	7
PC	18				15				12				17				15				16			
NC	1				1				–				1				–				–			

CM c-mitosis, *B* anaphase bridge, *BN* binucleated cell, *S* stickiness, *CB* chromosome break, *DAT* disturbed anaphase-telophase, *PC* positive control, *NC* negative control
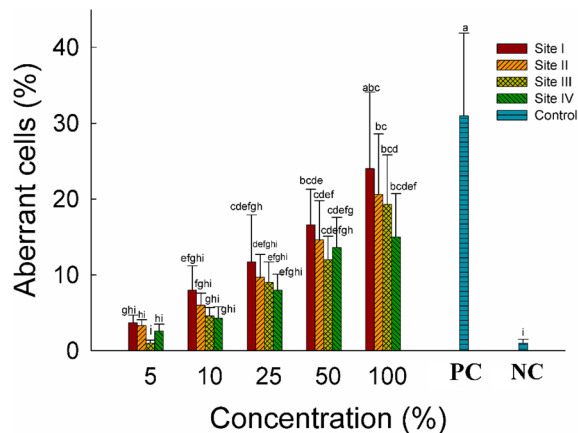

Fig. 4 Aberrant cells (%) induced in root meristems of *Allium cepa* by extracts of soil collected from agricultural fields near Dera Bassi (Punjab, India). PC positive control, NC negative control. Values represented as mean ± standard deviation. Different lowercase letters represent significant difference at $P \leq 0.05$ applying Duncan multiple range test
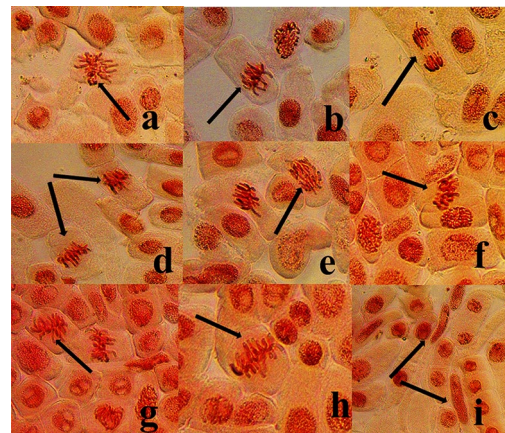

Fig. 5 Chromosomal abnormalities in *Allium cepa* root-tips caused by extracts of soil collected from agricultural fields near Dera Bassi (Punjab, India). **a** Spindle disturbance. **b** Sticky chromosomes. **c** Laggard, **d** Sticky chromosomes. **e** Mitotic bridge. **f** Vagrant chromosome. **g** Diagonal spindle. **h** c-mitosis. **i** Morphological changes. All micrographs have the same magnification (bar = 50 μm)

Discussion

Soil is the most important non-renewable biological resource that supports plant life and is essential for the subsistence of humans. However, the increasing population coupled with rapid industrialization has put it under tremendous pressure (FAO & UNEP, 2021). In the present study, the pH of soil samples was found to be nearly neutral. Effluents with too high or low pH upon entering the soil change soil pH, hence making soil infertile. Changed pH of soil can alter the solubility of the pollutants, influence the availability of micro-nutrients, and can enhance their toxicity level (Odjadjare & Okoh, 2010). High levels of organic carbon, phosphate, NH_3-N, NO_3-N, and available K were observed for site I and site II soil samples, which could be due to the high volume of wastewater received by these sites when compared with site III and site IV (Osakwe, 2012).

Industrial waste generally contains hazardous heavy metals and organic compounds. High concentration of heavy metals such as Cr and Cd can cause serious morbidity and mortality. Cu and Cd are used in corrosion prevention, galvanizing iron and steel, metal plating, and motors and generators. Hence, the presence of such metals in soil may be ascribed to the untreated effluents discharged by the industries using these metals (Singh et al., 2009). The presence of Cr, Ni, Co, Cu, and Cd in the agricultural soil observed during the current investigation agreed with the earlier reports from the northwestern regions of India (Dheri et al., 2007). In the present study, GC-MS analysis of soil samples revealed the presence of esters, acids, ketones, and phenols of benzene, hexadecane, dodecane, pentadecane, pyrimidine, and hexadecanoic and octadecanoic acid. The findings corroborate previous reports, which identified similar compounds in industrial wastewater (Liang et al., 2018; Yadav et al., 2019). The derivatives of phthalate and benzene are known for carcinogenicity (Dixit et al., 2015), phenolic compounds are endocrine disruptors (Kumari et al., 2016), and hexadecanoic and octadecanoic acid are probable carcinogen and mutagens (EPA, 2004). From agriculture lands, these contaminants may enter the food chain and bioaccumulate. Abegunrin et al. (2016) argued that irrigation using wastewater could be valuable as it increases nutrients and soil fertility, which in turn enhance crop growth. However, it should be reused with caution to protect soil quality and crop yield. Keeping in mind the possible adverse effects of industrial wastewater used for irrigation, Central Pollution Control Board (CPCB, India) has advised to use treated effluents for irrigation with caution (Aggarwal, 2019).

SOS response in bacteria is a DNA repair process that is induced in response to mutagenic/DNA-damaging chemicals. Though plants do not have any SOS repair mechanism, there exist homologies in the proteins (enzymes and amino acids) involved in DNA repair in eukaryotes (Janion, 2008). Accordingly, it has been used as an important test to evaluate genotoxic impacts of mutagenic and DNA-damaging chemicals, including pollutants (Kenyon & Walker, 1981). In the present investigation, the importance of $recA^+$ and $lexA^+$ genes to control the harmful effects of pollutants was studied, and DNA damage in the exposed cell revealed the sensitivity of recA and lexA mutants to the test samples. In general, the substances which induce SOS response in bacteria are genotoxic to higher eukaryotes, and this is the ground for various bacterial assays for determining genotoxic and carcinogenic potential (Maron & Ames, 1983).

We evaluated both cyto- and genotoxicity in terms of several CAs including breaks, bridge formation, sticky chromosomes, and MI. Extracts of polluted soil from all the four sites significantly ($P \leq 0.05$) declined MI in a dose-response pattern. According to Tedesco and Laughinghouse (2012), chromosomal assay provides an easy, good, and reliable tool to determine cytotoxicity of a pollutant/chemical. Occurrence of a cytotoxic effect was concluded based on the change in the proportion of mitotic phases and a decline in the mitotic activity after the treatment. Numerous checkpoints in the mitotic cycle make sure of the proper distribution of genetic material. A decline in mitotic activity may either be due to DNA damage or inhibition of DNA synthesis resulting from G2 phase blocking in cells (Selmi et al., 2014). Increase in prophase might be due to the occlusion of the dividing cells at checkpoint between prophase and metaphase if microtubule structure is altered (Scolnick & Halazonetis, 2000).

Based on the results of the *A. cepa* test, we suggest that the soil samples contained chemical substances with cytotoxic, aneugenic, and/or clastogenic potential. Stickiness and anaphasic bridges and *c*-metaphase were the most frequent CAs observed in the study. These suggest chromatin dysfunction (thus disruption

of chromatin organization) or spindle failure. The formation of bridges during anaphase and telophase is indicative of clastogenic effects, whereas *c*-metaphase suggests enhanced aneuploidy (Chandel et al., 2019). Our findings are corroborated by previous studies documenting mutagenic/genotoxic effects of soil collected from residential areas (Watanabe et al., 2008), biodiesel-polluted soil (Leme et al., 2012), pesticide-polluted soil (Datta et al., 2018), and fields with sugarcane vinasse (da Silva Souza et al., 2013).

In summary, our study reveals that the agricultural site near the industrial hub, Dera Bassi, is contaminated with genotoxic and mutagenic compounds, as indicated using different bioassays such as *E. coli* SOS response and *A. cepa* root tip assay. Further, genotoxic and chemical tests are required to evaluate toxicity profile of soil samples from agricultural sites in this region. Suitable measures should be taken to prevent the discharge of untreated wastewater in the agricultural fields or its use for irrigation purposes to avoid the entry of contaminants into the food chain.

Availability of data and materials The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests The authors declare no competing interests.

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