Pollution load assessment of agricultural soil samples of some villages from Ludhiana district, Punjab.

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Abstract

Aims Continuous use of inorganic fertilizers, pesticides, wastewater discharge, and leachates causes soil degradation, contamination of potable water and food ultimately leading to soil pollution and ill effects on human health. The current study involves monitoring of soil quality of agricultural soil samples collected from four different agricultural fields in Ludhiana, Punjab (India) near Buddha Nullah, a tributary of the Sutlej river.

Methods Physico-chemical characteristics and heavy metal content of soil samples were estimated following the standard protocols. The genotoxic potential of soil samples were evaluated by using *Allium cepa* root chromosomal aberration assay.

Results The agricultural soil samples were found to be slightly basic. Soil nutrients such as nitrate, phosphate and potassium ranged from 0.072 - 0.075 mg/g, 0.029 - 0.070 mg/g and 0.055 - 0.158 mg/g respectively. The contents (mg/kg) of heavy metals cadmium, chromium, cobalt, copper, and lead analyzed in five of the soil samples were observed to be above the permissible limits. *Allium cepa* root chromosomal aberration assay used for genotoxicity studies has shown that Hambran (HBN), induced maximum genotoxic effect, i.e. 46.7% in terms of percent aberrant cells in *Allium cepa*.

Conclusions The statistical analysis evaluated the positive correlation of heavy metals (Cu, Co, Ni) with the percentage chromosomal aberration induced in *Allium cepa* test. The Pearson correlation indicated that genotoxicity had a significant positive correlation with the content of Cu and Ni at $p \leq 0.05$ and with Co at $p \leq 0.01$.

Introduction

Physico-chemical characteristics of soil hold a vital position for the evaluation of soil quality as well as to designate its basic functions to support ecosystem and fertility. Soil resources are essential since they are the primary source of livelihood in the terms of agriculture and any degradation of soil quality results in the decline of productivity of agricultural lands (Belayneh et al. 2019). Soils get contaminated via accumulation of different contaminants such as pesticides, fertilizers, heavy metals etc. from various anthropogenic activities (Wuana et al. 2011). Chemicals such as iron, lead, mercury, copper, zinc, cadmium, cyanides, acids and alkalies are found in industrial wastes and reach the soil either directly or indirectly through water and air (Adimalla et al. 2020). Numerous studies have reported the widespread heavy metal contamination in urban soils and the health risk of heavy metals contamination (Maanan et al. 2015; Wang et al. 2014). Heavy metals are released into the environment by various anthropogenic activities including coal fired power plants, mine tailings, wastewater irrigation, application of fertilizers and pesticides, cement klins, trash incinerators, direct disposal as well as atmospheric deposition.

Heavy metals and metalloids are of global concern due to their potential to induce direct toxicity such as leaf chlorosis as well as alteration of activities of various key enzymes of metabolic pathways (Chileshe et al. 2020). Industrial waste containing metals like lead, zinc, arsenic, copper, cadmium and mercury adversely affect humans and other animals through intake of contaminated groundwater, contact with contaminated soil and direct ingestion. Apart from this, the heavy metal polluted sites also adversely affect the diversity of soil organisms which otherwise have a positive role in crop production. Some of the metals like copper (Cu), lead (Pb), nickel (Ni), and zinc (Zn) can inhibit soil organic matter decomposition as well as nitrogen mineralization processes (Gowd et al. 2010). Due to mutagenic and carcinogenic nature of some heavy metals like arsenic (As), chromium (Cr), cadmium (Cd), lead (Pb) and mercury (Hg), even low concentration in ecosystems, can impose serious health issues (Chen et al. 2020). Heavy metals are harmful because they can cause chronic degenerative changes in the body (Jaishankar et al. 2014). Heavy metal poisoning in animals causes neurological and gastrointestinal problems. Heavy metal toxicity is thought to be caused by changes in gene expression caused by environmental factors, notably dietary components that influence gene regulation (Alissa et al. 2011). In comparison to their absorption rate, heavy metals have a slow excretion rate once inebriated (Everett et al. 2008).

Plants are particularly vulnerable to fluctuating metal concentrations in the environment because principal interaction point for heavy metal ions is a plant's roots. The molecular process by which poisonous heavy metals cause cell death in plants is poorly understood (Tchounwou et al. 2012). Heavy metals, are thought to interact with enzymes (and maybe structural proteins) through metal sensitive groups such as SH- or histidyl groups, causing catalytically active or structural proteins to become inactive (Nagajyoti et al. 2010). Plants growing in contaminated areas mainly demonstrate visible symptoms on plant surfaces because of the heavy metal accumulation in plant parts (Ozturk et al. 2017). Growth reduction as a result of changes in physiological and biochemical processes in plants growing on heavy metal polluted soils has been recorded (Oancea et al. 2005).

Water-soluble heavy metals are easily absorbed by plants and animal tissue. After being consumed by organisms, heavy metals tend to interact with biomolecules (nucleic acids and proteins), altering their functions and perhaps changing their genetic material (Oves et al. 2016; Pohren et al. 2013). The mutagenic substances cause genotoxicity in the organisms that are exposed to them. For the examination of organism reactions to complex combinations of environmental pollutants, biological assays to assess toxicity, genotoxicity and mutagenicity are required (Honma 2020; Pohren et al. 2013). Higher plants such as *Allium cepa, Vicia faba, Zea mays, Tradescantia, Nicotiana tabacum, Crepis capillaris* and *Hordeum vulgare* have characteristics that make them suitable genetic models for assessing pollutants in the environment and therefore, frequently utilize in monitoring studies (Grant et al. 1994). Furthermore, *A. cepa* has been identified as a suitable species for studying chromosome damages and abnormalities in the mitotic cycle, owing to the presence of ideal chromosome conditions (Khan et al. 2020; Leme et al. 2009).

The present study was undertaken in order to estimate the physico-chemical parameters and content of heavy metals cadmium, copper, chromium, cobalt, nickel, lead and zinc of agricultural soil and to assess genotoxicity of collected soil samples using *A. cepa* root chromosomal aberration assay.

Material And Methods

Study area

The present study concerns the analysis of four soil samples taken from the paddy fields of Ludhiana Punjab, India for determination of various physico-chemical parameters including heavy metals (cadmium, copper, chromium, cobalt, nickel, zinc, and lead) and genotoxicity using *A. cepa* root chromosomal aberration assay. Soil samples were collected from four different agricultural sites (Aliwal, Hambrahn, Malakhpur and Balloke) in the Ludhiana district of Punjab, adjacent to Bhudda Nullah, a rivulet of river Sutlej. The soil samples were collected by digging soil to depths of 15–20 cm. The samples were transported to the laboratory, dried at room temperature for 72 h, ground into fine powder and stored for further analysis (Cabrera & Rodriguez 1999).

Physico-chemical analysis

Aqueous soil extracts (1 : 5 w/v) were prepared for physico-chemical analysis using distilled water. 50 g of soil was suspended in 250 ml of distilled water, shaken for 12 h at room temperature on mechanical shaker, the solution was filtered through *Whatman* No. 1 filter paper. Filtrate was referred to as soil extract and was used for further analysis. Different physico-chemical parameters such as pH, alkalinity, calcium content, magnesium, sodium, potassium, nitrates, phosphates and heavy metals were estimated following the protocols given by Trivedi et al. (1985) with slight modifications. Parameters such as calcium, magnesium, alkalinity were titrimetrically calculated and nitrates and phosphates were estimated using spectrophotometer (Kaur et al. 2014). The contents of sodium and potassium were estimated by flame photometer (model CL 26 D, make ELICO) and water holding capacity by Brass box method (Rao 1998).

Digestion and heavy metals analysis

Heavy metals were extracted from soil samples using a hot aqua regia digestion method. For which 1 g of each soil sample was weighed and taken in digestion beakers. 10 ml of aqua regia (1:3 HNO_3: HCl) was added to the soil sample. Aqua-regia was evaporated on a hot plate till brown fumes turned colorless with an indication of complete reaction. The digested samples were filtered and final volume was made up to 50 ml with distilled water. Estimation of heavy metals was done using atomic absorption spectrometer (model 240 FS; make Agilent).

Allium cepa for genotoxicity test

The genotoxic potential of soil samples were estimated by using *A. cepa* root chromosomal aberration assay. Healthy and young equal sized onions were selected. Without harming root primordial, outer dead scales of onion bulbs were removed. Denuded onion bulbs of uniform sized were allowed to root directly in the agricultural soil samples contained in pots for 24–48 h and in acid-washed sand (negative control) for 3 h. After the roots grow to size of 0.5–1 cm in length, the root tips were thoroughly washed with distilled water, cut and stored in Farmer's fluid (3:1:: ethanol : acetic acid glacial). For slide preparation, the fixed root tips were hydrolyzed in 1N HCl with intermittent heating for 1 min and then transferred to mixture of 1N HCl and aceto-orecin (1 : 9) for 15–20 min with intermittent heating. Root tips were then squashed in 45% acetic acid and slides were observed under a microscope to determine various forms of chromosomal aberrations. The genotoxic effects were evaluated in all the mitosis stages (prophase, metaphase, anaphase and telophase). Chromosomal aberration was categorized into physiological aberrations comprising laggards, vagrants, stickiness, delayed anaphases and c-mitosis while chromatin bridges and chromosomal breaks were included in clastogenic aberrations.

Results

Physico-chemical properties

Table 1 shows the results of physicochemical parameters. pH is a crucial soil parameter that determines whether the soil is acidic or alkaline. In the present study, pH was found slightly alkaline (7.92–8.38) in studied samples. The electrical conductivity of the samples ranged from 23.33 and 35.00 mS/cm. Water holding capacity (WHC) in all the sites was found to be low (35.57–36.32%). Various soil nutrients such as sodium (Na), potassium (K) and phosphate (P) ranged from 0.45–0.61 mg/g; 0.05–0.15 mg/g; and 0.02–0.70 mg/g, respectively.

Table 1
Physico-chemical parameters of agricultural soil samples collected from different villages of Ludhiana, Punjab (India).

S.No.	Sample code	pH	WHC (%)	Electrical conductivity (mS/cm)	Alkalinity meq/100g	Nitrates (mg/g)	Phosphates (mg/g)	Sodium (mg/g)	Potassium (mg/g)	Calcium (mg/g)	Magnesium (mg/g)	Chloride (mg/g)
1	AWL	8.21 ± 0.081	36.09 ± 0.184	35.00 ± 0.577	6.33 ± 0.882	0.08 ± 0.000	0.05 ± 0.000	0.62 ± 0.003	0.06 ± 0.000	1.02 ± 0.194	1.41 ± 0.417	0.08 ± 0.037
2	HBN	8.03 ± 0.018	36.32 ± 0.035	33.33 ± 0.882	4.00 ± 1.527	0.07 ± 0.007	0.07 ± 0.000	0.47 ± 0.024	0.14 ± 0.001	1.63 ± 0.119	0.44 ± 0.058	0.15 ± 0.103
3	MKR	7.92 ± 0.017	35.66 ± 0.149	30.33 ± 1.202	3.33 ± 1.202	0.07 ± 0.002	0.04 ± 0.004	0.46 ± 0.003	0.16 ± 0.000	1.11 ± 0.058	0.55 ± 0.096	0.09 ± 0.053
4	BLE	8.38 ± 0.044	35.57 ± 0.443	23.33 ± 0.882	4.67 ± 0.882	0.07 ± 0.005	0.03 ± 0.001	0.45 ± 0.009	0.10 ± 0.000	0.80 ± 0.116	0.59 ± 0.264	0.26 ± 0.173
All values are Mean ± Standard error (S.E.) with n = 3 for each parameter												
AWL: Aliwal; HBN: Hambran; MKR: Malakhpur; Baloke: BLE												

Heavy metals concentrations in soil

The present study showed the contamination of soil samples with that of heavy metals (Table 2). The mean concentration of Cr was found to be the highest among the heavy metals in soil samples. The value of Cr metal was found to be in the range between 1.55–115.75 (mg/kg). Content of Cr (165 mg/kg) was found higher in the Hambran (HBN) soil sample then the safe limit. The mean concentration of Cd and Pb in soil sample ranged from 1.61–3.35 (mg/kg) and 3.5–31.5 (mg/kg) respectively. The concentration of essential metals such as Co (6.35–10.16 mg /kg), Cu (2.605–39.45 mg/kg), Ni (6.25–18.45 mg/kg) and Zn (15.75–49.87 mg/kg) were found within the acceptable levels for agricultural soils. One way ANOVA revealed that heavy metal concentration significantly varied at $p < 0.01$ for each site.

Table 2
Heavy metal content of agricultural soil samples collected from different villages of Ludhiana, Punjab (India).

	Heavy metal content (mg/kg)						
Sample code	**Cd**	**Cr**	**Co**	**Cu**	**Ni**	**Pb**	**Zn**
AWL	1.61 ± 0.093[c]	1.55 ± 0.029[d]	6.35 ± 0.058[c]	2.60 ± 0.058[d]	6.25 ± 0.087[d]	2.66 ± 0.333[c]	15.75 ± 0.029[d]
HBN	2.55 ± 0.029[b]	115.75 ± 0.029[a]	10.16 ± 0.167[a]	39.45 ± 0.058[a]	18.45 ± 0.104[a]	14 ± 1.732[b]	48.35 ± 0.006[b]
MKR	3.35 ± 0.058[a]	10.85 ± 0.058[b]	8.75 ± 0.104[b]	30.75 ± 0.104[b]	13.25 ± 0.029[b]	31.5 ± 0.578[a]	49.87 ± 0.004[a]
BLE	1.7 ± 0.104[c]	8.63 ± 0.044[c]	6.35 ± 0.029[c]	6.95 ± 0.029[c]	8.5 ± 0.289[c]	3.5 ± 1.041[c]	19.21 ± 0.006[c]
Maximum permissible limit (MPL) mg/kg							
Agarwal (2009**)**	0.06	100	8	20	-	10	50
Indian-Awasthi (1999)	3–6	-	-	135–270	-	250–500	300–600
European Union (2009)	1.0	100	50	100	-	100	300
WHO (2020)	0.003	0.1	-	-	-	0.1	-

Mean Value ± S.E. of four experiments; AWL: Aliwal; HBN: Hambran; MKR: Malakhpur; Baloke: BLE; MPL: Maximum permissible limits.

Concentrations of heavy metals in soil samples (mean ± S.E). Different letters above bar indicates significant differences and bar followed by same letter are not significantly different ($p < 0.01$).

Genotoxic potential

The results of genotoxicity effect of the agricultural soil samples evaluated using the *Allium cepa* assay are shown in Table 3. *Allium cepa* has a long history of being used to detect the effects of numerous genotoxic chemicals as it has a number of advantages, including clear mitotic phases, visible, karyotype stability and a rapid response to harmful toxins. Chromosome abnormalities were observed to be higher in root tip cells treated with different soil samples than the control (acid-washed sand). Among the soil samples examined, HBN exhibited the maximum (46.7%) percentage of total chromosomal anomalies, followed by MKR (40.9%), AWL (32.0%), and BLE (31.0%). Delayed anaphase was the most prevalent physiological aberration, followed by c-mitosis while chromatin bridge was the most common clastogenic aberration. Sticky cells, laggards and vagrants were also observed. The spectrum of clastogenic abnormalities included chromosomal breakage, bridges, and ring chromosomes. Mitotic activity was measured as mitotic index (MI, number of mitosis per 100 nuclei) to indicate the levels of cytotoxicity of the soil samples. Mitotic index (MI) exhibited significant negative correlation with Cu, Co and Ni. The frequency of chromosomal abnormalities in soil samples differed considerably from the control.

Table 3
Genotoxic potential of different Agricultural soil samples of Ludhiana, Punjab (India) using *Allium cepa* root chromosomal aberration assay.

Chromosomal aberrations in *A. cepa* root tip cells																			
				Physiological aberration (PA)									**Clastogenic aberration (CA)**					**TAC**	
Sample code	**TC**	**TDC**	**MI**	**Cm**	**Da**	**Lg**	**St**	**Vg**	**Aa**	**Am**	**Total PA**		**Bg**	**Cb**	**Rc**	**Total CA**		**PA + CA**	
			%								**No.**	**%**				**No.**	**%**	**No.**	**%**
CNT	2512	566	22.6^{a}	4	4	-	2	-	-	-	10	1.7	2	-	-	2	0.3	12	2.1^{d}
AWL	3160	537	17.1^{b}	32	57	-	29	3	11	16	148	27.5	22	3	-	25	4.6	173	32.0^{c}
HBN	5571	603	10.8^{c}	53	95	-	15	5	11	49	228	37.7	50	3	1	54	8.9	282	46.7^{a}
MKR	5354	690	12.9^{c}	44	89	2	33	2	7	57	233	33.8	46	3	1	49	7.1	282	40.9^{b}
BLE	3729	583	15.6^{b}	37	53	1	6	4	6	23	126	21.5	53	1	1	55	9.4	181	31.0^{c}

Cb: chromatin bridge; Lg: Laggard chromosome/s; Am : abnormal metaphase; Aa: Abnormal anaphase; Vg: Vagrant; St: stickiness; Da: Delayed anaphase; Cm: C-mitosis; Rc: ring chromosome; Cb: chromosomal break; TAC: total aberrant cells

Different letters above bar indicates significant differences and bar followed by same letter are not significantly different (Tukey test, $p < 0.01$).

AWL: Aliwal; HBN: Hambran; MKR: Malakhpur; Baloke: BLE; CNT: Control

Discussion

Physico-chemical properties

The important soil property that determines acidity or alkalinity is pH and it is considered as critical parameter for healthy plant growth. During the present study soil samples studied were found to be slightly alkaline (7.92–8.38). Vanita et al. (2014) observed that agricultural soil samples from Amritsar were slightly alkaline with pH ranging from 7.4 to 7.8. Water holding capacity of soil samples ranged from 35.57–36.32% during present study. The content of calcium (mg/g) in agricultural soil samples varied from 0.80 (BLE: Baloke) to 1.63 (HBN: Hambran). All the soil samples showed magnesium content above the safe limits of 0–500 mg/kg as presented by Awashthi (1999), Indian Standard Institution (1983) and Alghobar and Suresha (2017) except for the Hambran (HBN) site which has magnesium content as (0.44 mg/g).

Potassium contents was observed to be low in comparison to calcium and magnesium. It might be due to the potential of monovalent cations (K^{+} and Na^{+}) leaching from the system while divalent cations (Ca^{2+} and Mg^{2+}) can be strongly adsorbed to soil particles when soil moisture content rises during rice cultivation. Application of magnesium fertilizers such as magnesium nitrate and magnesium sulfate could be the cause of high magnesium levels in the sites studied. Similar results were reported earlier by Kaur et al. (2022) in roadside soil samples collected from the vicinity of Buddha Nullah, Ludhiana, Punjab where the contents of calcium and magnesium were found in the range of 0.12–3.13 mg/g and 0.032–0.609 mg/g. Concentration of sodium in all soil samples ranged from 0.45 mg/g (BLE) to 0.62 mg/g (AWL). High content of Na in soil might be due to runoff of pesticides, fertilizers and other soil amendments. Electrical conductivity (EC) of the soil is a key indicator of its health. EC was found in the range of 23–35 mS/cm which was higher than the permissible limit i.e. 4 mS/cm (Alloway 1990). EC was observed to cause salinity issue in agricultural soil over the years (Bashir et al. 2019). The quantity of accessible nitrogen in the soil is measured in the form of nitrate-nitrogen (NO_3-N) and its concentration varies from crop to crop. However in general, a preferred concentration range of 0.01–0.05 mg/g is required. High level of nitrates can form carcinogens and can accelerate eutrophication in surface waters. The levels of NO_3-N can vary greatly with fluctuation of soil water. In the present study, nitrate content ranged from 0.072–0.075 mg/g. In the soil samples, phosphorous as phosphate was observed to be in the range of 0.029–0.070 mg/g where optimal phosphorous levels stimulate robust root and shoot development (Lambers 2022).

Heavy metal contents in soil

The concentration of essential micronutrient such as zinc in all the soil samples was found to be within permissible limits while Cd, Cr, Co, Cu and Pb exceeded the permissible limits. Extensive use of pesticides and fertilizers all over the countries, could pose severe risk of heavy metal contamination of soil. The occurrence of heavy metals in soil samples during present study can be correlated to the same along with the used irrigated water from Bhuddha Nullha, flowing nearby.

The high amount of heavy metal in soil samples were also reported by Kaur et al. (2014) in agricultural soils of Punjab where Cadmium (Cd) was found much higher (9.70–30.0 mg/kg) as compared to its safe limits (3–6 mg/kg) required for any agricultural soil as given by Awashthi (1999) and by Dheri et al. (2007) in agricultural soil samples of other parts of Punjab. Chromium (Cr) is a potentially toxic element (PTE) that is found mostly in areas of high human activity, such as steel and alloy manufacture, leather tanning, electroplating, wood preservation, printing and dyeing, chemical production, among others and is bound to influence plants and animals (Yang et al. 2021). It is the most commonly found in trivalent (+ 3, chromic) or hexavalent (+ 6, chromate) forms. Potassium chromate and potassium dichromate are two commercially accessible forms of hexavalent chromium (Cr(VI)). Potassium chromate is used as a fungicide to kill insects and protect plants against fungal infestations in agriculture (Gavris et al. 2014). Chromium causes the degradation of photosynthetic pigments in plants which interrupts the photosynthetic process. High level of hexavalent chromium (40 ppm – 500 ppm) in soil reduced seed germination upto 48% in bush bean *Phaseolus vulgaris* and by 23% in Lucerne (*Medicago sativa*) (Parr et al. 1982; Peralta et al. 2001). The concentration of chromium in the agricultural soil samples in the study area varied from 1.55–115.75 mg/kg and was less than the safe limits of 100 mg/kg as given by Agarwal (2009), except at one site i.e. Hambran (HBN) where the value was 115.75 mg/kg. The main reason for the high chromium content in the study area could be attributed to various anthropogenic actions, i.e., waste disposal from different industrial units, along with sampling sites in the study area.

Among the heavy metals, Cd is regarded as a serious toxicological concern (Dhaliwal et al. 2020). The maximum concentration of Cd (3.35 mg/kg) was observed at site Malakhpur (MKR) while minimum concentration of Cd (1.61 mg/kg) at site Aliwal (AWL). The use of water from the nearby flowing polluted Bhuddha Nullah, for irrigation purposes could be responsible for the high levels of Cd in the study locations exceeding the permissible limit of 0.06 mg/kg Agarwal (2009). The findings are comparable to those of Kaur et al. (2022) where Cd content (0.03 to 0.46 mg/kg) in most of the roadside samples were above the safe limit. Farid et al. (2015) reported that Cd enters the soil through the use of phosphate fertilizers, Cd-contaminated sewage sludge and manure with phosphate fertilizers, being the primary source of Cd pollution, in agricultural soil. In the current study, Pb contents in soil samples HBN (14 mg/kg) and MKR (31.5 mg/kg) were found to be above than the typical soil range i.e 10 mg/kg and below the typical range in sample AWL (2.66 mg/kg) and BLE (3.5 mg/kg) given by Agarwal (2009). Liang et al. (2011) had also reported the higher Pb concentration (44.9 mg/kg) in soil in relation to the present study. Rubber industries in the area could be accountable for the elevated Pb levels in the area. The rubber automotive tire usage was considered to be the source of contamination of soil which released manganese, iron, cobalt, nickel, copper, zinc, cadmium and lead which are used in the tire tread (Staszak 2018). Lead frequently gets used in the production of paints, putties, and pesticides as well as in arsenical pesticides such as lead arsenate (Wuana et al. 2011).

Cobalt (Co) is a component of vitamin B_{12}, and is beneficial to humans, yet excessive amounts of cobalt can be harmful to human health (Mahey et al. 2020). The concentration (mg/kg) of cobalt in the agricultural soil samples in the study area varied from 6.35–10.16 and was less than the safe limit of 8 mg/kg (Kaur et al. 2022) except for one site, i.e., Hambran (HBN) where the value was 10.16 mg/kg. The main sources of cobalt contamination in the environment have been identified as rechargeable lithium-ion batteries, compounds containing cobalt sewage effluents, and urban and agricultural runoff (Juraszek et al. 2020). Cobalt toxicity reduces plant growth, biomass, and chlorophyll content by interfering with antioxidant enzymatic activities, photosynthetic pigments and nutrient status (Salam et al. 2022). Copper (Cu) in soils exists in different forms, and its availability to plants varies substantially. Copper is considered as a micronutrient for plants, although it is also potentially toxic. Xiong et al. (2006) observed shorter root length, fewer leaves, negative impact on nitrogen metabolism and decrease in plant biomass with increasing Cu concentrations in Chinese cabbage. Main sources of Cu in agricultural soil were reported to be application of biocides or fungicides (Liscakova et al. 2022). In this study, the Cu concentrations were found 39.45 mg/kg in Hambran and 30.75 mg/kg in Malakhpur which were above the maximum permissible limits (20 mg/kg) as given by Agarwal (2009).

During the present study at all study sites, the content of zinc was observed to be less than the maximum permissible limit of 50 mg/kg (Agarwal 2009). Similar observations were also made by Chen et al. (2022). Zinc is a micronutrient that both plants and animals require. Zn deficiency results in yellowing of leaf, reduced plant growth and cause senescence (Kumar et al. 2022; Asati et al. 2016). Zn enters the environment through a variety of pathways, including mining, smelting, industrial and municipal wastes, urban runoff and most notably erosion of Zn-containing soil particles (Noulas et al. 2018). Nickel (Ni) is a micronutrient that is required for plant development and for the activity of several enzymes in low concentrations and is toxic at high concentrations. The presence of Ni in soil can be attributed to mining operations, smelter emissions, coal and oil combustion, sewage, phosphate fertilizers and pesticides (Asati et al. 2016). The critical toxicity limit of Ni for sensitive, moderately sensitive and resistant plants was suggested to be greater than 10 mg/kg, 50 mg/kg, and 1000 mg/kg dry matter, respectively (Dehdezi et al. 2021). Wuana et al. (2011) observed that the connection between soil and water contamination and metal uptake by plants is influenced by a variety of chemical and physical soil factors as well as crop physiological properties. Setia et al. (2021) reported diffuse sources of metals in soil, to be fertilizers, pesticide, sediments runoffs and higher concentration of metals in soil along trans-boundary areas of Sutlej river (Punjab). Tariq and Rashid (2012) reported that nitrate fertilizers were the most significant sources of Cd and Co while fungicides and pesticides for Pb and fertilizers for Zn, Cu, Fe, and Mn (Kaur et al. 2022). Furthermore, the sources of heavy metals like Cd, Pb and Co in agricultural soil samples, in the present study may be mainly originated from pesticides, fertilizers and industrial or domesticated sewage as well as irrigated water from Bhuddha Nullha.

Genotoxic potential

Chromosomal abnormalities are termed as the clear indication of DNA damage that cannot be easily repaired (Zeyad et al. 2019). Since the *Allium cepa* is a eukaryotic system, it can provide a higher degree of proximity when compared to the expected impacts on biota exposed to harmful substances such as heavy metals, pesticides and compounds discharged from industries like dyeing, electroplating, textiles etc (Batista et al. 2016; Mazzeo et al. 2011). Changes in chromosomal structure or chromosomal number are examples of chromosomal aberrations. Breaks in DNA, suppression of DNA synthesis and changes in DNA replication can all cause changes in chromosomal structure. Various chromosomal aberrations, such as chromosomal breaks, bridges and ring chromosomes indicate clastogenic aberrations (Sabeen et al. 2020). During DNA replication clastogenic abnormalities can occur. Physiological abnormalities caused by spindle anomalies include c-mitosis, vagrant, stickiness, delayed anaphase, and laggard. Different cytological parameters such as mitotic index and chromosome abnormalities such as breaks in chromosomes, lagging chromosome, c-mitosis, bridge formation, and stickiness were used to determine the genotoxicity of agricultural soil samples.

The induction of toxicity in soil samples by lowering the mitotic index and increase in chromosomal aberration was observed in all studied soil samples in the *A. cepa* test. In present study, mitotic index was found least for sample Hambran (HBN: 10.8%) having Cr more than permissible limit reported by Agarwal (2009). Singh et al. (2015) also reported significant decrease in mitotic index and in root length of *Allium cepa* as the concentration of Cr during the experiment increased. Metal stress causes disruptions in the cell cycle or chromatin disfunction which results in a significant reduction in mitotic index (Kopliku et al. 2013). Patnaik et al. (2013) observed that Cr (VI) at higher concentrations (100 or 200 µM) was toxic and resulted in nuclear disintegration or pycnosis in root cells of *Allium cepa*. The genotoxic effect can also be observed through the significant increase in the frequency of chromosomal aberrations for all the soil samples. Amongst all the agricultural soil samples, HBN (Hambran) sample showed maximum (46.7%) whilst sample BLE (Baloke) showed minimum (31%) percentage of chromosomal aberrations. The treatment with control (acid-washed sand) showed 2.1% total chromosomal aberration. The frequency of cells with delayed anaphase was found to be highly elevated followed by chromosomal bridge formation, c-mitosis, abnormal metaphase and stickiness. When the two anaphasic chromosomal groups are close to each other near the equatorial plate, delayed anaphase occurs. The loss of the protein covering that shields the DNA in chromosomes, as well as the breakage and exchange of basic folding fibre units in chromatids, can cause chromosome stickiness (Kumar et al. 2021). Disruption of spindle fibre lead to the occurrence of c-mitosis during the mitotic phase (Seth et al. 2008). The presence of harmful substances in the growth media of plants could be indicated by C-mitosis (Bonciu et al. 2018). Chromosomal bridges could be formed as a result of chromosome adhesion. Which was a sign of a toxic consequence and was considered irreversible (Marcano et al. 2004; Kumari et al. 2009). Similar findings were made by Soodan et al. (2015) and Becaro et al. (2017).

The usage of a wide range of organic and inorganic pesticides, herbicides and fertilizers by the farmers, in the current study, might have contributed to the soil samples genotoxicity. Insecticides are a class of chemical compounds that have a wide spectrum of toxicity and as a result, pose a risk to the environment (Anjum and Malik 2013). Kalefetoglu Macar (2021) reported cytotoxic and genotoxic effects such as decline in mitotic index of pesticide (abamectin) in non-targeted organisms following *Allium cepa* root chromosomal assay. Heavy metals, pesticidies and a variety of other pollutants present in soil were responsible for decline in mitotic index and ultimately cell death in roots of *A. cepa* (Cortes-Eslava et al. 2018; Carita and Marin-Morales 2008). Apart from this, high genotoxicity could possibly be attributable to the application of waste water to agricultural land from Bhuddha Nullah, which is located near the test sites under investigation. Our findings are consistent with some previous study of Pohren et al. (2013) who evaluated genotoxic potential of soil contaminated with heavy metals and reported different chromosomal abnormalities following *Allium cepa* root chromosomal aberration assay. Other studies that revealed cytotoxicity and genotoxicity of metal like chromium in plant cells also supported the current findings (Panda et al. 2002). According to Kwankua et al. (2012), heavy metal exposure hindered cells from entering cell division stages, resulting in a decrease in mitotic index.

Statistical analysis

Statistical analysis was performed using SPSS Statistics software (IBM SPSS Statistis for window, version 21). One-way ANOVA was applied to find significant differences between values of heavy metals and genotoxicity parameters like mitotic index (MI) and total aberrant cells (TAC %) for collected agricultural soil samples. One-way ANOVA and post hoc analysis using the Tukey test have showen significant differences in the observed parameters. Pearson correlation analysis was carried out using different agricultural soil quality parameters which revealed that the content of Cd, Cr, Cu, Co, Ni, Pb and Zn were strongly affected by pH. Soil pH is inversley correlated with these metals and the highest correlation coefficient was found for Zn ($r = -0.889$). Significant positive correlations were observed betweeen Cd and Pb in soil samples ($r = 0.981$, $p \leq 0.01$), which suggested the similar pollution sources of these two heavy metals. Similarly, significant positive correlation were observed between Cu and Co ($r = 0.989$, $p \leq 0.01$) and between Cu and Ni ($r = 0.981$, $p < 0.05$) and between Cu and Zn ($r = 0.973$, $p < 0.05$). These strong positive correlation between these heavy metals, suggested the similar origin of these metal pairs probably from agrochemicals used in the farm. Ihedioha et al. (2016) have reported strong positive and significant correlation between Cr, Ni and Mn in soil from Ada field, Nigeria and has implied that the metals had the same pollution sources. Mitotic index exhibited negative correlation with total aberrant cells. Cu, Co and Ni showed significant negative correlation with mitotic index, and significant positive correlation with TAC, indicating genotoxic nature of these metals.

Conclusion

The findings reveal that different contaminants in the soil can cause genetic damage such as genetic mutations and chromosomal abnormalities in agronomically significant plants. Heavy metals and other pollutants being cytotoxic can damage DNA and therefore, they have genotoxic effects as well. In the current study Cu, Co and Ni exhibited significant positive correlation with total aberrant cells. The current study clearly demonstrated that heavy metals were a prominent factors governing genotoxicity in *A. cepa* root tip cells inducing various mitotic aberrations also revealed the presence of genotoxic compound in the soil samples. These findings provide important information to policymakers and environmentalists for constructive, well-informed dialogue across municipal, industrial and agricultural sectors in order to take collective responsibility for developing strategies to reduce contamination of soil. As a matter of fact, steps must be taken to ensure the adoption of more environmentally friendly agricultural practises.

Declarations

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Conflict of Interest

Author declare no conflict of interest.

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Table

Table 4 is available in the Supplementary Files section.

Figures

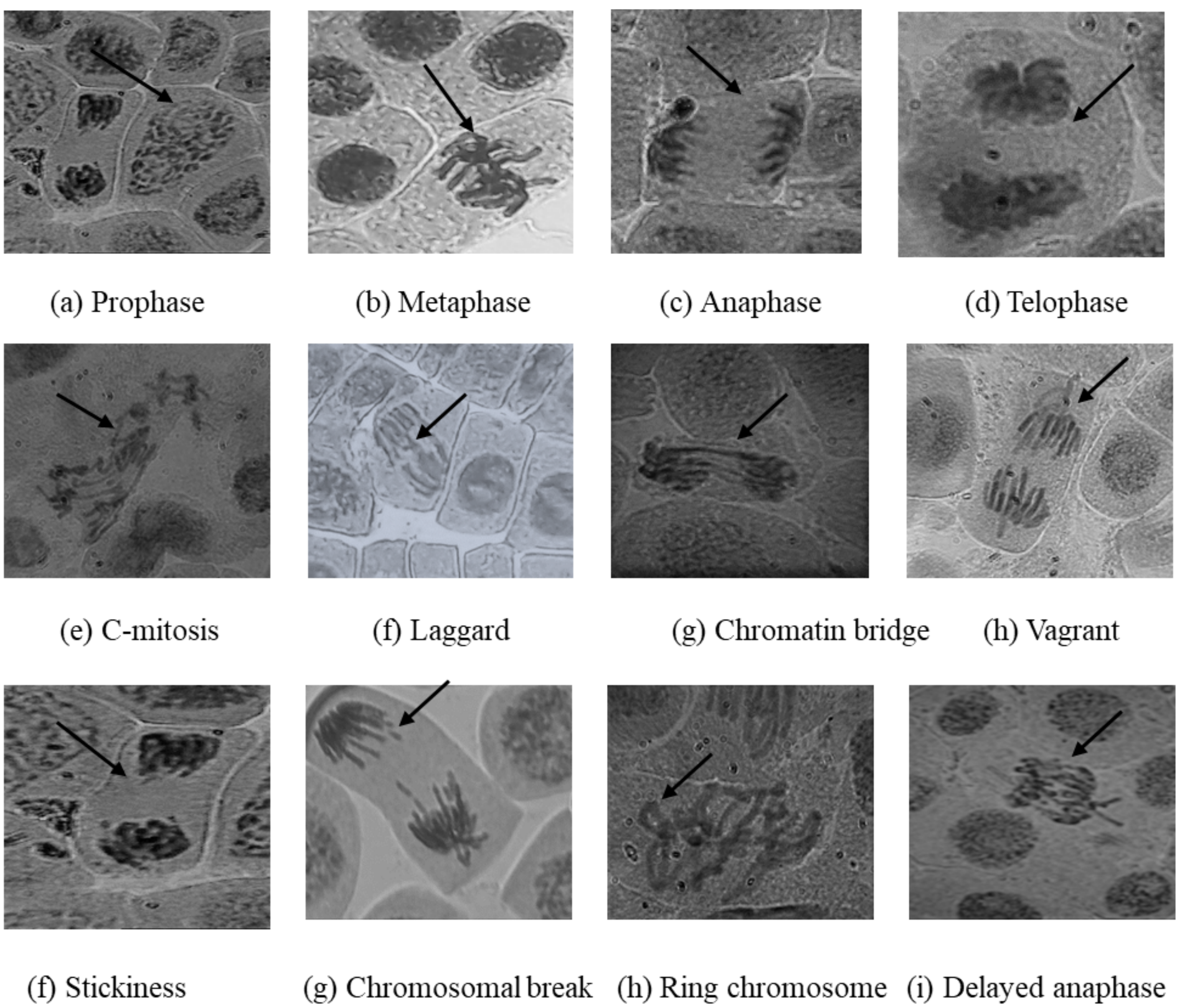

Figure 1

Normal cells showing different phase of mitotic division (a-d) and cells with chromosomal aberrations (e-l) in root tips of *A. cepa*

Supplementary Files

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- Table4docx.docx