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Research article

Analysis of physico-chemical parameters, genotoxicity and oxidative stress inducing potential of soils of some agricultural fields under rice cultivation

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Abstract: In India, agricultural soil has been deteriorated by various on-going practices involving application of chemical fertilizers, pesticides and effluents. Presently, Amritsar (Punjab), an agricultural land, is undergoing rigorous cultivation of wheat and rice crops which consequently increased the application of chemical pesticides and fertilizers for high yield. These agricultural practices are not only pulling out the essential nutrients from the soil but also adding up huge quantity of heavy metals and other dreadful contaminants. Keeping this in view, the present study was planned to assess the physico-chemical parameters and genotoxic potential of soil of four agricultural fields of Amritsar, India by employing *Allium cepa* root chromosomal aberration assay. The responses of different antioxidative enzymes in *A. cepa* on exposure of *Allium* bulbs to different soil samples were also analyzed. In case of physico-chemical parameters cadmium was found more (9.70–30.0 $mg^{-1}kg^{-1}$) than the typical range (3–6 $mg^{-1}kg^{-1}$). The genotoxicity in *A. cepa* (treated with agricultural soil samples) revealed induction of different types of chromosomal aberrations in both modes of treatment (*in situ* and root dip). Among anti-oxidative enzymes, the activities of superoxide dismutase (SOD) and ascorbate peroxidase (APX) were low and gluthione-S-transferase (GST) and dehydro-ascorbate reductase (DHAR) were high in treated bulbs as compared to control *A. cepa* bulbs. Moreover, the results obtained in this study clearly show harmful consequences of agricultural soils of Amritsar in terms of mitotic abnormalities as well as their harmful effect on antioxidant defense system of crop plants cultivated at that particular field.

Keywords: Agricultural soil - Heavy metals - Anti-oxidative enzymes - Root chromosomal aberration assay - *Allium cepa*.

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INTRODUCTION

Soil is found to be one of the key elements which sustain life on earth. It acts as an important part of all terrestrial systems, providing habitat for micro-organisms, plants, and animals (Deyn & Van der Putten 2005); and also act as a storage system for several natural resources (Achazi 2002). Soil is mainly composed of minerals, organic matter having different texture, structure, consistency, colour, chemical, biological and other features. It forms a loose covering of mineral particles that finely cover the earth's surface (Birkeland 1999). Soil has important ecological functions in recycling resources and has purification property as well. Soil supports life through main five processes, biomass productivity, detoxification of pollutants, cycling of C, N, P, S, and H_2O; and also acts as carbon sink (Hansen *et al*. 2008, Blakeslee 2010, Lal 2004).

Enormous studies have revealed that human beings are accidentally or mistakenly exposed to different kinds of contaminants in soil, water, air and food by different direct and indirect routes of exposure like inhalation,

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ingestion and dermal contact which result in different acute and chronic health problems (Bhatnagar 2001, Rekha & Prasad 2006). There are about 3 million cases registered worldwide every year for pesticide poisoning out of which 220,000 are fatal (Bolognesi 2003). Pesticide poisoning results in dreadful ailments like cancer, chronic kidney diseases, sterility among males and females, endocrine disorders, suppression of the immune system, neurological and behavioral disorders, especially among children (Agnihotri 1999). The increase in chromosomal damage and reproductive abnormalities has been reported in agricultural workers (Lander *et al*. 2000, Bhatanagar 2001). Many other reports confirmed the alarming level of pesticide residues in water, air, soil, food commodities and even in biological materials like human blood, fat, milk etc (Gupta 2004). The microbial populations of soil also get affected due to drastic changes in soil pH, alkalinity and organic matter (Zwietan 2004). Keeping this in mind, the present study was planned to analyse four soil samples collected from rice cultivated fields of Amritsar, Punjab, India, as per following objectives:

1. Estimation of physico-chemical parameters including heavy metals *viz*, cadmium, chromium, nickel, zinc, manganese and lead of the soil samples.
2. Genotoxicity assessment of soil samples using *A. cepa* root chromosomal aberration assay
3. Analysis of effect on activity of different anti-oxidative enzymes in *A. cepa* bulbs treated with soil samples.

MATERIAL AND METHODS

The present study pertains to analysis of four soil samples collected from rice cultivated fields of Amritsar, Punjab, India, with respect to their physico-chemical parameters including heavy metals (cadmium, chromium, nickel, zinc, manganese and lead), genotoxicity by *A. cepa* root chromosomal aberration assay and potential to influence activity of anti-oxidative enzymes in *A. cepa* bulb.

Site description and Collection of soil samples

Soils under rice cultivation from four different sites (agricultural fields) of Amritsar district of Punjab were collected. Four sites included Site 1 (S1) Vill. Heir, Verka Block, Amritsar; Site 2 (S2) Vill. Akalgarh, Dhapian, Teh., Baba Bakala, Amritsar; Site 3 (S3) Dera Ramdass, Amritsar and Site 4 (S4) Vill. Chabba, Amritsar (Fig. 1). Random sampling method was adopted for soil collection**.** Soil was collected from 5–6 different sites of each agricultural field by digging soil to depth of 15–20 cm (10×10×20 cm^3 approximately) and pooled together to form one representative sample. The samples were brought to laboratory, dried at room temperature for 72 h and finally ground into fine powder (Cabrera & Rodriguez 1999a). Washed sand was considered as negative control for further analysis.

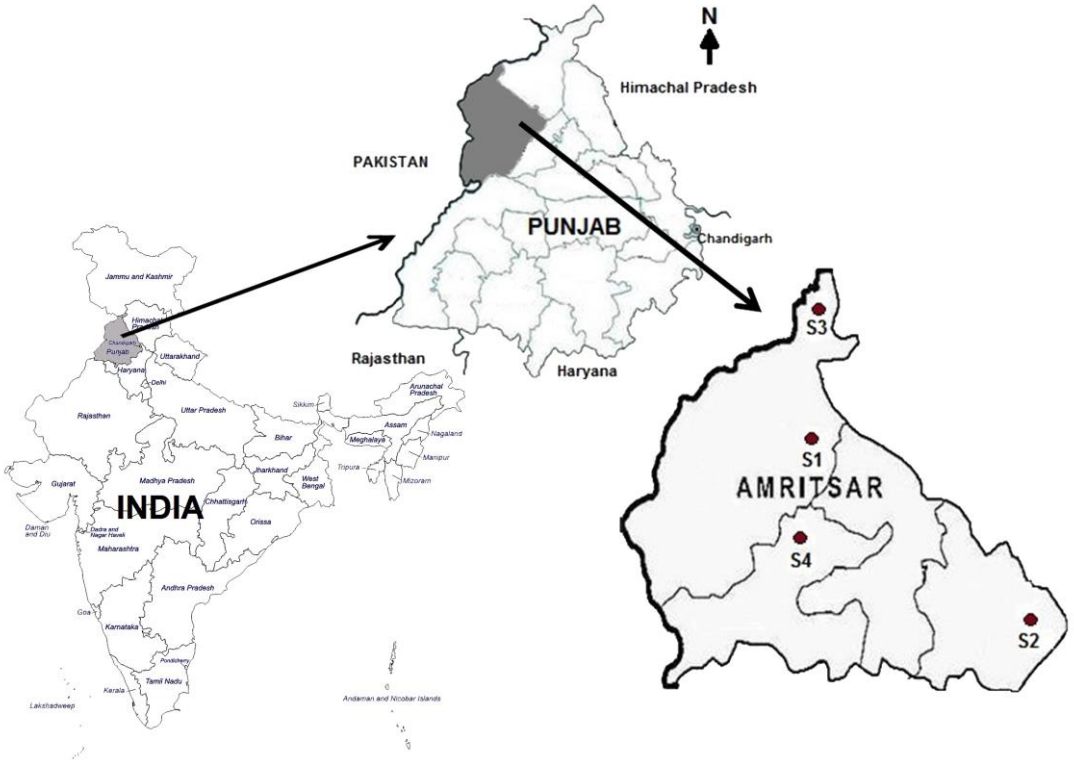

Figure 1. The location of the study area and distribution of the sampling sites. (S1- Verka Block; S2- Baba Bakala; S3- Dera Ramdass; S4- Chabba Village).

Physico-chemical analysis

For physico-chemical analysis, the soil extract was prepared by suspending soil in distilled water in ratio of 1:5 (w/v), shaken on mechanical shaker for 12 h at room temperature. The physico-chemical properties of soil samples were determined by following standard protocols given in Trivedi *et al*, (1985) with slight modifications. pH was measured by pH meter model µ pH system 361 make Shimadzu. The parameters like calcium, magnesium and alkalinity were determined titrimetrically whereas, nitrates and phosphates were estimated using spectrophotometer (model 2202, make Systronics). The contents of sodium and potassium were estimated by flame photometer (model CL 26 D, make ELICO).

Heavy metals analysis

Heavy metal analysis in soil samples were carried out in triplicate as given below. 1 g of soil was digested in glass digestion tube of 250 ml along with 15 ml of nitric acid (HNO_3) at 140˚C and the content was evaporated till dryness. The dried sample was further treated with 3 ml of perchloric acid ($HClO_4$) for oxidation from the sample solution for 30 min at 245˚C. The content was cooled down after digestion, filtered and final volume was made up to 50 ml with distilled water. The heavy metals measurement was performed at Institute of Himalayan Bioresource Technology IHBT Palampur with a Shi-madzu model AA 6300 Atomic Absorption Spectrophotometer (Tokyo Japan). The radiation source was Hallow cathode lamps (HAMA- MATSU PHOTONICS K.K. JAPAN) of metal (Chand *et al*. 2011).

Genotoxic potential

For estimation of genotoxic potential, the soil extract was prepared by suspending soil in distilled water in ratio of 1:2 (w/v), shaken on mechanical shaker for 12 h at room temperature (Cabrera & Rodriguez 1999b). The genotoxic potential of soil extracts was estimated by using *A. cepa* root chromosomal aberration assay. Fresh and young onions were purchased from local market. The primary roots of uniform sized onion bulbs were removed with the help of forceps. For *In situ* treatment, the denuded bulbs were grown directly in small pots containing soil whereas in root dip treatment, the bulbs were placed on couplinjars filled with distilled water for 24–36 h for rooting. After 24–36 h, *A. cepa* bulbs with freshly emerged roots of size 1–2 cm were treated with five concentrations (20, 40, 60, 80 and 100 %) of the soil extract and distilled water (negative control) for 3 h. After treatment, the bulbs were thoroughly washed, root tips were plucked and fixed in Farmer's fluid (glacial acetic acid and ethanol; 1:3). At least 9 root tips were squashed in aceto-orecin to prepare slides. The slides were screened under microscope to score different types of aberrations taking approximately 900 dividing cells. The chromosomal aberrations were apportioned into physiological (attributed to spindle inhibition) and clastogenic (attributed to direct breaking action on chromosomes). Different types of physiological aberrations included laggards, vagrants, stickiness, delayed anaphases, and c-mitosis while clastogenic aberrations included chromatin bridges and chromosomal breaks. Some physiological aberrations such as deviation of chromosome from poles at anaphase, asteroid structure at anaphase, deviation of alignment of chromosome at metaphase, which could not be included among any of the categories, were counted as abnormal metaphase and abnormal anaphases under other physiological aberrations.

Anti-oxidative enzymes

The denuded bulbs were placed on different soil samples contained in small pots for 72 h under saturated conditions. Anti-oxidative enzymes activity was checked by preparing supernatant of 1 gm of treated onion bulb, homogenized in pestle and mortar in 3 ml of chilled phosphate buffer (0.01 M, pH 7.6) and centrifuged (7000 rpm at 4°C for 15 min). The supernatant was used for estimating total protein content (Lowry *et al*. 1951) and activities of different anti-oxidative enzymes spectrophotometrically. The activity of catalase was determined by method of Aebi (1984); Superoxide dismutase activity was estimated according to the methodology of Kono (1978); the level of GST was determined according to protocol of Habig *et al*. (1974); the enzymatic activity of DHAR was measured by method of Dalton *et al*. (1986) and APX activity was measured according to the protocol of Nakano & Asada (1987).

Statistical Analysis

Arithmetic means of activation of antioxidant enzymes: CAT, SOD, GST, APX and DHAR and mean concentrations of physico-chemical parameters and heavy metals Cd, Cu, Ni, Mn, Pb, and Zn in soil samples were calculated. The dependence of activity of antioxidant enzymes in *A.* bulbs and concentrations of physico-chemical parameters including heavy metals in soils were calculated by correlation matrix (significance level at $p<0.05$). Correlation matrix was developed using Microsoft excel 2007.

RESULTS

Table 1. Physico-chemical parameters of soil samples of four different agricultural fields of Amritsar.

Parameter		Sample codes*			
		S1	S2	S3	S4
Bulk density (g cc^{-1})		1.88±0.006	1.89±0.010	1.01±0.003	1.00±0.003
Water holding capacity (%)		31.93±0.56	24.96±1.74	31.08±1.58	36.17±1.33
pH		8.20±0.058	8.20±0.000	8.25±0.000	8.14±0.003
Alkalinity (meq 100 g^{-1})		1.23±0.033	2.33±0.203	2.33±0.033	1.20±0.000
Calcium (mg g^{-1})		90.6±2.667	80.16±0.00	80.16±0.00	53.33±2.66
Magnesium (mg g^{-1})		189.3±2.66	259.80±0.0	446.50±6.6	406.70±2.66
Nitrates (mg g^{-1})		0.015±0.00	0.016±0.00	0.009±0.00	0.004±0.00
Phosphates (µg g^{-1})		0.614±0.03	0.65±0.012	0.74±0.025	0.76±0.032
Potassium (mg g^{-1})		0.35±0.005	0.26±0.004	0.12±1E-09	0.32±0.002
Sodium (mg g^{-1})		0.13±0.000	0.39±0.015	0.10±0.004	0.07±0.002
Soil texture (%)	Sand	74.70±0.13	69.10±0.21	55.60±0.38	69.92±0.05
	Silt	0.77±0.319	0.33±0.127	0.39±0.035	0.28±0.139
	Clay	24.53±0.23	30.56±0.34	44.01±0.34	29.78±0.34
Heavy metals (mg kg^{-1})	Cd (3-6)**	30.0±1.48	25.3±1.78	22.0±0.71	9.70±1.08
	Cu (135-270)**	58.1±0.12	19.2±0.33	28.4±0.15	20.8±0.08
	Ni (ND)**	24.7±0.50	27.2±0.14	26.7±0.43	27.9±0.74
	Zn (300-600)**	96.5±0.11	98.1±0.34	90.6±0.20	61.6±0.46
	Mn (ND)**	345.5±2.2	282.5±1.34	422.1±3.6	318.5±1.64
	Pb (250-500)**	24.8±0.95	19.6±0.36	25.0±0.62	22.1±0.36

All values are Mean ± SD. of 3 observations for each parameter

*S1- Soil sample from Vill Heir, Amritsar; S2- Soil sample from Vill, Akalgarh, Dhapian, Teh. Baba Bakala, Amritsar; S3- Soil sample from Dera Ramdass, Amritsar; S4- Soil sample from Chabba Village, Amritsar; Cd- Cadmium; Cu- Copper; Ni- Nickel; Zn- Zinc; Mn- Manganese; Pb- Lead

** Indicates safe limits, India for heavy metals in agricultural soils (mg kg^{-1}) (Awasithi, 2000).

Physico-chemical parameters

The results for different physico-chemical parameters are shown in table 1. Bulk density of all soils studied was found in the optimal range (1–1.89 g cc^{-1}). Water holding capacity (WHC) was found to be low (24.96–36.17 %) in all the sites. pH was found to be slightly alkaline (8.14–8.25) in all the soil samples. The contents of calcium (53.33–90.6 mg kg^{-1}), magnesium (189.3–446.50 mg kg^{-1}) and phosphates (0.614–0.76 µg kg^{-1}) were found in permissible/safe limits while nitrates (0.004–0.016 mg kg^{-1}) were found to be less in all samples. The potassium content was found in the range of 0.12–0.35 mg g^{-1} while sodium ranged from 0.07–0.39 mg g^{-1} in all soil samples studied. Soil textural composition of different soil samples showed higher concentration of sand (55.60–74.70 %) followed by clay (24.53–44.01 %) whereas the concentration of silt (0.28–0.77 %) was found to be very low.

Heavy metals

Soil samples collected from different agricultural fields showed varied levels of heavy metals. The content of manganese (Mn) ranged from 282.5–422.1 mg kg^{-1}. Following manganese, Zinc (Zn) was the second most abundant metal determined in range of 61.6–98.1mg kg^{-1}. The concentration of Copper (Cu) (19.2–58.1 mg kg^{-1}), lead (Pb) (19.6–25 mg kg^{-1}) and nickel (Ni) (24.7–27.9 mg kg^{-1}) were found to be in safe limits required in agricultural soils while cadmium was found much higher (9.70–30 mg kg^{-1}).

Genotoxic potential

Genotoxicity of all agricultural soil samples was assessed by employing *A. cepa* root chromosomal aberration assay. Different types of physiological (stickiness, delayed anaphase, vagrants, laggards etc.) and clastogenic (chromosomal breaks, bridges etc.) chromosomal aberrations were observed following treatments

Anti-oxidative enzymes

Among all samples, the onion bulbs treated with soil of Site 4 (S4) showed maximum content of proteins (0.15 mg g^{-1}) whereas bulbs treated with soil of Site 2 (S1) showed minimum content (0.075 mg g^{-1}). Catalase activity was found low in two sites (S3 and S4) of the four sites while higher in other two sites (S1 and S2). The activities of superoxide dismutase (SOD) and ascorbate peroxidase (APX) were found lower and activity of gluthione-S-transferase (GST) and dehydro-ascorbate reductase (DHAR) were found to be higher as compared to control in *A. cepa* bulbs treated with all agricultural soil samples (Table 2).

Table 2. Activity of different anti-oxidative enzymes in onion bulbs treated with different agricultural soil samples.

Sites	Catalase (CAT) (U min^{-1} mg^{-1} g^{-1} $protein^{-1}$)	Superoxide Dismutase (SOD) (U min^{-1} mg g^{-1} $protein^{-1}$)	Glutathione-S-Transferase (GST) (U min^{-1} mg^{-1} g^{-1} $protein^{-1}$)	Ascorbate Peroxidase (APX) (U min^{-1} mg^{-1} g^{-1} $protein^{-1}$)	Dehydroascorbate Reductase (DHAR) (U min^{-1} mg^{-1} g^{-1} $protein^{-1}$)
NC*	0.23±0.031	1091±98.84	0.26±0.055	0.60±0.175	0.37±0.062
S1	0.50±0.024	459.40±58.58	0.87±0.054	0.16±0.012	0.86±0.013
S2	0.39±0.035	493.40±104.4	0.47±0.018	0.11±0.023	0.59±0.025
S3	0.15±0.011	917.60±370.6	0.52±0.010	0.47±0.185	0.64±0.022
S4	0.15±0.009	845.50±242.4	0.35±0.027	0.16±0.016	0.42±0.031

Note: NC- Negative control (washed sand); U- Units; S1- Soil sample from Vill Heir, Amritsar; S2- Soil sample from Vill, Akalgarh, Dhapian, The. Baba Bakala, Amritsar; S3- Soil sample from Dera Ramdass, Amritsar; S4- Soil sample from Chabba Village, Amritsar.

DISCUSSION

Physico-chemical parameters

Vast application of chemicals like pesticides and chemical fertilizers results in deterioration of soil and crop quality of a particular field. Bulk density of all soils studied was found in the optimal range (1–2 g cc^{-1}) which is required for better growth of plants (1.0–2.0 g cc^{-1}). Increase in soil bulk density due to deforestation and subsequent cultivation period was earlier reported by many scientists (Mulugeta *et al*. 2005, Mojiri *et al*. 2012). Water holding capacity depicts good physical condition of soil. In present study, water holding capacity (WHC) was found to be low (24.96–36.17 %) in all the sites as compared to suitable range (60–80%) obtained in other soils studied (Castillo & Torstensson 2007). The low WHC in present study can be due to its sandy texture which results in limited storage of water. The similar results were reported earlier by Longwell *et al*. (1963) in Tennessee soils. pH is an important property which can directly affect the solute concentration and absorption in the soil and also assure maximum availability of essential nutrients to plants required for growth and development. The pH values for different soil samples observed in the present study ranged from 8.14-8.25. These results are in conformity with earlier studies on the soils of Kano Urban agricultural land (Dawaki *et al*. 2013) and agricultural soil of Vishakapatnam (Srinavas & Kumar 2001). However in some studies, this range has been considered higher as compared to the ideal range for rice cultivated soils *i.e.* 5.5-6.5 (Focht 1979, Bandara *et al*. 2005). The contents of calcium, magnesium and phosphates were found under permissible limits while nitrates were found to be less in all samples as shown in table 1. Decrease in nitrate content in our study can be attributed to its sandy texture as reported earlier (Gaines & Gaines 1994). Other important factor which can be responsible for low nitrates in agricultural soils can be successive cultivation as reported by Eyayu *et al*. (2009). Also, high nitrate contents in different agricultural soils were reported in previous studies (Rai *et al*. 2011). The potassium content was found in the range of 0.12–0.35 mg g^{-1} while sodium ranged from 0.07–0.39 mg g^{-1} in all soil samples studied. Almost similar results for potassium and sodium were earlier reported by Udotong *et al*. (2008) in soils of wetlands of Eket, Nigeria. Soil textural composition of different soil samples showed higher concentration of sand followed by clay whereas the concentration of silt was found to be very low. The same variation in soil texture of different soils has also been reported earlier (Mohapatra *et al*. 1996). Similarly, many other authors also reported lower clay content in cultivated lands (Eyayu *et al*. 2009, Mojiri *et al*. 2012) might be due to selective removal of clay from the surface by erosion.

Heavy metals

The total concentrations of the heavy metals in agricultural soils are shown in table 1. These are important and very essential micronutrients required for healthy plant growth (Delbari & Kulkarni 2011). Both manganese and zinc were found high but under safe limits (Awashthi 2000). Similar results were reported in agricultural

soils of Abobo area, Western Ethopia (Yitbarek *et al*. 2013). The concentration of Copper (Cu), lead (Pb) and nickel (Ni) were found to be in permissible limits. Cadmium (Cd) was found much higher as compared to its safe limits required for any agricultural soil as given by Awashthi (2000). As cadmium is highly soluble in soil and is extremely toxic in its nature so its presence in the soil is totally undesirable and harmful. In present study, the increase in cadmium can be due to excessive use of phosphate based fertilizers containing high content of Cd as earlier reported by McLaughlin *et al*. (1996). Moreover, many scientists in the past studies had witnessed significant increases in cadmium in fertilized soils as compared to unfertilized ones (Williams & David 1973, Mann *et al*. 2002). High cadmium toxicity/stress may sometimes leads to death of plants as reported in seedlings of bean (Mo & Li 1992). High amounts of heavy metals especially Cd and Pb in the plants adversely affect the absorption and transport of essential elements, disturb the metabolism, and showed direct impact on growth and reproduction (Xu & Shi 2000). Metal uptake by plants can be affected by metal concentration in soils, soil pH, cation exchange capacity, organic matter content, types and varieties of plants, and plant age (Alloway & Davies 1971).

Genotoxic potential

In case of genotoxicity study, different types of physiological (stickiness, delayed anaphase, vagrants, laggards) and clastogenic (chromosomal breaks, bridges) chromosomal aberrations were observed following treatments (in-situ and root dip) with different soil samples studied using *Allium cepa* assay (Fig. 2 & 3). Lah *et al*. (2008) also evaluated the genotoxicity of soil from six different sites of agricultural and industrial areas using *Tradescantia* MCN assay. In present study, genotoxicity has shown negative correlation with lead and positive with sodium content in the soil (Table 3). Since, lead is not soluble in water, thus it can be possible that lead is not fully translocated into plant system. Due to increase in sodium content, lead can be possibly replaced by sodium (important nutrient) which reached plant easily. Besides, the mobility, solubilty and bioavailability of lead in soil is largely controlled by complex interactions governed by several biogeochemical factors (Dumat *et al*. 2006, Kopittke *et al*. 2008, Lawal *et al*. 2010, Vega *et al*. 2010, Arias *et al*. 2010, Bi *et al*. 2010, Liu *et al*. 2010), which may resist the availability of lead in the plants. So this may be the main reason that present soils under study do not showed lead toxicity and thus induced moderate genotoxic effect in all soil samples.

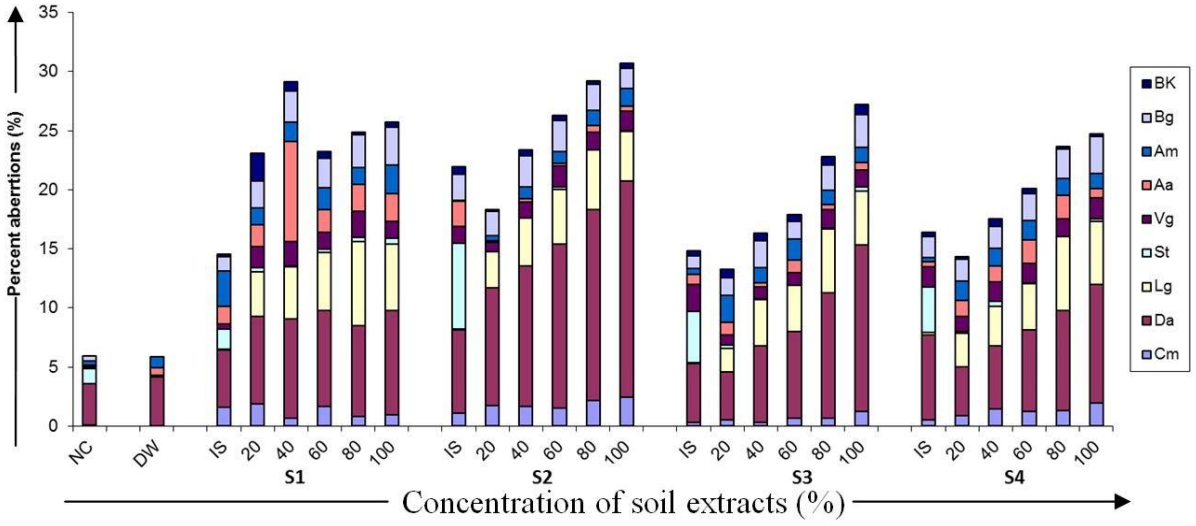

Figure 2. Percent aberrant cells in *Allium cepa* root tips following *in situ* and root dip treatment.

It was reported that heavy metals show deleterious effects on cell division of plants (Mo & Li 1992). Duan & Wang (1995) observed in his study that when beans were treated with low doses of Cd, Pb and Zn, the period of cell division elongates while with increased dose period remain shorter. Genotoxicity caused by heavy metals in plants affects the synthesis and the duplication of DNA and chromosomes by inducing different chromosomal aberrations. Pohren *et al*. (2013) also reported different types of chromosomal abnormalities in barley and *A. cepa* under heavy metals stress respectively. In our study cadmium was found higher than the safe limits as compared to other toxic metals. Cadmium generally has a capability to bind with the nucleotides causing direct damage to DNA by modifications in base structure and ultimately leads to lesions, DNA strand breaks, exchanges of sister chromatids, destruction of DNA-proteins crosslinks, effect on activity of different anti-oxidative enzymes and inhibition of DNA repair enzymes (Badisa *et al*. 2007, Lin *et al*. 2007, Markovska *et al*. 2009, Unyayar *et al*. 2010). The same results were observed by Zhao & Mo (1997) in his study where he found

that continuous exposure of beans, garlic and *A. cepa* with Cd, Pb, Hg resulted in different mitotic abnormalities *viz.*, polyploidy, C-karyokinesis, chromosomal bridges, rings, fragments, chromosomal fusion, micro-nucleated cells and nuclear decomposition.

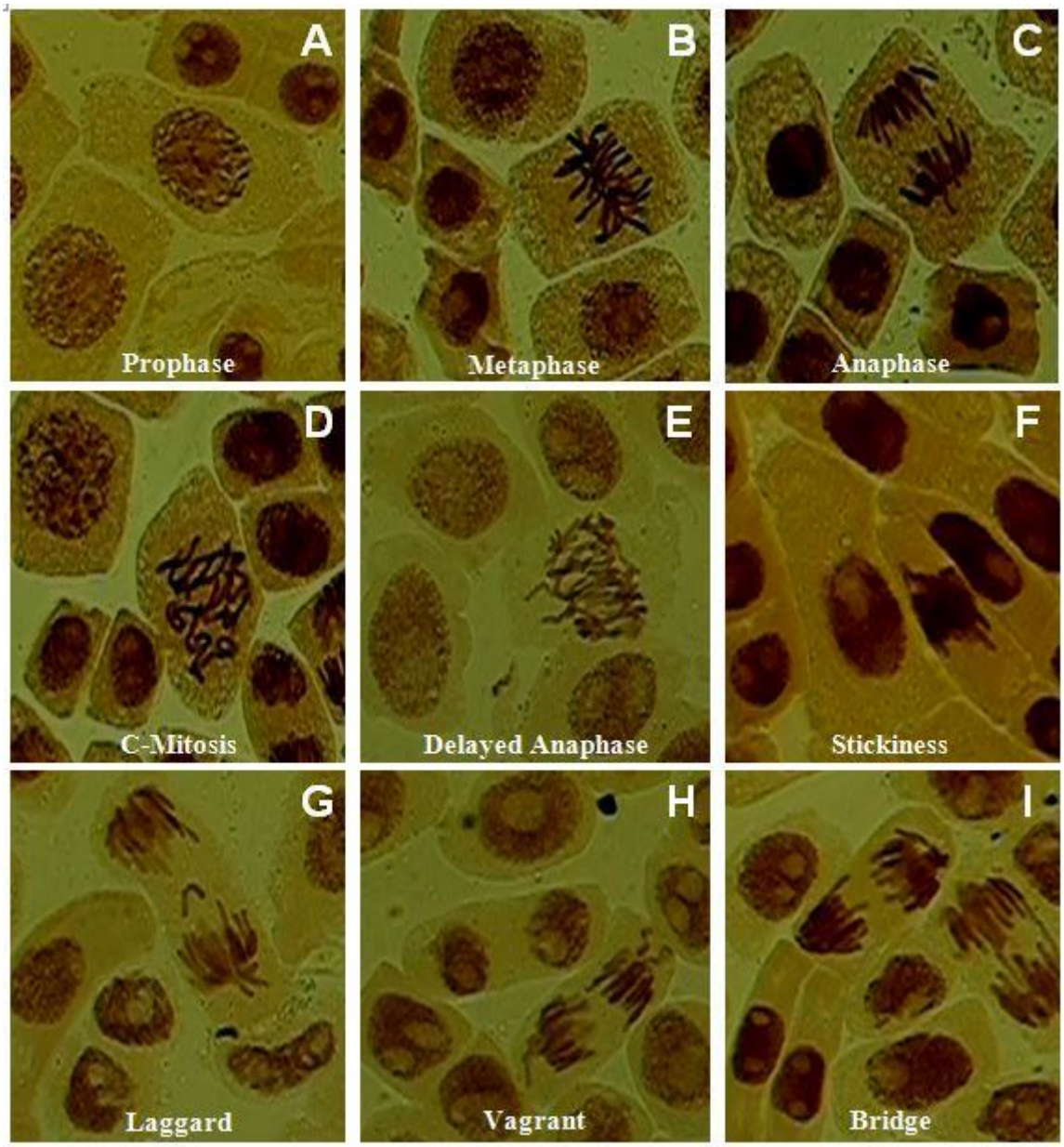

Figure 3. *Allium cepa* root tip cells treated with different soil samples showing spectrum of aberrations: **A–C**, Normal stages of cell division; **D–I**, Different types of chromosomal aberrations.

Anti-oxidative enzymes

A very common deleterious effect of pollutants is to produce high amounts of free radicals and other reduced oxygen species. In plants, the main sources of ROS (reactive oxygen species) production include pathogens, heavy metals, herbicides, air pollutants, drought and UV-B rays. ROS has been identified as the superoxide radical (O_2-), hydroxyl radical (-OH), hydroperoxyl radical (HO_2*), hydrogen peroxide (H_2O_2), alkoxy radical ($RO^{\cdot}$), singlet oxygen ($^1O_2$) and excited carbonyl (RO*) which are found to be highly cytotoxic to plants (Karuppanapandian *et al*. 2011, Vellosillo *et al*. 2010). ROS production and removal must be controlled strictly in order to avoid oxidative stress in plants. High levels of these reactive oxygen species can cause irreplaceable damage to biomolecules such as lipids, proteins and DNA. Plants possess complex anti-oxidative defence system which comprised of enzymatic (SOD, CAT, APX, GST, DHAR, MDHAR, detoxifying lipid peroxidation (LP) products like ascorbate and glutathione) and non-enzymatic (tocopherols, carotenoids and phenols) components which play very important role in scavenging these ROS. These systems are mostly located in organelles like chloroplasts, mitochondria and peroxisomes. Enzymatic components can convert the

Table 4. Correlation matrix among the physico-chemical properties, anti-oxidative enzymes response and In-situ simulation of different agricultural soils of Amritsar (Punjab).

	BD	WHC	pH	Alk.	Ca	Mg	NO3	PO4	K	Na	Cd	Cu	Ni	Zn	Mn	Pb	APX	CAT	DHAR	GST	SOD	In-Situ
BD	1																					
WHC	**-0.65**	1																				
pH	**0.07**	**-0.49**	1																			
Alk.	**0.02**	**-0.76***	**0.71***	1																		
Ca	**0.67**	**-0.58**	**0.72***	**0.31**	1																	
Mg	-0.95*	**0.41**	**0.07**	**0.24**	**-0.62**	1																
NO_3	0.93*	**-0.80***	**0.42**	**0.32**	**0.85***	**-0.82***	1															
PO_4	-0.97*	**0.55**	**-0.17**	**0.04**	**-0.79***	0.96*	-0.92*	1														
K	**0.47**	**0.27**	**-0.76***	**-0.81***	**-0.12**	**-0.65**	**0.12**	**-0.44**	1													
Na	**0.69**	-0.92*	**0.12**	**0.57**	**0.32**	**-0.47**	**0.72**	**-0.52**	**0.003**	1												
Cd	**0.78***	**-0.63**	**0.62**	**0.27**	0.98*	**-0.73***	0.92*	**-0.87**	**-0.005**	**0.42**	1											
Cu	**0.44**	**0.17**	**0.19**	**-0.48**	**0.65**	**-0.61**	**0.41**	**-0.63**	**0.39**	**-0.31**	**0.64**	1										
Ni	**-0.56**	**0.07**	**-0.39**	**0.25**	**-0.82***	**0.67**	**-0.60**	**0.74**	**-0.25**	**0.11**	**-0.81**	-0.96*	1									
Zn	**0.72***	**0.80***	**-0.73***	**0.53**	0.95*	**-0.58**	0.92*	**-0.77**	**-0.22**	**0.57**	0.95*	**0.40**	**-0.62**	1								
Mn	**-0.54**	**0.30**	**0.67**	**0.20**	**0.23**	**0.52**	**-0.28**	**0.35**	**-0.67**	**-0.63**	**0.07**	**0.25**	**-0.26**	**0.08**	1							
Pb	**-0.30**	**0.50**	**0.44**	**-0.25**	**0.33**	**0.15**	**-0.17**	**0.06**	**-0.21**	**-0.77***	**0.21**	**0.67**	**-0.60**	**0.07**	**0.86***	1						
APX	**-0.62**	**0.13**	**0.72***	**0.45**	**0.13**	**0.67**	**-0.31**	**0.49**	**-0.87***	**-0.45**	**-0.02**	**-0.04**	**-0.008**	**0.07**	0.94*	**0.65**	1					
CAT	0.96*	**-0.46**	**0.06**	**-0.16**	**0.72***	-0.99*	**0.87***	-0.99*	**0.55**	**0.48**	**0.81***	**0.65**	**-0.73***	**0.68**	**-0.42**	**-0.08**	**-0.57**	1				
DHAR	**0.61**	**-0.25**	**0.53**	**-0.06**	0.92*	**-0.67**	**0.71***	**-0.78**	**0.12**	**0.02**	**0.90***	**0.89***	-0.97*	**0.76***	**0.27**	**0.54**	**0.06**	**0.75***	1			
GST	**0.60**	**-0.08**	**0.34**	**-0.27**	**0.81***	**-0.71**	**0.62**	**-0.77**	**0.30**	**-0.08**	**0.81***	0.96*	-0.99*	**0.62**	**0.20**	**0.56**	**-0.05**	**0.77***	0.97*	1		
SOD	-0.98*	**0.54**	**0.06**	**0.11**	**-0.59**	0.98*	**-0.86***	0.95*	**-0.59**	**-0.62**	**-0.71***	**-0.47**	**0.56**	**-0.62**	**0.60**	**0.31**	**0.71***	-0.97*	**-0.58**	**-0.60**	1	
In-Situ	**0.44**	**-0.74***	**-0.15**	**0.49**	**-0.05**	**-0.24**	**0.40**	**-0.22**	**0.03**	0.92*	**0.04**	**-0.60**	**0.46**	**0.22**	**-0.75***	-0.94*	**-0.52**	**0.20**	**-0.34**	**-0.42**	**-0.40**	1

*significant at $p<0.05$ level; '-' indicates negative correlation

Note: BD-Bulk density (g cc^{-1}); WHC-Water holding capacity (%); Alk-Alkalinity (meq/100ml); Ca-Calcium (mg g^{-1}); Mg-Magnesium (mg g^{-1}); NO_3-Nitrates (mg g^{-1}); PO_4-Phosphates(mg g^{-1}); K-Potassium (mg g^{-1}); Na-Sodium (mg g^{-1}); Cd-Cadmium (mg kg^{-1}); Cu-Copper (mg kg^{-1}); Ni-Nickel (mg kg^{-1}); Zn-Zinc (mg kg^{-1}); Mn-Manganese (mg kg^{-1}); Pb-Lead (mg kg^{-1}); APX- Ascorbate peroxidase (U min^{-1} mg^{-1} g^{-1} protein); CAT-Catalase (U min^{-1} mg^{-1} g^{-1} protein); DHAR-Dehyro-ascorbate reductase (U min^{-1} mg^{-1} g^{-1} protein); GST- Gluthione-S-transferase (U min^{-1} mg^{-1} g^{-1} protein); SOD-Superoxide dismutase (U min^{-1} mg^{-1} g^{-1} protein).

potentially harmful superoxide radical and hydrogen peroxide to water and molecular oxygen, thus preventing cellular damage (Scandalios 2005).

Catalase showed low activity in two sites (S3 and S4) while high in other two sites (S1 and S2) as compared to control. Heavy metal stress leads to accumulation of H_2O_2 that can result in inactivation of catalase activity (Olteanu *et al*. 2011). Decrease in CAT activity with high content of cadmium was reported by Fornazier *et al*. (2002) and Sandalio *et al*. (2001). Dey *et al*. (2007) also reported a decline in CAT activity in wheat seedlings grown in the presence of cadmium chloride and lead nitrate and *Allium* cultivars under the stress of soil moisture respectively. Vitoria *et al*. (2001) determined increase in CAT activity in radish following exposure to high cadmium concentrations. Increase in CAT activity is adapted possibly to overcome the damage caused to tissue metabolism by toxic levels of reducing H_2O_2 (Karuppanapandian *et al*. 2011). These two reports confirmed that in present soils studied, cadmium may be the main culprit which leads to this type of CAT nature. Other reports stated that other than heavy metals, salinity and drought conditions may be the other reasons for decrease in CAT activity (Boo & Jung 1999, Karuppanapandian & Manoharan 2008, Hojati *et al*. 2010).

Superoxide dismutase activity was found lower in present study. Similar reduction in activity of SOD was earlier reported in pine roots due to cadmium toxicity (Schutzendubel *et al*. 2001). Contradictory to our results, Sharma *et al*. (2010) found increase in activity of SOD in plants of family Brassicaceae in response to heavy metal stress whereas Dixit *et al*. (2001) reported similar findings in pea roots and leaves. It was reported that cadmium increases the binding of metal ions to sulphydryl group of enzymes which ultimately increase the phytotoxicity of metals (Van Assche & Clijsters 1990) and resulted in low activity of SOD (Somashekaraiah *et al*. 1992, Guan *et al*. 2009). In another study, inefficiency of SOD in ROS scavenging has reported in rice crops under water deficit conditions (Boo & Jung 1999). Therefore in our study cadmium can be one of the factors that played a major role in inducing low activities of SOD.

The activity of gluthione-S-transferase (GST) was found high as compared to control in the present study. According to Marrs (1996), GST is involved in detoxification of herbicides, heavy metals and pathogen attack. Fatima & Ahmad (2005) also determined increase in GST activities in *A. cepa* under high stress of heavy metals. During stress conditions, lipid peroxidation products like hydroperoxides, epoxides, organic hydroperoxides and oxidative products of DNA degradation acts as substrates for GST. All these products get conjugated to glutathione and ultimately detoxified and get stored in vacuoles of cytosol. High activity of scavenging these toxic peroxidation products in barley vacuoles via GST were described earlier (Tommasini *et al*. 1993). Apart from this, ascorbate peroxidase also mediated this conjugation and detoxification of oxidized glutathione to unsaturated phenylpropanoids (cinnamic and coumaric acids) in plants (Dean & Devarenne 1997).

All soils studied showed low activity of ascorbate peroxidase (APX). Perioxidase catalyses the oxidation of phenols, amines and act as biomarkers of sublethal toxicity caused by heavy metals so called as stress enzyme (Zhang *et al*. 2007). Low activity of APX with exposure to high content of cadmium in pine roots was reported earlier (Schutzendubel *et al*. 2001). According to Schutzendubel & Polle (2002), cadmium induced inhibition can be associated with high H_2O_2 accumlation and growth retardation as he reported in popular roots. Fatima & Ahmad (2005) observed increase in APX activity in *A. cepa* under high stress of heavy metals. Boo & Jung (1999) reported low activity of APX in detoxification of ROS in rice under water deficit conditions. Increase in DHAR levels as compared to control were observed in our study. Increase in DHAR activity was previously reported in many plants in response to stresses like drought, metal toxicity and chilling effect (Sharma & Dubey 2005 & 2007, Yoshida *et al*. 2006, Maheshwari & Dubey 2009). Contradictory to our findings, Fatima & Ahmad (2005) reported no significant change in DHAR activity in *A. cepa* under high stress of heavy metals.

CONCLUSION

Present study showed clearly that cadmium act as one of the major factor governing genotoxicity in *A. cepa* root tip cells by induction of different mitotic abnormalities and showed significant variations in the antioxidant enzymes of *A. cepa.* It can be emphasized that the *A. cepa* test system responds to contaminants that are existing in the areas of study. *A. cepa* test system was found to be quick, simple, highly sensitive and capable of identifying genotoxicity of soil samples. This test system can be used as useful biomarker/ indicator for the detection of pollutants in the any ecosystems *viz*., air, water and soil. This information can be used for generating developmental strategies for soil management and as well as in implementation of risk assessment procedures in future.

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