Chapter-5

Discussion

Here are the opinions on which my facts are based

-Anon
DISCUSSION

Soil is one of the most dynamic ecological factors that support life on earth and have always been vital to human beings and their health. Soils are increasingly becoming sinks for a wide range of hazardous pollutants like pesticides, chemical fertilizers, industrial wastes etc. from various anthropogenic activities like industrial, domestic and intensive agricultural practices (White and Claxton, 2004; Ping et al., 2011; Pohren et al. 2013). These omnipresent compounds get accumulated in the soil matrix due to their persistent and hydrophobic nature. Among different pollutants, contamination of soil by heavy metals have attained focus from scientific community worldwide to assess their potential risks. Accumulation of heavy metals in agricultural soils can lead their entry into human beings via various routes of exposure such as consumption of contaminated drinking ground water and food crops; dermal contact and through food chain. Therefore, it is necessary to acquire better understanding of soil contamination in a comprehensive manner in order to evade potential risks linked with contaminated agricultural soils and associated food chains. Hence, there is an urgent need to have information regarding this important component of environment (Majer et al., 2002; Motelay-Massei et al., 2004; Feng et al., 2013).

Apart from being toxic, the danger of heavy metals being mutagenic, genotoxic as well as carcinogenic poses a problem of great concern. It is mandatory to evaluate agricultural soils for their potential risks in biological systems as physico-chemical analysis alone could not evaluate the quantitative risk of soil pollution (Bierkens et al., 1998; Maxam et al., 2000; Monarco et al., 2002; White and Claxton, 2004; Visioli et al., 2013). The genotoxicity assessment of the contaminated soils using well established bioassays have been widely documented (Kwasniewska et al., 2012; Feng et al., 2013; Rodriguez-Ruiz et al., 2014).

Among various bioassays, plant bioassays have gained a great attention due to the fact that plants are readily available and it is easier to handle the plant material as compared to animals. Chromosomal aberration assays using various plant systems
including *Allium cepa*, *Tradescantia* and *Vicia faba* has been validated by International Programme on Chemical safety (IPCS) under the auspices of World Health Organization (WHO) and United Nations Environment Programme (UNEP) to estimate genotoxicity of various compounds, wastewater samples, soil solutions and agricultural soils contaminated with pesticides and heavy metals (Cabrera and Rodriguez, 1999a,b; Chandra *et al*., 2005; Fernandes *et al*., 2007; Ferreira *et al*., 2011; Masood and Malik, 2013; Souza *et al*., 2013). Among different higher plant bioassays, *Allium cepa* root chromosomal aberration assay has been recommended as it is simple, less expensive, requires least facilities, has large and less number of chromosomes, chromosome morphology similar to that of mammals and has unique adaptation for *in situ* studies. Apart from the genotoxic responses, biochemical changes/enzyme alterations also play a significant role to indicate the magnitude of toxicity.

Enzymes of the detoxification machinery serve as important markers of environmental pollution (Filho *et al*., 2001). The efficacy of antioxidant enzymes as biomarkers of contaminants, especially that of heavy metals has been established by several researchers (Ahmad *et al*., 2000; Geret *et al*., 2002, 2003; Nadgorska-Socha *et al*., 2013). Several studies indicated that heavy metals caused oxidative stress by intervening the activities of antioxidative enzymes (Fatima and Ahmed, 2005; Hu *et al*., 2007; Tabrez and Ahmed, 2009a,b,c,d; Tang, 2012; Yu *et al*., 2013; Irfan *et al*., 2014).

Presently in India, ongoing rigorous agricultural practices are pulling out the essential nutrients particularly in wheat and rice crop fields. The district Amritsar of Punjab (India), an agricultural land, is under intensive cultivation of wheat, rice and some vegetable crops. In order to have high yield, vast varieties of pesticides and fertilizers, both organic and inorganic, are being used by the farmers which ultimately result in soil pollution. Apart from this, the direct use of sewage sludge, industrial wastes and waste water to agricultural land as source of plant nutrients aroused serious concern as they are known to contain many toxic metals along with useful nutrient elements. Keeping in view the alarming consequences of contamination of agricultural soils of Amritsar, Punjab (India), the present study was carried out to evaluate the genotoxic potential of different soil samples collected from rice and wheat fields.

Estimation of various physiochemical parameters *viz.* pH, alkanity, soil texture, calcium, magnesium, nitrates, phosphates, sodium, potassium and some of the heavy metals were also carried out. The study also involves estimation of total protein content and analysis of responses of certain antioxidative enzymes *viz.* ascorbate peroxidase (APX), catalase (CAT), dehydroascorbate reductase (DHAR), glutathione reductase (GR), glutathione-S-transferase (GST), guaicol peroxidase (POD) and superoxide dismutase (SOD), in *A. cepa* bulbs exposed to these soils. For convenience of study, the results have been discussed under different headings: physico-chemical analysis of soil, genotoxicity studies in *Allium cepa* root chromosomal aberration assay and biochemical parameters

5.1. **Physico-chemical analysis of soil**

Analysis of physico-chemical characteristics included parameters like soil texture, pH, alkalinity, contents of calcium, magnesium, nitrates, phosphates, sodium, potassium and content of heavy metals (cadmium, chromium, copper, iron, lead, manganese, nickel and zinc). Soil samples were collected from different agricultural fields under rice cultivation during September, 2009 (r1) and September 2010 (r2); and under wheat cultivation during March 2010 (w1) and March, 2011(w2).

Soil texture refers to relative proportion of different sized particles (sand, silt and clay) that make up the soil. These particles are grouped according to their size into soil separates. The size of particles varies for sand from 0.075 mm to 1.5 mm, silt from 0.075 mm to 0.02 mm and clay from 0.02 mm to 0.0015 mm. Soil texture classification is based on fractions of soil separates present in a soil. In the present study, analysis of textural composition of soil samples collected from different agricultural fields under rice and wheat cultivation revealed that content of sand in all soil samples was highest followed by clay. The content of silt was very low and was observed to be less than < 3%. The content of sand, silt and clay particles in all the samples collected during both samplings r1 and r2 from rice cultivated agricultural soils ranged from 56.80 - 65.71 %; 0.43 - 1.96 %; 32.81 - 41.49 % and 53.12 - 61.44 %; 0.96 - 1.35 %; 37.42 - 44.94 %
Discussion

respectively. Among soil samples (w1 and w2 sampling) of wheat cultivated fields the content of sand, silt and clay particles ranged from 56.21 - 65.71 %, 1.00 - 2.84 % and 33.29 - 42.34% and 54.72 - 64.78 %, 1.05 - 2.82% and 33.94 - 44.23 % respectively. All the soil samples studied were observed to be in the category of sandy clay loam based on Soil Textural Triangle as shown below which gives names to the soil depending upon various combinations of sand, silt and clay sized particles.

**SOIL TEXTURAL TRIANGLE**

*Source: http://www.nrcs.usda.gov/*

Several reports have mentioned the soil textural analysis throughout the world and supports our present study. Vidhya *et al.* (2001) analyzed textural composition of soils irrigated with effluents from a small scale chemical industry and found that content of sand, silt and clay particles ranged from 52 - 79 %, 4.5 - 26 % and 3.0 - 9.0%. Gokalp *et al.* (2010) analyzed textural composition of saline and alkaline grassland soils of Kayseri, Turkey and found that the content of sand, silt and clay
content to vary from 17.2 - 93.4, 3.15 - 67.0 and 0.62 - 73.4%, respectively. Ashraf et al. (2012) estimated physico-chemical characteristics of the grassland soils of Yusmarg Hill Resort (Kashmir, India) and found that major proportion being comprised by the sand fraction and soils were found to have a sandy silt character. Chen et al. (2014) studied soil texture of heavy metals polluted soil of Jiangsu province of China, traditionally cultivated under rice and winter wheat rotation crops. Soil particle fractions of different sizes were obtained and the content of coarse sand, fine sand, silt and clay in the studied soil was found to be 20.5 %, 33.6 %, 31.9 % and 14.0 %, respectively. Jansa et al. (2014) estimated various physico-chemical soil properties of crop fields of Switzerland and reported the content of clay and sand to range 8.2 - 55.5 % and 4.3-71.4%, respectively. The soil texture for different sampling sites was recorded to vary from heavily clayey to very sandy. Nakase et al. (2014) also reported the textural composition of agricultural soils collected from central Arizona, USA, affected due to prehistoric human activity and eolian deposition. The content of sand, silt and clay were found to range from 7.0 - 17.7%, 40.8 - 66.0% and 21.5 - 52.2%, respectively.

pH is an important parameter which measures hydrogen ion concentration and depends largely on the relative amount of the absorbed hydrogen and hydroxyl ions. It indicates the chemical composition and acidic or alkaline nature of soil. Availability of macronutrients to plants tend to decrease in soils with low pH whereas micronutrients tend to be less available in soils with high pH. Moreover, availability of nutrients is also directly influenced by pH of the soil (Adhikary, 2014). Various anthropogenic activities result in change in pH of soil and also enhance toxicity of soil. pH of all the soil samples collected from the fields under rice and wheat cultivation ranged from 7.32 to 8.51. In this study, pH of all the agricultural soil samples was found to be alkaline in nature and the results were similar to previous report by Zaiad (2010) who reported the alkaline range of pH of soil samples collected from sides of Al-Khums city between 8.1 - 8.6. Joshi and Kumar (2011) also evaluated agricultural soil of Sanganer region of Jaipur, Rajasthan for various physico-chemical parameters and reported pH to range from 7.6 - 9.2. Pujar et al. (2012) analyzed the physico-chemical characteristics of soil in Bijapur taluka, Karnataka and found that pH of soil ranged from 7.9 to 8.4. Several other studies have also reported the alkaline nature of different soil samples collected
from different parts of the world and indicated the pH value of more than 7 (Masakorala et al., 2013; Moghimi et al., 2013; Wagh et al., 2013; Ma et al., 2013; Masakorala et al., 2014).

Alkalinity is a measure of the acid neutralization capacity of a solution which gives stoichiometric sum of bases in the solution (Katnoria et al., 2011). In the natural environment, the most common sources of alkalinity include carbonates, bicarbonates, borates, phosphates, silicates, nitrates and hydroxides etc. Carbonates make up most of total alkalinity due to the presence of carbonate rocks and carbon dioxide in the environment. In the present study, the alkalinity of all the agricultural soil samples collected ranged from 0.23 mEq/100g to 3.30 mEq/100g. Katnoria et al. (2008) estimated physico-chemical characteristics of four soil samples collected from different agricultural fields of Amritsar (Punjab). The alkalinity of soil samples ranged from 0.13 to 0.96 mEq/100g. Prrveen et al. (2012) estimated alkalinity of soil collected from different sites of Nanded city, Maharashtra. The alkalinity of soil samples was observed to range between 1.0 to 3.5 meq/100g. Devdatta and Shashikant (2014) also analyzed soils along the estuarine area of Bhayander and Naigaon, Thane, Maharashtra for various physico-chemical parameters and reported the content of alkalinity in the range of 1.21-4.37 meq/100. Differences in content of alkalinity of different soil samples have been reported in enormous number of studies (Kelly-Quinn, 2003; Bhat et al., 2011; Pujar et al., 2012).

Calcium and magnesium elements have been identified as essential plant nutrients which serve as raw material for growth and development of plants. Calcium in the form of calcium pectate is an essential component required for the cell wall formation. Apart from its nutrient value, calcium in soil also helps to regulate the transport and retention of other nutrients like phosphorus, nitrogen and molybdenum. However, excess of calcium decrease the uptake of potassium and magnesium (Helper, 2005; Caffall and Mohnen, 2009). Magnesium is an essential element in biological systems and forms the vital component of chlorophyll and photosynthesis. It helps in the uptake of minerals such as nitrogen and phosphorus and plays a significant role (Huber and Jones, 2013; Verbruggen and Hermans, 2013). In the present study, all the agricultural soil samples collected from rice and wheat cultivated fields showed calcium
content to range from 21.37 mg/g - 101.50 mg/g and 30.44 mg/g - 74.82 mg/g respectively while the content of magnesium was found to range from 86.61 mg/g - 318.80 mg/g and 101.50 mg/g - 279 mg/g, respectively. Enormous studies from other regions have also reported the content of calcium and magnesium in different soil samples (Jodral-segadoj et al., 2006; Udotong et al., 2008; Doi and Ranamukhaaracchhi, 2009; Acosta et al., 2011; Kebir and Bouhadjera, 2011; Johnson et al., 2012; Tamminen et al., 2012; Upadhyay et al., 2013). Doi and Ranamukhaaracchhi (2009) estimated content of calcium of soil under paddy cultivation from different villages (Udom Sup village, Wang Nam Kiao district, Nakhon Ratchasima Province) of Thailand. The study revealed that content of calcium ranged from 1.47 to 5.44 mg/g in all the soil samples studied. Moraetis et al. (2011) also analyzed physico-chemical characteristics of soil samples from cultivated and uncultivated areas located in the region of Peloponnese in Greece affected due to olive mill wastewater irrigation. The content of calcium and magnesium was observed to be 917 mg/kg, 899 mg/kg and 482 mg/kg, 669 mg/kg for cultivated and uncultivated soil samples, respectively. Panwar et al. (2011) examined agricultural soils of Jalpaiguri district of humid subtropical India (West Bengal) for their physico-chemical characteristics. Range of calcium and magnesium content observed was 0.621 - 0.729 cmol/ kg and 0.236 - 0.351 c mol/ kg, respectively. Ahmed et al. (2012) studied the effect of industrial effluents on physico-chemical properties of agricultural soils collected from Bhairavgarh, Ujjain, MP, India. The content of calcium in contaminated and uncontaminated soil was found to range between 189 to 273 mg/kg and 63 to 94.5 mg/kg while content of magnesium was found to be 8.50 to 45.9 mg/kg and 3.08 to 6.99mg/kg, respectively. Ganorkar and Chinchmalatpure (2013) studied soils from Rajura bazar in Amravati district of Maharastra (india) for their physicochemical characteristics. The content of calcium and magnesium were found to range from 0.07 - 0.16 % and 0.842 – 0.895 %.

Soils are the main terrestrial reservoir of nutrients and their evaluation forms an important aspect for sustainable agricultural production (Quinton et al., 2010). Nitrogen, phosphorus and potassium are important soil elements that control soil fertility and yield of crops (Singh and Mishra, 2012). Nitrogen is one of the essential
nutrients for healthy plant growth. It acts as fundamental component of different proteins, enzymes and metabolic processes of growing plants. The vast majority of the total nitrogen in soil (> 98%) is unavailable to plants and is in the form of organic matter. It is available to the plants in the inorganic form of ammonium, nitrite and nitrate by the action of symbiotic nitrogen fixing microorganisms like \textit{Nitrosomonas}, \textit{Nitrosospiras} and \textit{Nitrobacter} species present in the soil (Gordon \textit{et al.}, 2001; Rubio-Asensio \textit{et al.}, 2014). Depending upon the soil type, microbial activity, use of agrochemicals etc. the content of nitrates in soil varies both spatially and temporally. Enormous number of surveys and reviews have reported variation in the levels of nitrate content of soil from few hundred micromolar to milimolar concentrations (Wolt, 1994; Reisenauer, 1996; Miller and Smith, 1992, 2008; Dechorgnat \textit{et al.}, 2011).

In the present study, the content of nitrates in rice cultivated soil samples was found to range from 0.30 mg/g - 2.20 mg/g while soil samples from wheat cultivated fields showed the nitrate content of 0.10 mg/g - 1.94 mg/g. Our results were in conformity with study by Chaudhuri \textit{et al.} (2009) who estimated the content of nitrates in soil of mangroves of the Andaman affected by tsunami and found the content to be in the range of 2.0 - 2.85 mg/g. Bahuguna \textit{et al.} (2011) also evaluated physico-chemical parameters of polycyclic aromatic hydrocarbons contaminated soils of Uttarakhand, India and the content of nitrates was reported to vary from 0.221 - 7.112 μg/g. Masakorala \textit{et al.} (2013) reported nitrogen content of 6.60 - 20.81 mg/kg in the soil contaminated with total petroleum hydrocarbon from DaGang oil field at southeast of Tianjin, China. Ma \textit{et al.} (2014) reported nitrogen content in the range of 0.59 - 1.84 g/kg in soil samples collected from the different sites of Daqing, China. On the contrary, few studies have shown high content of nitrates for example Bhat \textit{et al.} (2011) analyzed the physico-chemical parameters of soils collected from two different sites of Chandur Bazar tehsil of Amravati district and reported the content of nitrates to be 179.2 mg /kg and 153.0 mg/kg. Yao \textit{et al.}, (2013) studied the influence of sewage irrigation on agricultural soils of China and found the mean content of 653.18 mg/kg and 514.17 mg/ kg for waste water and regular water irrigated fields, respectively.

Phosphorus is one of the most limiting macronutrients for plant growth. It plays an important role for the development of healthy roots and fruits and also provides
disease resistance. It is present at levels of 400 – 1,200 mg/kg of soil. Phosphorus is present in soil as, organic and inorganic phosphates (Igual et al., 2001; Rodriguez et al., 2006; Thuynsma et al., 2014). It is available to the plants exclusively in the form of inorganic phosphates. Low availability of phosphorus due to its slow diffusion and high fixation in soils make it as major limiting factor for plant growth. Although in the last few decades, application of chemical phosphorus fertilizers and animal manure have improved soil production but have also caused damage to environment (Richardson et al., 2009; Shen et al., 2011). Phosphorus fertilizers are often over-applied to obtain maximum crop yield, which lead to degradation of soil and water eutrophication (Vance et al., 2003; Conley et al., 2009; Wu et al., 2013). The phenomenon of phosphorus fixation and precipitation in soil is generally dependent on pH and soil type (Hayat et al., 2010). In the present study, phosphate content of different soil samples from rice and wheat cultivated field ranged from 0.54 mg/g to 2.57 mg/g and 1.12 mg/g to 2.06 mg/g, respectively. The content of phosphorus was very low as compared to several other reports from different regions of the world. Katnoria et al. (2008) estimated different physico-chemical parameters of four soil samples collected from different agricultural fields of Amritsar, Punjab and reported the concentration of phosphates as 0.75 mg/g to 6.90 mg/g. Rabah et al. (2010) also determined physico-chemical parameters of soil contaminated with effluents of Sokoto metropolis, Nigeria and found the content of phosphorus to be 5.60 mg/g. Bahuguna et al. (2011) reported the content of phosphate in the range of 0.030 - 0.499 mg/g in polyaromatic hydrocarbon contaminated soils of Uttarakhand, India. Content of phosphates was reported by Khan et al. (2011) who conducted a study on six agriculturally important, water eroded soil series of Sharkul area of district Mansehra, Hazara Division, Khyber Pakhtunkhwa in Pakistan. The phosphate content in surface and subsurface layers of slightly eroded soil samples (Dosera and Girari), moderately eroded soil series (Nakholi and Sharkul) and severely eroded soil series (Ahl and Banser) was found to be 4.40, 4.6 mg/kg and 2.3, 2.7 mg/kg; 3.77, 3.45 mg /kg and 1.25, 1.34 mg/kg; 2.77, 2.84 mg /kg and 0.82, 0.96 mg/kg respectively.

High content of phosphate was reported by Rai et al. (2011) in soils irrigated with sewage water and canal water of Dehradun city, India. The content was reported to
be 108.44 mg/kg and 23.43 mg/kg, respectively. Ashraf et al. (2012) estimated the content of phosphorus in soils collected from Yusmarg hill resort, Kashmir and found it to range from 12 µg/g to 36 µg/g in different soil samples. Pandeeswari and Kalaiarasu (2012) studied physico-chemical parameters of the soil from different locations of tsunami affected sites of Cuddalore district of Tamil Naidu and reported the content of phosphates from 6.00 kg/ha to 14.53 kg/ha. Velmurugan et al. (2012) also estimated soil samples collected from different (red soil and sandy loam) sunflower fields contaminated with chemical fertilizers and organic manure treatment and showed phosphorus content of 92.23 kg /ha to 184.731 kg/ha and 95.41 kg /ha to 186.61 kg/ha for red and sandy sunflower growing soil, respectively. Peng et al. (2013) studied eroded rhizosphere soils under revegetation from the towns of of Zuolin (gully bed) and Yuanma (slope area) in the Yuanmou dry-hot valley, China. The content of total phosphorus and available phosphorus was observed to be 0.24 - 0.31; 0.15 - 0.24 g/kg and 21.8 - 23.2; 19.0 - 21.5 mg/kg, respectively. Waterlot et al. (2013) estimated phosphorus content in kitchen and lawn soil samples contaminated with effluents of lead and zinc smelters in France. The content was reported to be 1.3 g/kg and 0.5 g/kg, respectively. Masakorala et al. (2014) reported the content of extractable phosphorus in the range of 14.38 mg/kg to 19.67 mg /kg in the contaminated soil samples from DaGang oil field in China.

Potassium is the third essential and most abundant element that assists in water absorption and retention, improves plant growth (strong roots and sturdy stems) and provides resistance against drought and crop diseases by providing longer shelf life to plants. Potassium is supplied to plants by soil minerals, organic material and fertilizers (Romheld and Kirby, 2010; Dreyer, 2014). Potassium also accounts for the micro structural stability of soil and sustains the network between soil particles by forming new linkages between them. Consequently, potassium preserves and improves the structure of soil and provides resistance against shearing and loading forces thereby enhancing the storage capacity of soil for plant available water (Holthusen, et al., 2010, Ingo, 2014). The content of potassium in the upper 0.2 m of most agricultural soils ranges between 10 and 20 g/kg. Mineral soils have potassium of 0.04 - 3% yet, most of the potassium content of soil (90 - 98%) is incorporated in the crystal lattice structure of
minerals and thus not directly available for plant uptake (Jackson, 1964; Sparks, 1987; Zorb et al., 2014). Availability of potassium differs with different soil types and is affected by physico-chemical properties of the soil. Soils with acid sandy, waterlogged and saline characteristics showed deficiency of potassium content (Rengel and Damon 2008; Mengel and Kirkby 2001). In the Indian subcontinent progressive decline of crop yields was attributed to interruption of recycling of organic matter and mineral nutrients especially potassium in soil. As a result, decline in content of potassium and organic matter of soil was supposed to be a major cause of lower crop yields (Benbi et al., 2006; Brar, 2009; Romheld and Kirby, 2010). The present study showed content of potassium to range from 0.008 mg/g - 0.135 mg/g in rice cultivated soil samples while the content was found to be 0.44 mg/g to 0.194 mg/g in wheat cultivated soil samples. Our results are in conformity with several other reports. Katnoria et al. (2008) reported the potassium content of 0.16 - 0.25 mg/g in agricultural soil samples of Amritsar, Punjab. Joshi et al. (2009) conducted some physico-chemical analysis of four farm site soils in an area surrounding Rajkot, Gujarat, India and reported the potassium content of 0.21 - 0.29 meq/l. Attah (2010) estimated the potassium content of cereal cultivated rhizospheric soils of Ambo, Woreda, Westshoa and Euthopia. The content of potassium was found to be high in all the soil samples i.e. from 240 - 496 mg/kg. Rai et al. (2011) reported potassium content of 121.66 mg/kg and 81.66 mg/kg in soil irrigated with sewage water and canal water of Dehradun city, India respectively. Ashraf et al. (2012) also reported content of potassium (5.0 mg/100g - 9.35 mg/100g) in grassland soils of Yusmarg Hill Resort, Kashmir, India. Pujar et al. (2012) conducted soil characterization of samples collected from various localities of Bijapur taluka, Karnataka. The content of available phosphorus was found to range from 8.0 - 10.1 kg/ha. Peng et al. (2013) studied eroded rhizosphere soils under revegetation from the towns of Zuolin (gully bed) and Yuanma (slope area) in the Yuanmou dry-hot valley, China and the content of available potassium was found to range as 115.3 - 122.7; 52.8 - 115.3 mg/kg, respectively. Ma et al. (2014) also reported the available potassium content of 235.97, 137.24, 162.80 and 112.40 mg/kg in soil samples from four different sites of an oil producing region of China.
Discussion

Sodium is a functional nutrient beneficial to plants with potassium deficiency. It is involved in regeneration of phosphoenolpyruvate (an anion with high energy phosphate bond involved in biosynthesis of various aromatic compounds) in Crassulacean Acid Metabolism (CAM) in C4 plants (Maathuis, 2014). It helps in cation-anion exchange processes in soils. Sodium can have beneficial effects on growth of plants due to its osmotic function and thus regarded as functional nutrient (Wakeel et al., 2011). Soil salinity due to high concentration of sodium cause plant growth inhibition. Sodium ions compete with the potassium ions in the soil to find their entry into the plant cells because of their similarity in ionic radius and hydration energies resulting into sodium toxicity and potassium deficiency (Rubio et al. 1995; Gorham et al., 1997; Maathuis and Amtmann 1999; Schachtman and Liu, 1999; Schachtman 2000; Maser et al. 2001; Elumalai et al., 2002; Wang et al., 2004; Zhang et al., 2010 a,b,c). Our results showed range of sodium content to vary from 0.038 mg/g to 0.349 mg/g in rice cultivated soil samples and 0.038 mg/g to 0.252 mg/g in soil samples collected from wheat cultivated fields. Several studies have reported varied range of sodium content in soils from different regions. Udotong et al. (2008) estimated potassium and sodium content of wetland soils of Eket, Nigeria for two seasons and found the content to range from 0.04 - 0.07 and 0.0 - 0.07 mg/g in wet season and 0.06 - 0.12 mg/g and 0.06 - 0.32 mg/g in dry season respectively. Ashraf et al. (2012) found the range of content of sodium to be in the range of 4.5 mg/100g to 11.7 mg/100g in soils of Kashmir, India. Stauffer et al. (2014) assessed physico-chemical properties of soil affected by short rotation coppice compared to soil samples collected from forest, grassland and arable land use located in the Aisne river valley of Rethel at north of France. The content of sodium in soil was reported to be 0.11cmol/kg, 0.09 cmol/kg, 0.13 cmol/kg, and 0.04 cmol/kg in top soil samples collected from short rotation crop fields, grasslands, foresst and agrosystems, respectively.

Heavy metals are ubiquitous in the environment and are categorized as the most hazardous class of anthropogenic environmental pollutants due to their persistent nature and higher toxicity. Although some are essential for normal plant growth and are constituent of proteins including enzymes but elevation in their concentration causes interactions at cellular and molecular stages. These interactions induce various
toxicity symptoms at different levels of morphological, physiological, biochemical and molecular processes. Some heavy metals also stimulate the inhibition of plant growth, formation of free radicals and reactive oxygen species causing oxidative stress (Hall, 2002). Soil is one of the most important sink of heavy metals and is exposed to a number of toxic heavy metals by various natural and anthropogenic activities which include rapid expansion of industrial areas, industrial emissions, excessive use of agrochemicals, fertilizers, pesticides, sewage sludge, waste water irrigation and atmospheric deposition. Heavy metal contamination of soil pose risk hazards to humans via direct ingestion or dermal contact, contaminated water and through food chains (soil-plant-human or soil-plant-animal-human) (Mclaughltn et al., 2000; Ling-yu et al., 2010; Zhang et al., 2010, Wuana et al., 2012). Due to accumulation of heavy metals in soil and their adverse effects on productivity of agricultural crops by affecting food safety and marketability, crop growth due to phytotoxicity and environmental health of soil fauna, the identification and quantification of these elements is of great concern. Consequently, the influence of plant metabolic activities cause the geological and biological redistribution of heavy metals through pollution of the air, water and soil (Nagajyoti, 2010). In the present study, we have estimated the content of chromium (Cr), cadmium (Cd), copper (Cu), iron (Fe), lead (Pb), manganese (Mn), nickel (Ni) and zinc (Zn) in agricultural soil samples under rice and wheat cultivation.

Cadmium is one of the most abundant and highly toxic trace pollutants of great concern to environment and ecosystem health. It has been recognized as a potent cytotoxic, mutagenic, genotoxic and carcinogenic agent that affects plants and animals (Santos et al. 2010; Aimola et al., 2012; Monteiro et al., 2012). Cadmium occurs naturally in soils in complex forms but anthropogenic emissions mostly due to non judicial use of phosphate fertilizers, industrial and mining activities, municipal and sewage effluents as soil amendment, burning of fossil fuels, atmospheric depositions and vehicular emissions are the significant sources of soil contamination (Wuana and Okieimen, 2011; Irfan et al., 2014; Anjum et al., 2014a,b). Cadmium exists in soils in several chemical forms with varying degrees of solubility and bioavailability and its uptake by plants is influenced by its concentration in the soil and various biotic and
abiotic factors. Range of cadmium in soil varies as 0-1 mg/kg in normal soils; 1-3 mg/kg in slight contaminated soils and 3-10 mg/kg for cadmium polluted soils (Irfan et al., 2014; Spence et al., 2014). Cadmium exposure to humans results in etiology of health effects which includes nephrotoxicity, hepatotoxicity, reproductive toxicity; osteoporosis, fibrosis cardiovascular diseases (Chedrese et al., 2006; Rennolds et al., 2010; Nair et al., 2013; Rani et al., 2014) Cadmium in plants affects crop productivity, efficiency of photosynthesis and content of pigments and interferes with various physiological processes of plants. Stress of Cadmium in plants leads to a battery of toxicity symptoms reflected in terms of necrosis, chlorosis, wilting, interference in mineral accumulation, oxidative stress, carbohydrate metabolism, cell progression and may contribute to reduced biomass production (Papoyan et al., 2007; Monteiro et al., 2009; Santos et al., 2010; Gill et al., 2012, Dias et al., 2013). Our study has shown the content of cadmium to range from 0.541 mg/kg (SWFIr2) to 16.90 mg/kg (NEFIr2) in soil samples from rice cultivated fields. Among all the samples from wheat cultivated soils, Cd was not detectable in four of the soil samples viz. NEFIIw2, SEFIw2, SEFIw2 and NWFIw2 whereas as for other samples, the content was found to range from 0.078 mg/kg (NEFIw2) – 21.26 mg/kg (NWFIw2). Mahanta and Bhattacharyya (2011) also evaluated Cd content in soils of Guwahati, Assam (India) under different types of land use, viz., residential, commercial, industrial, public utilities (parks, place of worship, etc.) and roadsides affected due to variety of natural and anthropogenic processes like atmospheric deposition of particles from industrial emissions and the application of fertilizers. The range of content of Cd was found to be 9.2 - 18.0 mg/kg, 8.0 - 14.0 mg/kg, 6.5 – 12.3 mg/kg, 6.9 - 11.9 mg/kg, 3.1 -15.9 mg/kg, respectively. Lee et al. (2012) reported the cadmium content of 11.27 mg/kg in agricultural soil (paddy) from an area adjacent to the Seosung mine in Korea. Al-Farraj et al. (2012) estimated Cd content in contaminated soil samples collected from an area bounded by Mahad AD’ Dahab gold mine from south-east part of Medina area, Saudi Arabia and revealed the content of Cd as 17.2 mg/kg and 18.1 mg/kg using Hossner and USEPA 3051 method, respectively. Afkhami et al. (2013) studied sediment samples collected from the local soil, drilling mud and the waste pit nearby Ahwaz oil field in Iran and content of cadmium was shown to be 5.40 mg/kg, 7.20 mg/kg and 7.90 mg/kg, respectively. Zhen-
Xing et al. (2014) estimated soil cadmium in agricultural soils from six villages viz. Yanghe, Xiaozhen, Shangba, Luqiao, Tangxin and Xinyi in vicinity of Dabaoshan Mine in Shaoguan, China and the content was reported to range from 1.13 - 1.94 mg/kg, 0.62 - 2.75 mg/kg, 0.97 - 3.29 mg/kg, 1.54 - 3.51 mg/kg, 1.47 -3.16 mg/kg and 0.48 - 4.42 mg/kg, respectively.

Chromium is a highly toxic non-essential metal which can enter the soil ecosystem mainly through anthropogenic depositions. It exists in several oxidation states but the most stable and common forms are Cr(0), Cr(III) and Cr(VI) species. Chromium exists in soil as combination of Cr(III) and Cr(VI) and persist there for years, especially if the soils are sandy or have low levels of organic matter. The concentration of chromium showed wide variation in soils and their content was found to range from 0.2 to 1000 mg/kg. Cr is toxic for agronomic plants at concentration of about 0.5 to 5.0 mg/l when present in nutrient solution and 5 to 100 mg/g in soil (McGrath, 1995; Oliver, 1997; Wuana and Okieimen, 2011; Oliveira et al., 2012; Dhal et al., 2013). Cr toxicity in plants depends on its valence state. Cr(III) is less mobile and not so toxic but can be harmful to human body and have teratogenic effects. Cr(VI) is highly mobile and shows stronger toxicity and has also have been considered as strong carcinogenic substance (Song et al., 2014). Chromium compounds are highly toxic and detrimental for growth and development of plants (Nagajyoti et al., 2010; Singh et al., 2013). Cr is taken up by plants through carriers of essential ions such as sulphates. Symptoms of Cr toxicity in plants are diverse and include decrease of physiological processes like seed germination (Zeid et al., 2001; Zhou and Li 2004; Lopez-Luna et al., 2009; Datta et al., 2011) decrease of growth (Ozdener et al., 2011; Tang et al., 2012), decrease of yield (Zou et al. 2006; Lakshmi and Sundaramoorthy 2010) impairment of photosynthesis and enzymatic activities (Speranza et al., 2009; Eleftheriou et al., 2012), oxidative stress (Redondo-Gomez et al., 2011; Fargasova, 2012), and mutagenesis (Shanker et al. 2009; Mangabeira et al., 2011). Present study showed varied content of chromium in the range of 2.35 mg/kg (NEFIr1) - 22.50 mg/kg (SEFIr2) in soil samples collected from rice cultivated fields and 8.22 mg/kg (NEFIw2) - 32.90 mg/kg (SWFIw2) in soils of wheat cultivated fields. Enormous studies have reported the content of chromium in soils from other regions of the world.
Discussion

(Chanda et al., 2011; Koz et al., 2012; Amuno, 2013; Krishna et al., 2013; Zhang et al., 2013).

Results from our study are in consistence with study of Aelion et al., 2009 who revealed the chromium content of 7.0 mg/kg, 4.4 mg/kg and 24 mg/kg in soils with different land covers collected from commercial, rural and urban sites of US census metropolitan statistical area, respectively. Sun et al. (2009) also estimated the chromium content in forested soils from site Huang Pu (HP) and Botanical garden (BG) of Guangzhou, China polluted due to various anthropogenic activities. The range of chromium content observed for HP and BG was 10 - 13 mg/kg and 2 - 5 mg/kg, respectively. Nganje et al. (2010) studied farmlands soils nearby mine and unmineralized areas of Middle Benue Trough, Nigeria and found the content of chromium to be 2 mg/kg. Soriano-Disla et al. (2010) also estimated chromium content in agricultural soils of Spain exposed to sewage sludges spiked with heavy metal salts. The content of Cr was found to range from 16.30 mg/kg to 57.0 mg/kg. Liu et al. (2011) also found chromium content of 41.78 mg/kg in soils from greenhouses and farmlands from four main vegetable production areas of Shandong province, China. Chopra and Pathak, (2013) estimated metals in soils from Spinacea oleracea cultivated microplots contaminated due to irrigation with sugar mill effluent collected from R.B.N.S. Sugar mill Ltd., Laksar (Uttarakhand). The content of chromium was reported to be 22.31 mg/kg.

Copper is an essential micronutrient required for growth and development of plants particularly for various electron transport reactions in photosynthesis and respiration. In plants, Cu is especially important in various physiological processes like seed production, nitrogen fixation and reduction, disease resistance, protein metabolism and regulation of water (Yruela et al., 2005; Fernandez-Calvino et al., 2009; Peng et al., 2012; Ruyters et al., 2013; Lesniewska et al., 2014). Increase in industrialization, waste deposition and various anthropogenic activities have contributed to the increasing level of copper in ecosystems which has emerged as one of the major environmental pollutants. Different human activities like excessive use of copper containing fertilizers, fungicides, herbicides and pesticides lead to further increase in its content in
agricultural soil (He et al., 2004; Ke-Lin et al., 2006; Bouazizi et al., 2010; Lukatkin et al., 2013)

Copper has a low mobility in soil and due to its non-degradable and persistent nature it may tend to accumulate in the upper soil layers and cause contamination of water resources through leaching. Range of copper in soil is very wide and it varies from 1 - 140 mg/kg, with average values in the range of 13 - 24 mg/kg (Mackie et al., 2012; Lesniewska et al., 2014). Copper toxicity in humans can cause anaemia, liver and kidney damage, and stomach and intestinal irritation (Wuana and Okieimen, 2011; Wang et al., 2013; Pal, 2014) Copper contamination poses a cytotoxic role, induces oxidative stress and causes injury to plants (Nagajyoti et al., 2010). Various toxicity symptoms include retardation of seed and plant growth (Muccifora and Bellani, 2013) leaf chlorosis (Toselli et al., 2009; Yang et al., 2011; Miotto et al., 2014) disturbance of metabolic pathways (Bouazizi et al., 2010; Sanchez-Pardo et al., 2012; Elleuch et al., 2013) and damage to macromolecules (Nagarani et al., 2012; Babu et al., 2014).

In the present study, the content of copper range from 11.61 mg/kg (SEFIr2) to 31.08 mg/kg (SWFIr2) in soil samples under rice cultivation and 9.62 (SEFIw2) mg/kg to 31.18 mg/kg (SWFIw2) in soil samples under wheat cultivation. Our results are in conformity with several other reports from different regions of the world. Bai et al. (2010) reported the copper content of 19.70 - 41.77 mg/kg, 29.82 - 45.08 mg/kg and 14.53 - 61.40 mg/kg in soils from uncultivated, cultivated wetland and cultivated wetland after abandonment collected from eastern region of Yilong lake in China. Cui and Du (2011) studied paddy field soils from the vicinity of an abandoned mine area polluted by heavy metals from the tailings of the Pb–Zn mine in Shangyu city of eastern China and the content was found to be 25.6 - 26.0 mg/kg. Al-Khasman et al. (2012) also reported copper content in soils of Jordan at 0 - 10 cm and 10 - 20 cm depth in the range of 8.60 mg/kg - 48.65 mg/kg and 10.51 - 24.30 mg/kg, respectively. Amuno (2013) found the copper content of 19.57 mg/kg in cemetery soils of Gisozi memorial centre at Kigali, Rwanda. Cai et al. (2013) also reported the copper content of 12.9 mg/kg, 77.4 mg/kg, 15.2 mg/kg, 60.9 mg/kg, 41.6 mg/kg and 116.3 mg/kg in soil samples of parks, roadside, residential, sport grounds, urban and dust soils, respectively collected from region of Guangzhou in China. Similar range of copper content (6.5 - 32.9 mg/kg) as
found in the present study was also observed by Novaes dos Santos and Alleoni (2013) who studied farm soils from Brazilian agricultural frontier of southwestern Amazon with naturally occurring heavy metal content in soil. Mackie et al. (2013) studied the effect of long term copper application in soil samples taken from an organic vineyard in Brackenheim, Baden Wurttemberg, Germany and revealed the content of copper to range from 43 mg/kg - 142 mg/kg.

Iron is an essential micronutrient which plays many physiological and biochemical functions in plants and animals. It is an integral part of several enzymes and also participates in various redox reactions (Nagajyoti et al., 2010; Nunez et al., 2012; Balk and Schaedler, 2014). In soils, iron exists as ferric hydroxides under aerobic conditions while in anaerobic soils it is reduced to ferrous ions (Fe^{2+}) which are taken up excessively by plants (Becker and Asch 2005; Shahid et al., 2014; Wu et al., 2014). It is required in several vital processes of photosynthesis, oxidative phosphorylation, nitrogen fixation, DNA replication and hormone synthesis. However, in excess it can induce the production and accumulation of reactive oxygen species causing oxidative stress and altering the physiological, morphological and biochemical characteristics of the plants. Various toxicity effects include protein oxidation, lipid peroxidation, damage of chlorophyll pigment and nucleic acids leading to apoptosis, reduction in photosynthesis and yield (Arora et al., 2002; de Dorlodot et al. 2005; Moller et al., 2007; Nagajyoti et al., 2010; Xing et al., 2010; Jucoski et al., 2013). In our study, the content of iron was found to be 14804.73 mg/kg (SEFIr1) to 20536.00 mg/kg and 13270.18 mg/kg (SEFIw2) to 19807.30 mg/kg (NWFIw1) in rice and wheat cultivated soil samples, respectively. Ahsan et al. (2009) also studied agricultural soils from floodplain agricultural fields of Faridpur (FD) and Dhamrai (DM) region of Bangladesh contaminated due to ground water irrigation rich with As and trace metals and reported iron content to be 48.37 mg/kg and 37.00 mg/kg, respectively. High content of iron in present work is in conformity with study by Basar et al. (2009) who estimated content of iron in agricultural soils of Turkey before and after irrigation with water from lake Iznik which has been polluted with industrial and municipal wastes. The iron content was found to be 4420 mg/kg and 41201 mg/kg, respectively. Bhuiyian et al. (2010) conducted metal analysis of soils of mine drainage and surrounding agricultural fields
of Barapukuria coal basin located in Dinajpur district of northern part of Bangladesh and reported iron content of 59853 mg/kg. Sehgal et al. (2012) reported iron in the range of 4431.5 – 4915.3 mg/kg in agricultural soil from fields along the course of river Yamuna near Wazirabad-Okhla barrage at New Delhi, India which is affected due to anthropogenic pollution of mining, industrial processing, agricultural run-off and sewage disposal. Nazzal et al. (2013) also reported high content of iron in roadside samples from 401, 400, 404 highway and Don valley parkway region of greater Toronto, Canada polluted due to vehicular emissions. The content of iron was reported to 51,784.2 mg/kg, 52,558.5 mg/kg, 44,799.6 mg/kg and 44,988.5 mg/kg in samples from 401, 400, 404 and Don valley parkway regions, respectively.

Lead is one of the most abundant toxic heavy metals of great concern. It is a widely distributed environmental and occupational contaminant known for its mutagenic, clastogenic and carcinogenic properties (Poreba et al., 2011; Green and Pain, 2012; Thompson et al., 2014). Lead contamination of soil and plants can be through exhausts of vehicular emissions, mineral wastes, industrial and atmospheric emissions, land application of animal manures, pesticides, fertilizers, use of wastewater and sewage sludge, coal combustion residues, spillage of petrochemicals (Khan et al., 2008; Zhang et al., 2010; Ali, 2014). Pb in the environment exists as an insoluble form. Ionic lead, Pb(II), lead oxides and hydroxides and lead metal oxy anion complexes are the general forms of Pb that are released into the soil, groundwater, and surface waters. Lead accumulates in the upper 8 inches of the soil and is highly immobile. Typical mean Pb concentration for surface soils worldwide averages 32 mg/kg and ranges from 10 mg/kg to 67 mg/kg (Kabata-Pendias and Pendias 2001; Nagajyoti et al., 2010; Wuana and Okieimen 2011). In the environment, lead is known to be toxic to plants, animals, and microorganisms. Within living systems, lead reacts or complexes with many biomolecules and adversely affects the reproductive, nervous, gastrointestinal, immune, renal, cardiovascular, skeletal, muscular and hematopoietic systems as well as developmental processes (Brent, 2006; Navas-Acien et al., 2007; Cleveland et al., 2008; Rastogi, 2008; Vij, 2009; Sanders et al., 2009; Flora et al., 2011; Flora and Gupta, 2012; Agrawal et al., 2014). Lead is not an essential element required by plants but they absorb it from the ambient environment mainly in soils contaminated due to automotive
emissions and excessive use of agrochemicals which contain heavy metal as impurities (Adriano, 2001; Lamhamdi et al., 2013). Lead affects the metabolism of plants and can cause a broad range of physiological and biochemical effects like inhibition of seed germination, impaired plant growth and root elongation. Lead also affects the processes of transpiration, chlorophyll production and cell division (Sharma and Dubey, 2005; Seregin and Kosevnikova, 2008; Lamhamdi et al., 2011; Akinci et al., 2010; Ali et al., 2013a,b; Lamhamdi et al., 2013; Antunes and Kreager, 2014; Shahid et al., 2014; Tian et al., 2014).

In our study, we observed that the content of lead ranged from 3.07 mg/kg (SEFIr2) to 17.31 mg/kg (NEFIIr1) in soil sample from rice cultivated fields and 8.10 mg/kg (SEFIw1) to 23.18 mg/kg (SWFIIw2) in soils samples from wheat cultivated fields. Results from present study are in conformity with several other studies where the content of lead in soils from other parts of the world. Content of lead was also observed by Tra and Egashira (2001) who studied agricultural soils (paddy and upland fields at different locations) of south east part of Medina area, Saudi Arabia and reported the content of lead in the range of 11 g/kg to 33 mg/kg. Neupane and Roberts, (2009) reported lead content of 7.6 - 20 mg/kg, 9.2 - 18 mg/kg and 6 - 16 mg/kg in soil samples from agricultural, adjacent forest and boundary soils affected due to different land-use activities, respectively. Similar content of lead has been reported in study by Iwegbue et al. (2009) who analyzed soils at two depths (0 - 15 and 15 - 30 cm from farmlands and adjoining swamps and creeks affected due to spillage of one of the biggest oilfields in the Nigeria Delta. The content of lead was reported to be 25.02 mg/kg and 23.90 mg/kg in top soil and sub soil samples, respectively. Baykara and Dogru (2010) also reported the content of lead to range from 1.05 - 11.64 mg/kg in soil samples affected due to natural or artificial radioactive nuclides of seismically active areas of Turkey. Ling-yu et al. (2010) estimated content of lead in soils of China under four land use patterns like greenhouse field (GF), uncovered vegetable field (UF), maize field (MF) and forest field (FF). The content was reported to be 17.98 mg/kg, 16.19mg/kg, 15.74mg/kg and 16.28 mg/kg in GF, UF, MF and FF soils, respectively. Kizilkaya et al. (2011) also analyzed agricultural soils of Bafraplain, Turkey from fields with application of high rates of phosphorus fertilizers and intense soil cultivation and revealed the lead content
to range from 4.45mg/kg to 33.33 mg/kg. Amuno (2013) also estimated the content of lead in cemetery soils from Gisozi memorial centre of Kigali, Rwanda and reported the content of lead to be 28 mg/kg. Novaes dos Santos and Alleoni (2013) studied agricultural soils of southwestern Amazon in Brazil, polluted with heavy metals for content of lead and the content was found to range from 5.2 mg/kg to 25.8 mg/kg.

Manganese is an essential micronutrient that plays an important role in various redox reactions of plants. It helps plants in water splitting and O₂ evolution system in photosynthesis, activation of several enzymes involved in various physiological reactions, protects the photosynthetic apparatus from deleterious action of reactive oxygen species, helps in formation and organisation of the lamellar system of chloroplasts (Zanao Junior et al., 2010; Broadley et al. 2012; Millaleo et al., 2013; Tewari et al., 2013). Commercial fertilizers containing synthetic manganese (Mn) chelates and complexes are the major source of manganese alleviation in agricultural soils. Reduced form of Mn (Mn II) in soil is readily available for plant acquisition (Akter et al., 2014; Lopez-Rayo et al., 2013). Toxicity of Mn adversely affects the physiological and biochemical functions of plants (Millaleo et al., 2013). Toxicity symptoms of manganese include necrosis and chlorosis of older leaves necrotic lesions, leaf browning and even death, loss of apical dominance and proliferation of axillary shoots (Fuhrs et al., 2009, 2011 Millaleo et al., 2010; Nagajyoti et al., 2010; Fuhrs et al., 2011; Kovacik et al., 2014).

In the present study, the content of manganese ranged from 207.91 mg/kg (SWFIIr1) to 539.70 mg/kg (NWFIIr2) in rice cultivated soil samples while in wheat cultivated soil samples the content ranged from 192.88 mg/kg (SEFIw2) to 383.94 mg/kg (NWFIIw2). Our results are in accordance with several other studies which reported the content of manganese in different soil samples from different regions. Chen et al. (2007) studied cultivated soils of Xijia village, contaminated due to sewage water irrigation from open canal receiving sewage from Huamu, Beicai, Zhangjiang, Tang and Heqing Town of China. The content of manganese was reported to range from 504 mg/kg to 531 mg/kg. Khalil et al. (2008) also studied agricultural soils in vicinity of polymetallic mine and revealed the content of manganese to range from 532 mg/kg - 683 mg/kg. Ahsan et al. (2009) reported manganese content of 449.68 mg/kg and
553.75 mg/kg in agricultural soils of two different locations of Bangladesh contaminated with polluted ground water irrigation. Moreno- Jimenez et al. (2009) also found manganese content of 353.6 mg/kg, 432.6 mg/kg and 427.8 mg/kg in cultivated soils in vicinity of mine dumps, mine drainage and unaffected areas of Spain, respectively. Singh et al. (2011) observed manganese content of 37 mg/kg in lake water irrigated agricultural soils of Gorakhpur, Uttar Pradesh, India. Kanman and Gandhimathi (2012) also reported the content of manganese in soil samples collected from and around the open dumpsite Ariyamangalam municipal solid waste at Tamil Naidu, India. In their study, they found content of manganese in the range of 42.07 mg/kg to 171.6 mg/kg. Mahanta and Bhattacharaya (2012) studied soils of Guwati, Assam, India under different types of land uses viz., residential, commercial, industrial, public utilities and road side and revealed the manganese content in the range of 194.8 - 456.6 mg/kg, 223.2 - 466.3 mg/kg, 300.8 - 636.0 mg/kg, 246.8 - 503.8 mg/kg and 321.8 - 512.2 mg/kg, respectively. Massas et al. (2013) studied the soils of Thriassio plains of Greece under mixed land uses (i.e., residential, agricultural, and industrial) between Elefsina and Aspropyrgos two major towns affected due to increase in industrialization (steel industries cement factories, petroleum recycling units, large warehouses, oil and many chemical industries). They found the content of manganese in range of 160.3 mg/kg to 588.5 mg/kg.

Nickel is recognized as an essential trace element which is distributed uniformly throughout the soil profile but gets accumulated at the surface soils by activities of industrial and agricultural depositions. Anthropogenic activities further release Ni into the soil by various sources such as vehicular emissions, metallurgical and electroplating industries, emissions of smelters, municipal and industrial wastes, application of agricultural lands with phosphate fertilizers, pesticides, organic manure and sewage sludges (Cempel and Nikel, 2006; Nagajyoti et al., 2010, Wuana and Okieimen, 2011; Hamner et al., 2013) Nickel in soil exists in various forms as: inorganic crystalline minerals or precipitates, free ion complexed or adsorbed on organic and inorganic cation exchange surfaces or soil solution. Its content in soil varies in a wide range from 10 to 1000 mg/kg (Izosimova, 2005). Exposure of humans to nickel polluted environments can cause number of pathological effects like dermatitis, immunotoxicity,
nephrotoxicity, hematological disorders, cancer of the respiratory tract and nickel poisoning (Sivulka, 2005, Pietruska et al., 2011, Clancy and Costa, 2012, Magaye and Zhao, 2012, Wang et al. 2013, Ahmad et al., 2013, Holmes et al., 2013, Miguel et al., 2013). Several physiological alterations and diverse toxicity symptoms were found in plants exposed to excess of nickel in soil (Boisvert et al., 2007, Chen et al., 2009, Yusuf et al., 2011, Sreekanth et al., 2013). Various toxicity symptoms included inhibition of seed germination (Bhardwaj et al., 2007), necrosis and chlorosis in different plant species (Rahman et al., 2005), decrease in water uptake (Gajewska et al., 2006; Gajewska et al., 2009), disorder of cell membrane functions due to impairment of nutrient balance (Sreekanth et al., 2013). Besides this excess of nickel also effects growth pattern and development of plants (Gajewska et al., 2009; Yusuf et al., 2011; Stanisavljevic et al., 2012; Hu et al., 2013; Pietrini et al., 2014).

In the present study, the content of nickel for all the soil samples from agricultural fields under rice and wheat cultivation ranged from 15.67 mg/kg (SEFIr2) to 34.51 mg/kg (NWFIr1) and 10.29 mg/kg (SEFIw2) to 31.31 mg/kg (NWFIIw1), respectively. Enormous studies throughout the world have shown varied content of nickel in different soil samples. Achiba et al. (2009) studied agricultural soils of farms of agronomic national institute of Tunisia (INAT) following history of 5 yr application of municipal solid waste compost and farmyard manure. The range of nickel content observed was found to be 31.5 mg/kg to 37.5 mg/kg for compost soils and 28.6mg/kg to 33.7 mg/kg for manure soils. Jian-Hua et al. (2009) also reported the range of nickel as17.58 mg/kg to 23.71 mg/kg in railroad side soils of Longxi-Haizhou railroad, China. Neupane and Roberts (2009) evaluated soil samples from agricultural, forested and boundary soils affected due to different land-use activities in Ohio, USA and reported the content of nickel in the range of 18 - 32 mg/kg, 21 - 56 mg/kg and 21 - 48 mg/kg, respectively. Stafilov et al. (2010) estimated nickel content of 42 mg/kg, 53 mg/kg, 78 mg/kg and 68 mg/kg in soils from cultivable, uncultivable, urban and polluted areas of Veles region from Republic of Macedonia. Koz et al. (2012) also reported nickel content of 28.74 mg/kg in surface soil collected from vegetated sites in vicinity of copper mining in east of Turkey’s black sea region. Jalali and Hemati (2013) conducted metal analysis of soils from three agricultural areas (paddy fields) affected due to
natural and anthropogenic inputs of metals and reported the content of nickel to range from 10.8 mg/kg to 18.4 mg/kg.

Zinc is an essential trace element that is required to maintain several metabolic processes of plants and animals. In animals, it is important in homeostasis, immune function, oxidative stress, apoptosis, aging, etc. (Chasapis et al., 2011). In plants, it is one of the important micronutrients essential for carrying out many physiological and biochemical processes like photosynthesis, respiration, chlorophyll biosynthesis, metabolism of carbohydrates, lipids, proteins and also plays a structural role in regulating gene expression and regulation (Chen et al. 2009; Disante, 2014). Zinc occurs naturally in soil but its concentration increases rapidly due to various anthropogenic activities like industrial processes, such as mining, steel processing, coal and waste combustion, sewage sludge, industrial waste products and application of zinc containing fertilizers. In soil, it exists in several forms with varying solubility but plants absorb zinc in its divalent form (Rehman et al., 2012; Disante, 2014). The common range of zinc is 10 - 300 µg/kg while in uncontaminated soils concentrations ranged from 17 - 160 µg/kg (Kiekens, 1995; Oliver, 1997; Rehman et al., 2012). Zn in the range of 150 - 300 mg/kg was reported in polluted soils (Devries et al. 2002; Warne et al. 2008; Nagajyoti et al., 2010). High concentration can produce a wide range of phototoxic effects like decrease in growth and development of plant, induction of oxidative damage, inhibition of many plant metabolic functions (Dazy et al. 2009; Disante et al., 2010; Disante et al., 2011; Sytar et al., 2013; Barrameda-Medina et al., 2014).

In the present study, the content of zinc ranged from 48.07 mg/kg (SEFIr2) to 87.55 mg/kg (NEFIr2) for soil samples collected from rice cultivated fields whereas in soil samples from wheat cultivated fields the content varied from 44.55 mg/kg (SEFIw2) to 108.99 mg/kg (NEFIw1). The results showed the contamination of all the soil samples under rice and wheat cultivation with Zn. El-Arby et al. (2006) studied agricultural soils from two sites of El-Sadat city at Minufiya Governorate, Egypt irrigated from well water and treated industrial wastewater and reported the zinc content in the range of 4.20 mg/kg to 11.72 mg/kg. Zinc content of 3.51 mg/kg to 246.44 mg/kg was also observed in soils under rice/paddy cultivation from Hangzhoue-Jiaxinge-
Huzhou plains of China affected due to rapid urbanization and industrialization by Liu et al. (2006). Martin et al. (2006) estimated the content of zinc in agricultural soil samples contaminated due to intense agricultural practices in region of Ebro basin in Spain and reported the content to be 17.53 mg/kg. Mico et al. (2006) also evaluated agricultural soils under intense agriculture activities from Alicante province in southeast Spain and found the zinc content of 52.8 mg/kg. Yan-Feng et al. (2007) reported the zinc content of 112.9 mg/kg in agricultural soils from industry-based peri-urban area under anthropogenic influence on heavy metal distribution in the soil of Wuxi city of Jiangsu province in China. Lu et al. (2009) estimated zinc in vegetated soils from four zones (V, VI, VII and VII) of Hangzhou city of China under cultivation of rice and economic crops affected by application of chemical fertilizers and other anthropogenic factors. The content of zinc was found to be 44.5 mg/kg, 43.5 mg/kg, 42.5 mg/kg and 42.5 mg/kg for zones V, VI, VII and VII, respectively. In another study by Soriano-Disla et al. (2010), content of zinc was estimated in agricultural soils of Spain exposed to sewage sludge and was found to range from 34.4 mg/kg to 96.0 mg/kg. Markovic et al. (2010) also reported content of zinc (75.3 mg/kg to 142 mg/kg) in agricultural soils contaminated with pesticides and heavy metals. Kizilkaya et al. (2011) studied agricultural soils from fields with application of high rates of phosphorus fertilizers and intense soil cultivation and reported the range of zinc content to vary from 20.51 – 90.13 mg/kg. Novaes dos Santos and Alleoni (2013) reported zinc content in the range of 1.2 mg/kg to 100.9 mg/kg in soils from agricultural frontier of southwestern Amazon in Brazil.

Metal concentrations showed significant variations amongst different agricultural soil samples. Comparing results from our study to those from other studies in the literature, it appears that in the present study the soil samples were slightly contaminated with one or the other heavy metals which could be because of excessive use of agrochemicals, existence of industrial activities as well as urban and vehicular emissions. Agricultural soil samples showed varied levels of metals and content of metals was found in the order as: Fe > Mn > Zn > Ni > Cu > Cr > Pb > Cd. Our results were in consistence with the study by Jalali and Hemati (2013) who studied soils of agricultural fields of Isfahan province in central Iran and reported that Fe (1240.4
Discussion

mg/kg) was the most abundant metal followed by Mn (95.7 mg/kg), Pb (51.6 mg/kg), Zn (23.8 mg/kg), Ni (13.4 mg/kg), Cu (7.0 mg/kg) and Cd (2.8 mg/kg). Present study revealed the moderate contamination of soils when compared to soils of other places of India like Varanasi (Sharma et al., 2007), Karnataka (Sehgal et al. 2012) and Delhi (Krishna et al. 2013). Chopra and Pathak (2013) also estimated metals in soils from Spinacea oleracea cultivated microplots contaminated due to irrigation with sugar mill effluent collected from R.B.N.S. Sugar Mill Ltd., Laksar (Uttarakhand). The content of heavy metals was reported to be Cr (22.31), Ni (213.27), Cd (8.72), Zn (49.64) and Fe (183.11). Concentrations of Cd and Zn however were found to be higher when compared with mean metal limits recommended by the UK Interdepartmental Committee for Restoration of Contaminated Land (ICRCL 1987) for Cd (1 mg/kg) and Zn (25 mg/kg) in soil used for agriculture and recreation.

5.2. Genotoxicity studies in A. cepa root chromosomal aberration assay

Soils are exposed to large number of contaminants from different industrial activities, discharge of untreated sewage, incomplete combustion of the fuel in the heating plants and automobiles and excessive use of chemical pesticides and fertilizers (Alam et al., 2009; Kaur et al., 2011). The study concerns estimation of genotoxic potential of soil samples of agricultural fields from different regions of district Amritsar, Punjab (India) in A. cepa root chromosomal aberration assay employing in situ and root dip mode of treatments. Soil samples were collected from different agricultural fields under rice cultivation during September, 2009 (r1) and September 2010 (r2); and under wheat cultivation during March 2010 (w1) and March, 2011 (w2).

In order to delineate the potential of agricultural soils to induce one or another kind of chromosomal aberrations, different kinds of induced aberrations were apportioned into physiological aberrations attributable to spindle abnormalities manifested by c-mitosis (Cm), delayed anaphases (Da), laggards (Lg), stickiness (St) and vagrant chromosomes (Vg) and clastogenic aberrations, attributable to the direct action on chromosomes resulting in the manifestation of chromosomal breaks (Bk), chromatin bridges (Bg) and ring chromosomes (Rc). Some aberrations such as asteroid structures, deviation of chromosomes from the poles at anaphase, deviation of
alignment of chromosomes at metaphase were counted as abnormal metaphase (Am) and abnormal anaphase (Aa). In our study, different types of chromosomal aberrations were observed in A. cepa root tips treated with different soil samples.

During in situ treatment, different types of chromosomal aberration were observed in root tip cells of A. cepa on treatment with different agricultural soil samples. The squash preparations from treated root tip cells of A. cepa bulbs revealed high percentage of chromosomal aberrations as compared to control (4.14%). A few cells with c-mitosis, delayed anaphases, stickiness and bridges were observed in control bulbs. No instance of Lg, Vg, Aa, Am, Bk and Rc was found in control bulbs. Among all the soil samples collected from rice cultivated agricultural fields of sampling r1 and r2, sample SWFIIr1 (23.79%) and SWFIIr2 (19.847%) showed maximum while soil sample NEFIIr1 (7.71%) and NWFIr2 (9.041%) showed minimum percentage of total chromosomal aberrations, respectively. Soil samples from wheat cultivated fields of both w1 and w2 sampling also resulted in appearance of both physiological and clastogenic aberrations. Among all soil samples of w1 and w2, sampling maximum percentage of aberrations was observed in soil sample SWFIw1 (20.21%) and SWFIw2 (15.54%) while minimum in sample SWFIw1 (7.40%) and NEFIw2 (7.75%). The frequency of root tip cells with physiological and clastogenic aberrations following treatment with different soil samples ranged from 7.18 % - 21.50 %; 0.43 % - 2.29 % for r1 sampling; 6.971 % - 15.48 %; 0.65 % - 4.36 % for r2 sampling; 6.86 % - 17.04 % ; 0.54% - 3.16 % for w1 sampling and 6.12 % - 13.36 %; 0.26 % - 3.25 % for w2 sampling, respectively.

During Root dip mode of treatment, the squash preparations of root tips of control A. cepa bulbs showed 3.46% of cells with chromosomal aberrations. Squash preparations from root tip cells of treated A. cepa bulbs showed increase in chromosomal aberrations with increase in concentration of soil extract for all soil samples. Among different rice cultivated soil samples, of r1 and r2 sampling again the samples SWFIIr1 (25.95 %) and SWFIIr2 (26.47%) showed maximum while samples NWFIr1 (12.51%) and NWFIr2 (13.49%) showed minimum percentage of aberrations at the highest concentration tested (100%). It was observed that soil sample SWFIw1
(27.09 %); SWFIw1 (12.75 %) and SEFIw2 (25.59 %); NWFIw2 (13.46 %) of w1 and w2 sampling showed maximum and minimum percentage of chromosomal aberrations at the maximum dose tested, respectively.

To evaluate genotoxicity of contaminated soils, plants are suitable systems (Cotelle et al. 1999; Knasmüller et al., 1998; Souza et al., 2009, 2013). Allium cepa is among the most widely and sensitive plant species to evaluate the genotoxicity of complex environmental matrices due to large size and quantity of its metacentric chromosomes (Ma et al., 1995). Appearance of anomalies during mitosis in the chromosomes of A. cepa meristematic cells is an easy method to study effect of several genotoxic and mutagenic compounds (Konuk et al. 2007; Leme and Marin-Morales 2008; Yildiz et al., 2009; Ozkara et al. 2011; Liman et al. 2012 ; Herrero et al., 2012; Liman et al. 2013), different environmental mixtures such as aquatic (Caritá and Marin Morales 2008; Radic et al., 2010; Bianchi et al. 2011; Dusman et al., 2014; Radic et al., 2014) as well as terrestrial habitats (Souza et al. 2009; Christofoletti et al., 2013; Souza et al., 2013)

Chromosomal aberrations are considered as end result of genotoxic effects of various physical and chemical agents and are also estimates of exposure of various organisms to these agents that impair human health (Pohren et al., 2013). The term c-mitosis was coined by Levan (1938) to describe the effects of some chemicals which act in a fashion similar to that of colchicine and prevents the assembly of spindle microtubules by dissociating disulphide bonds. In delayed anaphases the two anaphasic groups of chromosomes lie close to each other near equatorial plate. Chromosomal breaks observed in the present study are considered to involve the DNA molecule responsible for linear continuity of the chromosomes and may be due to unfinished or misrepair of DNA (Evans 1977). The formation of anaphasic chromatin bridges may be attributed to unequal exchanges resulting in formation of dicentric chromosomes which are pulled equally to both poles at anaphase (Sax and Sax 1968). According to Al-Najjar and Soliman (1980) in addition to unequal translocation or inversion of chromosome segments, the formation of chromatin bridges may be attributed to chromosome stickiness and subsequent failure of free anaphasic separation.
Genotoxicity of the soil samples in the present study could be attributed to excessive use of vast varieties of pesticides and fertilizers; both organic and inorganic by the farmers, which ultimately result in soil pollution. High genotoxicity in terms of chromosomal abnormalities observed could also be attributable to presence of various industries like paper industry, sugar mill industry and distillery in the vicinity of agricultural fields from where some soil samples were collected. Apart from this, the direct use of sewage sludge, industrial wastes and waste water to agricultural land as source of plant nutrients aroused serious concern as they are also known to contain many toxic metals along with useful nutrient elements.

In the present study, the chromosome aberrations induced could also be due to aneugenic agents, such as heavy metals, present in soil matrix which are found to be potentially mutagenic and are closely associated with environmental pollution and support findings of our study. Several studies have shown genotoxic potential of metals to induce alterations in chromosome structure, and number and disturbances in the mitotic apparatus (Fiskejo 1983; Minissi and Lombi 1997). Christofoletti et al. (2013) also reported the genotoxic and mutagenic potential of heavy metals in soil as demonstrated by increase in chromosomal aberrations like micronuclei and chromosomal break, metaphase with adherence, anaphase with chromosomal bridge, anaphase with chromosome loss and polyploidy. Heavy metals have been reported to inhibit mitotic index and results in appearance of chromosomal aberrations, micronuclei and binucleate cells in *Allium cepa* (Marcano et al., 2006; Konuck et al., 2007; Yi et al., 2007; Achary *et al.*, 2008,2010; Kumari *et al.*, 2011; Patnaik *et al.*, 2013; Pakrashi *et al.*, 2014). Our result on genotoxicity confirmed the use of *A. cepa* as a bioindicator of soil pollution and forms an important base for future application of such bioassays to evaluate the content of chemicals present in environmental matrix. Therefore, further comprehensive studies and regular surveys are recommended to carefully monitor heavy metals and trace elements of the region.

Enormous studies have shown different types of mitotic and chromosomal abnormalities indicating genotoxic potential of contaminated soil from different parts of the world (Kovalchuk *et al.*,1998; Kong and Ma 1999; Andrade *et al.*, 2008; Katnoria *et al.*, 2009; Masood and Malik, 2013; Souza *et al.*, 2013). Our results are in conformity
with an earlier study by Dragoeva et al. (2009) who evaluated genotoxic potential of agricultural soil and reported various chromosomal abnormalities like vagrant chromosomes, chromosomal fragments at anaphase and telophase and multipolar anaphases. Leme et al., (2012) also assessed the genotoxicity of contaminated soil matrix by using A. cepa root chromosomal aberration assay and reported various chromosomal abnormalities in merismatic cells of A. cepa.

Masood and Malik (2013) reported the cytotoxic and genotoxic potential of soil from various toxic metal contaminated agricultural fields in the vicinity of industrial area of Jajmau, Kanpur (India) in terms of chromosomal aberrations which included c-mitosis, anaphase bridges, laggards, stickiness, broken and unequal distribution of chromosomes. In another study by Pohren et al. (2013) chromosomal aberrations like multipolar anaphases, metaphases with adherence, anaphases with bridges, chromosomal losses, chromosomal breaks and micronucleated cells were observed in A. cepa root tip cells treated with soils contaminated from municipality effluents of Triunfo, state of Rio Grande do Sul in Brazil.

Souza et al. (2013) also reported the clastogenic potential of polluted cultivated soils using the A. cepa root chromosomal aberration assay. Different types of mitotic and chromosomal abnormalities were observed in A. cepa root cells which included anaphase with chromosome loss and chromosomal adherence, multipolar anaphases, chromosomal breaks and bridges. Arora et al. (2014) also evaluated genotoxic risks in soil with temporal imbalance of ammonium–nitrate ratio employing Allium cepa root tip chromosomal aberration assay. The cytological analysis of root tips showed dividing cells with different types of division anomalies which included mitotic abnormalities as well as interphase nuclear abnormalities.

5.3. Biochemical parameters

Agricultural soils throughout the world are found to be polluted with different heavy metals, such as arsenic (As), cadmium (Cd), cobolt (Co), chromium (Cr), copper (Cu), lead (Pb), molybdenum (Mo), nickel (Ni) and zinc (Zn). Toxicity by heavy metals cause deleterious biological effects due to their persistent and non-degradable nature. These metals find their way into the agricultural soils by application of fertilizers,
pesticides, sewage sludge, emissions of various industrial activities, automobile exhausts and bad watering practices for long-term utilization. These heavy metals can cause stress to the plants by increased production of reactive oxygen species (ROS) and cause interference with their physiological, biochemical and molecular mechanisms vital for metal tolerance and acclimatization (Liu et al., 2008a; Kaur et al. 2011; Kumchai et al., 2013; Martins et al., 2014; Srivastava et al., 2014).

Keeping this in view, the present study involved evaluation of the biochemical parameters such as content of total proteins and antioxidative enzymes viz., ascorbate peroxidase (APX), catalase (CAT), dehydroascorbate reductase (DHAR), glutathione reductase (GR), glutathione-S-transferases (GST), guaicol peroxidase (POD) and superoxide dismutase (SOD) in A. cepa bulbs exposed to agricultural soil samples from different fields under rice cultivation during September, 2009 (r1) and September 2010 (r2) and under wheat cultivation during March 2010 (w1) and March, 2011(w2).

Proteins are considered as the most important group of biomolecules and their types as well as quantity vary not only among different organisms but also in different parts of the same organism. In our study, total protein content of onion bulbs treated with different soil samples of r1 and r2 sampling varied from 1.08 mg/kg (NWFIr1) to 2.49 mg/g (SWFIIr1) and 1.04 mg/g (NWFIr2) to 2.65 mg/g (SWFIIr2), respectively. Agricultural soil samples from wheat cultivated fields of w1 and w2 sampling also revealed increase in content of protein varying from 1.06 mg/kg (SWFIw1) to 2.10 mg/kg (SWFIIw1) and 1.12 mg/kg (NEFIw2) to 3.30 mg/kg (SWFIIw2), respectively. The total protein content was found to be higher in A. cepa bulbs treated with all the soil samples as compared to untreated bulbs (control). Elevation in total protein content of Allium bulbs treated with polluted soils (due to excessive use of agrochemicals, heavy metals and industrial emissions) observed in present study is in consistence with report by Mahajan and Tuteja (2005) who also assumed that increase in protein content could be attributed to the fact that contaminants present in the soil caused stress, resulting in activation of some genes responsible for overcoming stress. Although both the expression and functions of such proteins are unclear, yet it indicates that there is a relationship between some forms of plant adaptation and tolerance to stresses and expression of stress-induced proteins (Gupta and Ahmad 2011). Our results are in
conformity with those of Olorunfemi and Lolodi (2011) who estimated total protein content in A. *cepa* treated with different concentrations (0 %, 0.2 %, 0.4 %, 0.8 %, 1 %, 2 %, 3 %, 4 % and 5 %) of fresh effluents from the cassava processing mills in Uselu Quarters, Benin City and found dose dependent increase in protein content up to four-fold at 1% as compared to control. After 1 % concentration, protein content was found to decrease. Sharma *et al.* (2011a) also reported higher content of total soluble proteins in *Raphanus sativus* seedlings under nickel stress as compared to control.

Different types of environmental stresses not only enhance the protein content but also trigger the active defense mechanisms in plants resulting in expression of various detoxifying enzymes (Mahajan and Tuteja, 2005). The balance between ROS production and detoxification is maintained by enzymatic antioxidative system which involves different enzymes such as APX, CAT, DHAR, GST, POD and SOD. It is well established that reactive oxygen intermediates (ROI) and antioxidative enzymes play a crucial role in the establishment of normoxia in biological systems and in resistance to oxidative stress. The dual role of ROI as toxic and signaling molecules are ensured by complex and elaborate system controlling intracellular ROI levels. The ROI are detoxified by single or a series of antioxidative enzyme reactions. It is clear that the capacity and activity of the antioxidative defense system are important in limiting oxidative damage and in destroying active oxygen species that are produced in excess of those normally required for metabolism. Consequently, the role of these antioxidative enzymes, such as SOD, CAT, POD, GR, APOX, MDHAR and DHAR becomes very important as all these enzymes act in a co-ordinated manner and constitute “Asada-Foyer-Halliwell pathway” (Noctor and Foyer, 1998; Arora *et al*., 2002; Asada, 2006; Sharma *et al*., 2011a,b).

In the present study, the activities of APX, CAT, DHAR, GST and SOD were found to be higher in A. *cepa* bulbs treated with all the soil samples as compared to control whereas activities of GR and POD were low. Our findings showed that increase in specific activity of APX (mol UA mg/g fresh protein) of onion bulbs treated with different soil samples of r1 sampling and r2 sampling varied from 0.42 (SWFIr1) to 1.57 (SWFIr1) and 0.51 SEFIr2) to 1.46 (NEFIr2), respectively as compared to untreated bulbs. Agricultural soil samples from wheat cultivated fields of w1 and w2
Discussion

Sampling also revealed increase in APX activity (mol UA mg/g fresh protein) varying from 0.36 (NWFIw1) to 0.93 (SWFIw1) and 0.30 (SWFIw2) to 1.15 (NEFIw2), respectively. Present study was observed to be in conformity with previous studies which have shown alterations in antioxidative enzymes under stress in plant systems. Bhardwaj et al. (2009) estimated effect of enhanced lead and cadmium in soil on physiological and biochemical attributes of *Phaseolus vulgaris* L. and found that activity of APX increased with increasing concentration of metals. Tepe and Aydemir (2011) reported increase in activity of APX in barley plants under boron toxicity. Mishra et al. (2013) studied antioxidative defense responses of *Glycine max* L. CV. Merrill under lead induced oxidative stress and revealed increase in activity of APX.

Catalase and other peroxidases are the major enzymes, required for the detoxification of H$_2$O$_2$ produced during photorespiration and are present in peroxisomes and different cell compartments such as chloroplasts, cytosol, peroxisomes and mitochondria (Tabrez and Ahmad 2011a,b). Present study showed slight increase in activity of CAT in *Allium* bulbs on treatment with different samples as compared to control. CAT activity (mol UA mg/g fresh protein) of onion bulbs treated with different soil samples of r1 sampling and r2 sampling varied from 0.005 (NEFIr1) to 0.012 (NWFIr1) and 0.005 (SEFIr2 and SWFIr2) to 0.008 (NEFIr2), respectively. Among bulbs treated with soil samples of w1 sampling, the sample NWFIw1 and SWFIw1 showed minimum CAT activity of 0.005 mol UA mg/g fresh protein whereas sample SEFIw1 showed maximum activity of 0.013 mol UA mg/g fresh protein. *A. cepa* bulbs treated with soil samples of w2 sampling also revealed increase in content of CAT varying from 0.005 - 0.012 mol UA mg/g fresh protein for samples SWFIw2 and NWFIw2, respectively. Our results are in conformity with earlier reports where increase in activity of CAT was found under metal stress. Bhardwaj et al. (2009) estimated effects of enhanced lead and cadmium in soil on physiological and biochemical attributes of *Phaseolus vulgaris* L. and found that activity of APX increased with increasing concentration of metal. Tepe and Aydemir (2011) found increase in activity of APX in barley plants under boron toxicity. Zou et al. (2012) also reported increase in activity of CAT in *Allium cepa Var. Agrogarium* under metal stress. Nadgorska-Socha et al. (2013) explored effects of soil contamination by selected
metals (cadmium, copper, nickel, lead or zinc) on the antioxidant response of *Vicia faba* plants and found increase in activity of CAT.

Dehydroascorbate reductase (DHAR) plays an important role in signaling and maintains the cellular level of ascorbic acid by regulating its redox state thereby, affecting cell response and tolerance to oxidative stress (Chen and Gallie, 2006; Gallie *et al.*, 2012; Anjum *et al.*, 2014a,b). Present study showed increase in activity of DHAR in all *A. cepa* bulbs exposed to agricultural soil samples as compare to untreated bulbs (0.12 mol UA mg/g fresh protein). Soil samples of r1 and r2 sampling showed DHAR activity (mol UA mg/g fresh protein) of 0.18 (NEFIr1) to 0.32 (SWFIr1) and 0.14 (SEFIr2) to 0.33 (SWFIr2), respectively. Agricultural soil samples from wheat cultivated fields of w1 and w2 sampling also revealed increase in specific activity of DHAR (mol UA mg/g fresh protein) varying from 0.18 (NEFIw1, SWFIw1 and NWFIIw1) to 0.27 (SWFIw1) and 0.17 (SWFIw2) to 0.38 (NWFIIw2), respectively. Our study is in conformity with several other reports where increase in activity of DHAR was reported. Arora *et al.* (2012) studied antioxidative defense system of *Brassica juncea* L. subjected to cobalt ion toxicity and reported increase in activity of DHAR. Karuppanapandian and Kim, (2013) also investigated the effects cobalt induced oxidative stress, as well as the role of antioxidant systems on the leaves of hyperaccumulating plant *Brassica juncea* L and reported the increase in activity of DHAR. *Oryza sativa* L. seedlings also showed increase in activity of DHAR on exposure to slight salinity by NaCl.

Glutathione reductase is a member of flavoenzyme family which catalyzes the NADPH dependent reduction of glutathione disulphide (GSSG) to glutathione (GSH) and it maintains glutathione in the reduced state which in turn reduces dehydroascorbate to ascorbate. The GR activity in agricultural soil exposed *A. cepa* bulbs declined with respect to control. Decrease in activity of GR was observed in *A. cepa* bulbs treated with different soil samples as compared to control. Bulbs treated with soil samples of r1 sampling and r2 sampling showed GR activity (mol UA mg/g fresh protein) of 0.03 (SWFIr1) to 0.42 (NEFIr1) and 0.07 (SWFIr2) to 0.44 (NEFIr2), respectively. Agricultural soil samples from wheat cultivated fields of w1 and w2 sampling also revealed decrease in specific activity GR (mol UA mg/g fresh protein) varying from
0.17 (NWFIIw1) to 0.41 (NEFIIw1) and 0.03 (SWFIIw2) to 0.37 (NWFIIw2), respectively. GR participates not only in H$_2$O$_2$ scavenging, but also favors a high GSH/GSSG ratio to maintain a proper cellular redox mechanism (Sharma et al., 2011a,b). Several other reports have mentioned decrease in activity of GR under abiotic stress (Gao et al., 2008; Karruppanapandian et al., 2009; Nouairi et al., 2009; Anna et al. 2011; Gangwar et al., 2011). In a study by Kumar et al. (2009), suppressed activity of GR was observed in maize plants colonized with Piriformospora indica. Similarly, Tabrez and Ahmad (2011d) reported decrease in GR activity under the effect of trichloethylene in A. cepa.

Glutathione-S-transferases (GSTs) are a super family of enzymes, principally known for their important role in detoxification reactions (Kumar et al., 2013; Dubey et al., 2014). The activity of GST in Allium bulbs treated with all the soil samples was found to be higher than control bulbs. GST activity (mol UA mg/g fresh protein) of onion bulbs treated with different soil samples of r1 and r2 sampling varied from 0.10 (SEFIr1) to 0.19 (SWFIIR1) and 0.10 (SWFIr2) to 0.22 (SWFIIR2), respectively. Among soil samples collected during wheat cultivation, the bulb treated with sample NWFIIw1 showed minimum GST activity of 0.11 mol UA mg/g fresh protein whereas sample SWFIIw1 showed maximum activity of 0.33 mol UA mg/g fresh protein. A. cepa bulbs treated with soil samples of w2 sampling also revealed increase in content of GST varying from 0.10-0.22 mol UA mg/g fresh protein for samples SWFIw2 and SWFIIw2, respectively. The findings of our study is in consistence with reports by Sun et al. (2007), Shukla et al. (2012) and Dubey et al. (2014) that glutathione levels in plant tissues are known to increase under metal stress. Similar increase in activity of GST has been reported by Gupta and Ahmad (2011) when they studied the effects of Mathura Refinery waste water (MRWW) in A. cepa bulbs. Significant increase in activity of GST was reported by Dubey et al. (2014) while studying the heavy metals induce oxidative stress and genome-wide modulation in transcriptome of rice. Increase in activity of GST and their role in detoxification is well documented by researchers throughout the world (Tabrez and Ahmad 2009; Tabrez and Ahmad 2011b; Sbartai et al., 2011; Perez-chaca et al., 2014)
POD also plays an important role in carrying out different physiological functions which include biosynthesis of cell wall components like lignin and suberin, oxidation of toxic compounds and various developmental processes (Sharma et al., 2011a). In the present study, POD activity in Allium bulbs treated with soil samples of r1, r2, w1 and w2 sampling showed a decrease as compared to control. Only A. cepa bulbs treated with two samples SWFIr1 and SWFIr1 in r1 sampling and one sample SWFIr2 of r2 sampling showed increase in activity of POD. Variation in activity of POD was also reported by Tang (2012) where enzyme activity showed an increase and then decrease in Mimusops elengi seeds experiencing intense oxidative stress following desiccation induced changes. The results were in consistence with other report where Kou et al. (2013) found a decrease in POD activity in Huang guan pears (Pyrus purifolia Nakai) that were treated with calcium chloride, chitosan and pullulan for prolonging the post harvest life of pears.

SOD is a family of metaloenzymes which is considered as the first line of defense against ROS generation because superoxide radical is considered as a precursor to several other ROS. Superoxide is considered as the central component of the signal transduction which activates the genes responsible for enzymes of defense system including SOD and could serve a very useful marker for metal stress. Superoxide radical generated by any means, is dismutated to H$_2$O$_2$ and oxygen by SOD, and this H$_2$O$_2$ generated is then removed by peroxidases. H$_2$O$_2$ is converted to water by APX in concert with oxidation of ascorbate and also by GPX with oxidation of GSH in the cytosol and chloroplasts (Fatima and Ahmad 2005; Tabrez and Ahmad 2011a, d). Bulbs treated with soil samples of r1 sampling and r2 sampling showed SOD activity (mol UA mg/g fresh protein) of 43.42 (NEFIr1) to 97.91 (SEFIr1) and 43.48 (SEFIr2) to 106.03 (SEFIr2), respectively. Agricultural soil samples from wheat cultivated fields of w1 and w2 sampling also revealed significant increase in specific activity SOD (mol UA mg/g fresh protein) varying from 43.48 (SEFIw1) to 106.03 (SEFIw1) and 45.22 (NEFIw2) to 138.01 (SEFIw2), respectively. Increase in SOD activity in the present study was consistent with various other studies. Fatima and Ahmad (2005) also reported
the utility of SOD in *A. cepa* as biomarker for detection of toxic metals in waste water collected from the industrial estate of Aligarh city, India. Shi *et al.* (2009) showed alleviated level of SOD in *Cannabis sativa* L. under Cd-induced oxidative stress. Significant enhancement of about 8 times in SOD activity was found with respect to control. Tepe and Aydemir (2011) also reported an increase in SOD activity of lentil and barley plants when exposed to boron stress. Saidi *et al.* (2014) reported increase in activity of SOD in seedlings of *Helianthus annuus* under Cd-induced oxidative stress. Thounaojam *et al.* (2014) studied activities of antioxidant enzymes in *Oryza sativa* L. under copper stress and reported dose dependent increase in activity of SOD. Several other studies have reported increase in activity of SOD in different plants under different abiotic stresses (Zou *et al.*, 2012; Lu *et al.*, 2013; Perez-Chaca *et al.*, 2014; Zhang *et al.*, 2014)

Results of the present study revealed that activities of APX, CAT, DHAR, GST and SOD were higher in *A. cepa* bulbs treated with soil samples as compared to control whereas activities of GR and POD were low. Metal analysis have shown the presence of one or the other heavy metal in all the soil samples tested and thus the increase in enzyme activities may be attributed to heavy metal induced oxidative stress indicating response of cells to cope up with increased level of ROS (Gupta and Ahmad, 2011). Similar increases in activities of antioxidative enzymes have been reported by several researchers (Zhang *et al.*, 2008; Nadgórksa-Socha *et al.*, 2010; Dubey *et al.*, 2014). In our study, the activity of APX, CAT, DHAR, GST and SOD in *Allium* bulbs was enhanced indicating the active involvement of these enzymes in detoxification of heavy metal induced production of ROS. These results are in consistent with several studies reporting the ability of plants to tolerate metal stress by enhancement of antioxidant defense system (Shi *et al.*, 2009; Duarte *et al.*, 2014; Lamhamdi *et al.*, 2013; Yu *et al.*, 2013; Sanchez-Pardo *et al.*, 2014).

Our findings suggest that *A. cepa* test model is a simple and sensitive system for monitoring the toxicity of soil samples polluted with contaminants from various anthropogenic activities. The study prelude the use of combination of physico-chemical
and analytical analysis along with biological systems to evaluate the potential toxicity of contaminated soils of Amritsar. The data from genotoxicity and oxidative stress studies using chromosomal aberration assay and antioxidative enzyme activities, respectively serve as useful biomarkers and provide significant information for soil monitoring.