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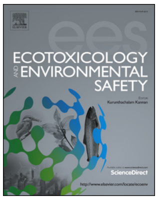

Potential ecological risks of metal(loid)s in riverine floodplain soils

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ABSTRACT

The quality of soils under different land uses is getting deteriorated throughout the world due to various anthropogenic activities. This deterioration is highly complex in riverine floodplain areas due to contamination by multiple point and non-point sources and change in seasons. Therefore, a study was conducted to analyze seasonal (pre and post-monsoon) variations in physico-chemical characteristics, contents of metal(loid)s (Al, As, Cd, Cr, Co, Cu, Fe, Mn, Mo, Ni, Pb, Sb and Zn) in riverine floodplain soils under three land uses (agricultural, riverbank and roadside) from areas around the rivers Beas and Sutlej in Punjab, India. Further, analysis was done to assess the ecological and genotoxic risks (*Allium cepa* genotoxicity assay) posed by metal(loid)s in these soils. It was observed that soil samples under the three land uses were slightly alkaline (pre-monsoon) to acidic (post-monsoon) in nature with sandy texture and low soil organic matter. The levels of most metal(loid)s increased in post-monsoon soil samples under the three land uses, which was attributed to increase in soil organic matter, silt and clay contents in post-monsoon samples due to precipitation, flooding and sedimentation. The ecological Risk Index (58.3–104.5) and Modified Risk Index (145.2–178.9) calculated to analyze the level of ecological risks of metal(loid)s revealed that As, Cd and Sb posed moderate to considerable ecological risks in the agricultural and roadside soils in both seasons. *Allium cepa* genotoxicity assay indicated that the metal(loid)s in studied soils can cause genotoxic effects in biological systems. Therefore, various steps such as reduction in use of agrochemicals, promotion of organic agricultural methods and decontamination of soils using techniques such as phytoremediation etc must be taken to ensure reduction and containment of metal(loid)s in such riverine floodplain areas.

1. Introduction

The soil environments worldwide are under threat of metal(loid) contamination due to human activities such as industrialization, urbanization, intensive agriculture, wastewater irrigation, vehicular traffic etc. (Brady and Weil, 2008; M. Kaur et al., 2014; Chandrasekaran et al., 2015). But, the metal(loid) contamination risk is far more complex in riverine floodplain areas because a conglomeration of metal(loid)s brought and deposited by rivers from distant areas can occur in the soils (Rennert et al., 2017; Iwegbue et al., 2018). The metal contents in riverine floodplain soils under different land uses are further impacted by changes in climatic conditions, because events like precipitation during rainy seasons (as monsoon season in India) causes flooding which significantly changes the soil characteristics and deposits metal (loid)s brought from various upstream sources. The soil characteristics such as pH, soil organic matter (SOM), soil texture etc. significantly affect the mobility, availability and toxicity of metal(loid)s in soils (Troeh and Thompson, 2005; Boluda et al., 2011). Therefore, the regular analysis of soil characteristics and metal(loid)s contents is necessary for determining their impacts on plants, animals and humans. But, analysis of metal(loid) contents alone does not indicate their potential risks in soil. Further different factors and indices such as Contamination Factor (CF) and Enrichment Factor (EF), Ecological Risk Index (RI) and Modified Risk Index (MRI) should be calculated to determine the potential ecological impacts of metal(loid)s in soils (Trujillo-González et al., 2016; Duodu et al., 2016; Tian et al., 2017; Bhatti et al., 2018). In order to examine the impact of contaminants (such as metal(loid)s) in soil on biological systems, rapid, sensitive and widely applied plant assays such as *Allium cepa* root chromosomal assay can be used (Rank et al., 2003; M. Kaur et al., 2014).

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Considering the risks posed by metal(loid)s in riverine floodplain soils, a seasonal study was conducted in floodplain areas of Punjab, India around its two main rivers Beas and Sutlej, to analyze a hypothesis that "Metal(loid)s pose ecological and genotoxic risks in soils under different land uses in riverine floodplain soils". The area selected for study was around Harike wetland which is a Ramsar site where Beas and Sutlej rivers merge. Although previous studies have been done on heavy metal(loid) contamination of soils around rivers from the study area and other parts of world (Bhatti et al., 2016, 2017; Rennert et al., 2017; Iwegbue et al., 2018), but few reports have depicted the seasonal changes in soil characteristics and ecological impacts of metal(loid)s contents in riverine floodplain areas. Therefore, in the present study soils under three land uses i.e. agricultural, riverbank and roadside were selected for analysis of soil characteristics, metal(loid)s contents, ecological risks posed by metal(loid)s and genotoxic potential (using *Allium cepa* genotoxicity assay) in two seasons i.e. pre-monsoon (April to June) and post-monsoon (October to January).

2. Material and methods

2.1. Study area

The study area comprised of six villages situated around the rivers Beas and Sutlej in Punjab in areas around Harike wetland. Fig. 1 shows the map of the studied area having the six villages and Supplementary Table 1 shows the details of villages. These villages have fertile lands with sandy loam and calcisolic soils. The annual rainfall in this area is 435.6 mm and has semiarid to sub-humid climate. Agriculture is the main occupation of this area and has two main crop seasons Rabi (pre-monsoon) and Kharif (post-monsoon). Wheat and rice are the two main crops grown in Rabi and Kharif season, respectively. Significant industrial and urban activities are prevalent in upstream areas like Ludhiana, Jalandhar, Kapurthala (J. Kaur et al., 2014).

2.2. Sampling and preparation

Soils were collected in the month of April 2013 (pre-monsoon) and October 2013 (post-monsoon). The monsoon season falls in the study area from July-September period in which precipitation occurs throughout north India. The precipitation causes high inflow of water in rivers from mountains and causes flooding and sedimentation in areas along almost all rivers of north India including Beas and Sutlej. Soil samples in triplicates were collected from each village as composite samples for each of the three land uses i.e. agricultural under wheat (pre-monsoon) and rice (post-monsoon) cultivation, river bank and roadside.

Five subsamples were pooled for each replicate to form the composite soil samples (approximately 2 kg) of the three land uses from

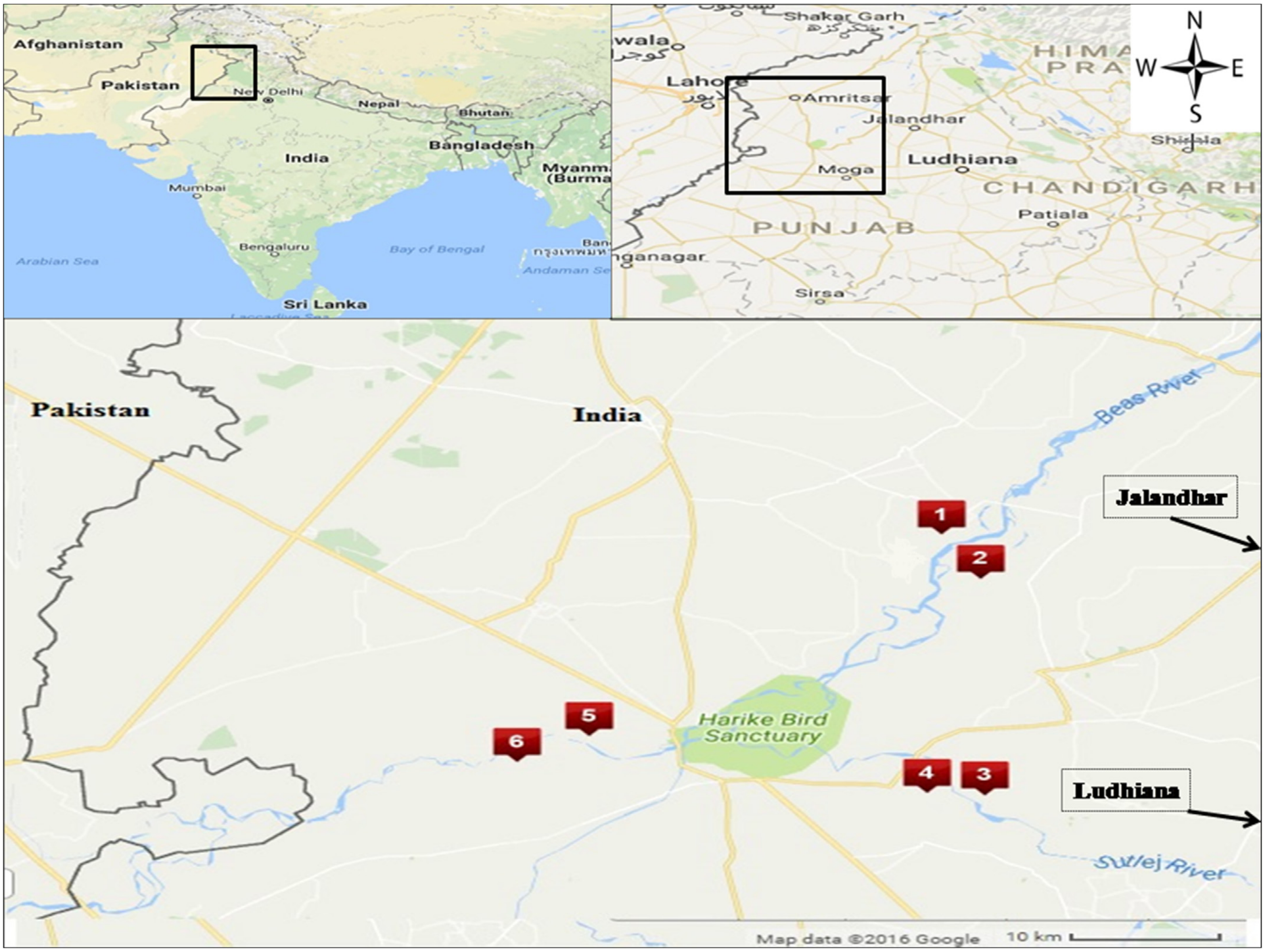


Fig. 1. Map of the sampling villages around Beas and Sutlej rivers, Punjab, India. *Six sampling villages are shown in red flags. Village names (full detail of villages given in Supplemetary Table 1): 1-Jalalabaad; 2-Rajewal; 3-Yousufpur; 4-Tibbi Taiba; 5-Doomniwala; 6-Gatta Badshah. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

different sites across the village. Surface soil samples were taken from a depth of 0–15 cm. Bulk density (BD) was determined using the core cylinder method (Jacob and Clarke, 2002). The soil samples were air-dried, ground, passed through 2 mm sieve and stored in clean polythene bags until further analysis (Kavianpoor et al., 2012).

2.3. Physico-chemical analysis of soils

The pH and conductivity of soils were determined in 1:5 (m/v) soil: water suspension (Rodriguez Martin et al., 2013). Walkley Black wet oxidation method was used for the determination of soil organic carbon content (Nelson and Sommers, 1982) and multiplied by a factor of 1.72 to estimate soil organic matter (SOM) contents of soils. Soil texture was determined by the Hydrometer method (Jacob and Clarke, 2002). Potentially available phosphorous (AP) and total nitrogen (N) in soils were determined by the Olsen method (Olsen et al., 1954) and Kjeldahl method (Bremner and Mulvaney, 1982), respectively, while, EDTA titration method was used for measuring calcium (Ca) and magnesium (Mg) (Lanyon and Heald, 1982). Sodium (Na) and potassium (K) were determined using flame photometric method. The carbonate content of soils ($CaCO_3$) was determined using an acid neutralization method (Hesse, 1971). Soluble salts were determined using a batch extraction method using 1:5 (m/v) soil/water ratio. Five grams of air dried soil was shaken with 25 ml water for one hour. Chloride and sulphate were analyzed using a Dionex ICS-2500 system with 2.0 mm AS16 anion separation column and hydroxide eluent generated on line followed by conductivity detection after chemical suppression by APHA method 4110 C (Rayment and Lyons, 2011). The solutions were then analyzed for non-metallic elements S and Si on an Agilent 5100 Inductively Coupled Plasma Mass Spectrometer by APHA method 3120.

2.4. Total metal(loid)s concentrations in soils

Total metal(loid) concentrations (Al, As, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Sb and Zn) in soils were determined using a concentrated acid closed vessel microwave digestion procedure (US EPA method 3051 A, 1998). Approximately 0.25 g of soil was weighted into 50 ml PFA digestion vessels and 10 ml of aqua regia added (3 ml (v/v) Hydrochloric acid (HCl): 1 ml Nitric acid (HNO_3)). The samples were digested in a microwave oven (Milestone) using the temperature and time program in which the samples were heated to 175 ^{0}C over 10 min and then kept at 175 ^{0}C for 10 min. The samples were then cooled for 20 min. After digestion, digest solutions were diluted to 50 ml using ultrapure deionized water (Milli-Q, Millipore), filtered to < 0.45 µm (Sartorius) and stored at 40 °C in a refrigerator until analysis. The digest solutions were analyzed for selected metal(loid)s (Al, As, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Sb and Zn) using iCAP 6000 series Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) from Thermo Fisher Scientific and Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) (Agilent 7700) at CSIRO, Australia. For standard quality assurance and quality control, certified reference material (MESS-3), in-house standards and duplicate samples were analyzed. Duplicate analysis was done for 10% samples for which the relative standard deviations for these replicate samples were less than 5%.

2.5. Level of anthropogenic metal(loid) contamination in soils

The level of anthropogenic metal(loid) contamination in soils was examined by calculating the Contamination Factor (CF) and Enrichment Factor (EF). These factors are dependent on the anthropogenic and reference/background metal(loid) concentration in soils (Pathak et al., 2015). There is no data currently available regarding the background metal(loid) concentrations in soils of the study area. Therefore, the concentrations of metal(loid)s in the earth's crust (Taylor and Mclennan, 1995) were used as done previously by Sakram et al. (2015). The CF and EF values were calculated using the following equations:

a) Factor: -Contamination Factor (CF) is a reflection of anthropogenic inputs of metal(loid)s in soils. The CF was defined by Hakanson (1980) as:

$$CF = C^i_0 / C^i_n \tag{1}$$

where, C^i_0 is the mean concentration of metal(loid) i from the sampling site, and C^i_n is the concentration of metal(loid) in reference/background soil environment (Taylor and Mclennan, 1995). The values of CF is classified into four pollution categories by Hakanson (1980) i.e. CF $<$ 1: low contamination; CF- 1 to $<$ 3: moderate contamination; CF- 3 to $<$ 6: considerable contamination; CF- $\geq$ 6: very high contamination

b) Enrichment Factor (EF) gives the enrichment of an element of interest against the concentration of a reference element (Sakram et al., 2015). An element geochemically distinguishing with high concentration in the environment and not capable of showing antagonism or synergism towards the examined element can be used as a reference element (Chandrasekaran et al., 2015). Iron (Fe) was selected as the reference element, as it had been widely used for normalization previously (Paul et al., 2015). The EF can be calculated as follows:

$$EF = (C_{sample} / Fe_{sample}) / (C_{reference} / Fe_{reference}) \tag{2}$$

where, C_{sample} represents the concentration of the metal(loid) in the soil sample, Fe_{sample} is the Fe concentration in sample, $C_{reference}$ is the concentration of the tested element in reference environment (Taylor and Mclennan, 1995) and $Fe_{reference}$ is the Fe concentration in the reference environment. Sutherland (2000) proposed five pollution categories of elements in soils: EF: $<$ 2 for minimal, EF: 2–5 for moderate, EF: 5–20 for significant, EF: 20–40 for very high and EF: $>$ 40 for extreme pollution in soils.

2.6. Potential ecological risk impact index

The potential Ecological Risk Index (RI) has been proposed to determine the potential ecological impacts of metal(loid) contaminants in soils (Hakanson, 1980) proposed. The RI is defined as the addition of the potential ecological risk factors (Er) of individual metal(loid) at one site. The Er of individual metal(loid) is calculated by multiplying CF of a metal(loid) observed for a site and toxicological response factor (Tr) of metal(oid). The toxicological response factor for different metal(loid) were 10 for As, 30 for Cd, 5 for Cu, Co and Pb, 3 for Ni, 2 for Cr and 1 for Mn and Zn (Hakanson, 1980; Zheng-qi et al., 2008; Duodu et al., 2016). The RI is calculated using the following equation:

$$RI = \sum_{i=1}^{n} Eri = \sum_{i=1}^{n} Tri \times CFi \tag{3}$$

where, *Eri* is the potential ecological risk of an individual metal(loid) *i*, *Tri* represents the toxic response factor of the metal(loid) and *CFi* represents the contamination factor of the metal(loid) *i*.

The RI is based upon CF which accounts for anthropogenic inputs of metal(loid)s into the soil environment, but does not account for background lithogenic and sedimentary inputs of elements of interest. In order to assess ecological risks of the anthropogenic as well as lithogenic inputs of metal(loid)s, the Enrichment Factor (EF) is substituted for the calculation of potential ecological risk index. The ecological risk index derived using EF for metal(loid)s in soils is called a Modified Risk Index (MRI) (Duodu et al., 2016; Bhatti et al., 2018). The MRI can be defined as the addition of the modified potential ecological risk factors (mEr) of individual metal(loid) at one site. The mEr of individual metal (loid) is based upon EF of metal(loid) at the site and its toxicological response factor (Tr) as described in case of CF. Thus, the MRI is calculated using the following equation:

$$\mathrm{MRI} = \sum_{i=1}^{n} \mathrm{mEri} = \sum_{i=1}^{n} \mathrm{Tri} \times \mathrm{EFi} \tag{4}$$

where, mEri is the modified potential ecological risk factor of an individual metal(loid) at a site, Tri represents the toxic response factor of the metal(loid) and EFi represents the enrichment factor of the metal (loid) at the site. The grading standards of RI and MRI are presented in Supplementary Table 2.

2.7. Genotoxicity analysis by *Allium cepa* genotoxicity assay

The genotoxicity of soil samples was examined using *Allium cepa* root chromosomal aberration assay. Healthy and young uniform sized onions (n = 16) of about 10–15 g wet weight, were purchased from the local market and grown directly in small pots containing soil samples (five onions for each replicate of soil sample). Distilled water was used as negative control as described by Rank (2003) and M. Kaur et al. (2014). The onions pots were kept for 48 h in a BOD (Biochemical oxygen demand) incubator at 20 ± 1 °C until roots were grown which were collected and preserved in Farmer's Fluid (glacial acetic acid:-ethanol at 1:3 ratio). The roots were then hydrolyzed in 1 M HCl for 1 min and kept in aceto-orcein stain and 1 M HCl for 35 min, washed in 45% glacial acetic acid for excessive dye removal, squashed under a cover slip on a glass slide and sealed with DPX (a mixture of distyrene, a plasticizer (tricresyl phosphate) and xylene). The slides were examined under a light microscope for determination of the Mitotic Index (MI) i.e. percentage of dividing cells in each slide and percent Aberrant Cells (AC) i.e. number of aberrant cells per 100 dividing cells. The slides were observed for chromosomal abnormalities such as abnormal metaphases, stickiness, c-mitosis, chromatin bridges etc.

2.8. Statistical analysis

The physico-chemical characteristics and metal(loid) content analysis of different soil samples from each village was done in triplicates, and the average of the values from the six villages was represented as mean ± SE. One way ANOVA followed by Tukey's HSD test as post hoc was used to compare the means of physico-chemical properties and metal(loid) contents in soils of the three land uses. The difference in soil characteristics and metal(loid)s between the soil samples of pre-monsoon and post-monsoon seasons was analyzed by Student's Independent *t*-test. The difference in genotoxicity parameters between the soil samples and negative control was also analyzed by Student's Independent *t*-test. Differences at $p < 0.05$ were considered statistically significant in both Tukey's HSD test and *t*-test. Pearson's correlation coefficients were calculated to analyze the correlation between physico-chemical properties, metal(loid)s and genotoxicity parameters (MI and AC) of the soil samples. Statistical analysis was done with the help of IBM SPSS version 16.0 (Chicago, IL, USA) and Minitab version 14.0 (Pennsylvania, USA) and PAST computer software programs.

3. Results and discussion

The average of physico-chemical characteristics and metal(loid) contents in soils under the three land uses analyzed in pre-monsoon and post-monsoon seasons from the six villages are given in Tables 1, 2, respectively. The genotoxic potential of the soil samples analyzed is represented in Table 3. Supplementary Table 3 presents the Pearson's correlation analysis of soil characteristics, metal(loid)s and genotoxicity parameters of soils, while Supplementary Table 4 presents the comparison of metal(loid) contents observed in soils under three land uses in the present study to metal(loid) contents observed in other parts of world.

3.1. Physico-chemical characteristics of soils

The physico-chemical characteristics are very important in identifying the soil fertility and mobility of metal(loid)s in soil (Paul et al., 2015). Soil pH-value which is a very important soil character was found to be slightly alkaline during pre-monsoon and slightly acidic during post-monsoon season (Table 1). Bulk density (BD) values of soil samples under the three land uses ranged from 1.1 to 1.2 g/cm^3 in pre-monsoon samples and 1.0–1.2 g/cm^3 in post-monsoon season. The range of pH and BD in the present study was similar to the range observed by Kumar et al. (2015) and M. Kaur et al. (2014) in soils of district Bathinda and Amritsar of Punjab, respectively in wheat sowing pre-monsoon season. The texture of soil samples under the three land uses was found to be sandy to sandy loam with maximum sand contents observed in riverbank soils. Soil organic matter (SOM) which is a major factor in controlling and stabilizing metal(loid)s in soil was found to be near desertification levels. Such low levels of SOM can be attributed to the sandy texture of the soil under the three land uses. Overall, the essential plant nutrients nitrogen (N), available phosphorous (AP) and potassium (K) were found to be higher in agricultural soils as compared to soils under riverbank and roadside land uses (with exceptions to K and AP contents in post-monsoon roadside soil samples) which can be attributed to application of mineral fertilizers in agricultural soils. The other soil nutrients i.e. calcium, magnesium and sodium were found to be in desired levels in both seasons. High $CaCO_3$ contents (4.3–6.8%) observed in soils in present study might be due to semi-arid conditions and calcareous nature of soils (Kumar and Babel, 2011). Sulphur, which is considered as the fourth major plant nutrient after N, P and K, (Prasad and Shivay, 2016) ranged from 180.9 to 201.3 mg/kg in pre-monsoon samples and from 124.7 to 275.1 mg/kg in post-monsoon samples. Selenium, an essential nutrient for humans and animals recommended by WHO was found to be significantly lower than Spanish maximum permissible limits of 85 mg/kg (Miguel et al., 2016) in the present study. Chlorine which occurs in soils in the form of chlorides (Troeh and Thompson, 2005) was found to be lowest in agricultural soil sample in the present study due to uptake of Cl^- by crop plants for nutritional requirements. The content of sulphates (SO_4^{2-}) which are important constituents of soil parent rock material (Brady and Weil, 2008) was also found to be lowest in agricultural soil samples and highest in roadside soil samples.

3.1.1. Seasonal analysis: physico-chemical characteristics

3.1.1.1. Agricultural soils. Statistically significant differences were observed in soils under the three land uses for all studied soil characteristics in post-monsoon samples in comparison to the pre-monsoon samples from the six villages (Supplementary Table 5). The decrease in levels of soil pH in post-monsoon samples can be attributed to decrease in the levels of bases such as $CaCO_3$, Na^+ and increase SOM contents which secrete organic acids (Brady and Weil, 2008). The decrease in EC in post-monsoon samples can be attributed to lowering of available soil ions (N, AP, Na etc.) in post-monsoon samples by leaching due to precipitation or flooding caused in these riverine floodplains (Troeh and Thompson, 2005). The EC of soils is in direct proportion to the concentration of various ions, which is represented by positive correlation between EC and these ions (Supplementary Table 3). Higher levels of silt and clay contents were observed in post-monsoon agricultural soils which can be attributed to flooding caused by precipitation in monsoon season which brings sediments of varying size in these riverine floodplain soils (Brady and Weil, 2008). The increase in silt and clay contents in soils also resulted in increase of various soil nutrients such as Ca^{2+}, Mg^{2+}, Na^+, Se, S etc. in post-monsoon samples, which are mainly lithogenic in origin. The significant positive correlation (Supplementary Table 3) between silt, clay and many of these minerals represents the similar source of origin. The levels of EC (< 1 mS/cm) and BD observed in soils were suitable for agriculture (USDA-NRCS, 2014a, 2014b; Kumar et al., 2015).

Table 1
Average physico-chemical characteristics of soil under the three land uses in pre and post-monsoon samples.

Parameters	Agricultural soil		Riverbank soil		Roadside soil	
	Pre-monsoon	Post-monsoon	Pre-monsoon	Post-monsoon	Pre-monsoon	Post-monsoon
pH	8.0 ± 0.2^{a}	$6.6 \pm 0.1^{A*}$	7.9 ± 0.2^{a}	$6.6 \pm 0.2^{A*}$	7.7 ± 0.1^{a}	$6.5 \pm 0.1^{A*}$
EC (mS/cm)	0.4 ± 0.1^{a}	0.3 ± 0.1^{A}	0.3 ± 0.0^{a}	0.3 ± 0.1^{A}	0.3 ± 0.1^{a}	$0.6 \pm 0.3^{A*}$
BD (g/cm^3)	1.1 ± 0.0^{a}	1.0 ± 0.1^{A}	1.2 ± 0.1^{a}	1.2 ± 0.1^{A}	1.2 ± 0.1^{a}	1.2 ± 0.1^{A}
Sand (%)	82.2 ± 3.2^{a}	82.0 ± 4.7^{A}	93.5 ± 2.0^{a}	91.0 ± 4.7^{A}	87.1 ± 7.7^{a}	88.7 ± 4.6^{A}
Silt (%)	12.3 ± 1.6^{a}	11.8 ± 2.8^{A}	3.7 ± 1.1^{a}	6.0 ± 3.7^{A}	9.0 ± 5.6^{a}	7.3 ± 3.5^{A}
Clay (%)	5.5 ± 1.7^{a}	6.2 ± 2.0^{A}	2.8 ± 0.9^{a}	3.0 ± 1.1^{A}	3.9 ± 2.2^{a}	4.0 ± 1.2^{A}
SOM (%)	2.8 ± 0.5^{a}	2.4 ± 0.6^{A}	1.8 ± 0.4^{a}	2.6 ± 0.4^{A}	1.9 ± 0.4^{a}	$2.9 \pm 0.3^{A*}$
Ca (meq/100 g)	1.0 ± 0.2^{a}	1.2 ± 0.3^{A}	0.6 ± 0.1^{a}	$1.0 \pm 0.2^{A*}$	0.7 ± 0.2^{a}	2.7 ± 0.4^{A}
Mg (meq/100 g)	1.0 ± 0.3^{a}	$1.9 \pm 0.3^{A*}$	1.1 ± 0.2^{a}	1.8 ± 0.5^{A}	0.9 ± 0.2^{a}	$2.9 \pm 0.8^{A*}$
Na (mg/100 g)	60.9 ± 12.0^{a}	$24.0 \pm 10.9^{A*}$	39.2 ± 9.0^{a}	26.1 ± 8.2^{A}	54.0 ± 23.2^{a}	31.3 ± 10.0^{A}
K (mg/100 g)	127.6 ± 10.6^{a}	126.5 ± 15.5^{A}	84.3 ± 14.7^{b}	120.4 ± 20.6^{A}	90.0 ± 10.9^{b}	$159.6 \pm 20.5^{A*}$
N (g/kg)	0.5 ± 0.2^{a}	0.2 ± 0.1^{A}	0.2 ± 0.0^{a}	0.2 ± 20.6^{A}	0.2 ± 0.1^{a}	0.2 ± 0.0^{A}
AP (mg/kg)	87.2 ± 21.7^{a}	$38.9 \pm 6.6^{A*}$	49.4 ± 12.8^{ab}	30.6 ± 5.6^{A}	44.5 ± 11.5^{b}	96.5 ± 49.5^{A}
CaCO$_3$ (%)	6.8 ± 0.9^{a}	6.5 ± 2.1^{A}	6.4 ± 1.3^{a}	4.3 ± 1.2^{A}	6.3 ± 1.2^{a}	6.4 ± 2.3^{A}
S (mg/kg)	180.9 ± 27.5^{a}	191.5 ± 42.3^{B}	188.1 ± 57.3^{a}	124.7 ± 16.5^{B}	201.3 ± 56.0^{a}	$275.1 \pm 81.2^{A*}$
Se (mg/kg)	0.5 ± 0.1^{a}	0.6 ± 0.0^{A}	0.4 ± 0.0^{b}	0.4 ± 0.1^{B}	0.4 ± 0.1^{ab}	0.4 ± 0.1^{B}
Cl (mg/kg)	24.8 ± 10.2^{b}	19.8 ± 19.1^{B}	149.9 ± 99.7^{a}	$47.6 \pm 58.5^{AB*}$	39.0 ± 22.0^{b}	$158.6 \pm 123.2^{A*}$
SO$_4$ (mg/kg)	61.9 ± 20.0^{b}	69.5 ± 25.0^{B}	245.0 ± 144.1^{a}	103.8 ± 77.2^{B}	108.1 ± 61.6^{b}	$220.2 \pm 142.7^{A*}$

Mean values of parameters followed by different letters (Lowercase for pre-monsoon, Uppercase for post-monsoon) in rows are significantly different (one-way ANOVA; Tukey's test, $p \le 0.05$) in soils under the three land uses.

* Difference between pre-monsoon and post-monsoon values of parameters statistically significant (Independent Student's *t*-test, $p \le 0.5$).

3.1.1.2. Riverbank soil. The statistically significant increase in silt and clay contents of post-monsoon samples was most evident in riverbank soils (Supplementary Table 5), which can be due to the maximum effect of flooding in monsoon seasons on these soils. The rivers Beas and Sutlej are the main factors affecting soil texture of these soils as these rivers have brought and settled weathered rock, sand, silt and clay particles from mountainous region to this area for thousands of years (Bhatti et al., 2017). There was significant increase in SOM in most post-monsoon riverbank samples which can be attributed to availability of higher amount of water in these soils due to precipitation during monsoon season and faster decomposition of organic litter (Troeh and Thompson, 2005). Statistically significant decrease in Cl contents was observed in most post-monsoon riverbank samples which can be due to leaching of freely available Cl to lower layers in these well-drained post-monsoon soils (Brady and Weil, 2008).

3.1.1.3. Roadside soils. In comparison to soils under other land uses in these floodplain areas, roadside soils were more affected by precipitation than flooding (Supplementary Table 5). There was significant increase in EC of post-monsoon roadside samples which can be attributed to very high increase in soil nutrients such as N and P. Such high increase in the levels of different nutrients in roadside soils can be attributed to factors such as deposition of nutrients from nearby agricultural fields due to overflow of water, lack of adequate vegetation for absorption and occasional deposition of sediments in cases of severe flooding. Very high increase (statistically significant) in SO_4^{2-} was also observed in post-monsoon roadside samples which might be due to oxidation and release of sulphates in these soils due to drainage caused in monsoon (Brady and Weil, 2008).

3.2. Total metal(loid) contents in soils

In the present study, despite the intensive agricultural practices and other anthropogenic inputs, the contents of the various metal(loid)s analyzed were found be below various national and international maximum permissible limits (Table 2). Also, the metal(loid) contents

Table 2
Average metal(loid) contents in soils under the three land uses in pre and post-monsoon samples.

Metal(loid) (mg/kg)	Agricultural Soil		Riverbank Soil		Roadside Soil		Indian limits[x] (mg/kg)	European Union limits[y] (mg/kg)
	Pre-monsoon	Post-monsoon	Pre-monsoon	Post-monsoon	Pre-monsoon	Post-monsoon		
Al ($\times 10^3$)	10.1 ± 1.2^{a}	10.4 ± 1.9^{A}	7.1 ± 0.5^{a}	7.8 ± 1.5^{A}	8.6 ± 2.0^{a}	8.8 ± 1.8^{A}	–	–
As	6.8 ± 1.2^{a}	8.4 ± 1.6^{A}	4.5 ± 0.4^{a}	5.0 ± 1.0^{A}	6.5 ± 1.5^{a}	6.2 ± 1.6^{A}	–	20
Cd	0.1 ± 0.0^{a}	0.1 ± 0.0^{A}	0.1 ± 0.0^{b}	0.1 ± 0.0^{A}	0.1 ± 0.0^{ab}	0.1 ± 0.0^{A}	3–6	1
Co	4.8 ± 1.1^{a}	5.1 ± 1.6^{A}	1.9 ± 0.6^{b}	3.1 ± 1.5^{A}	3.3 ± 1.4^{ab}	3.7 ± 1.4^{A}	–	50
Cr	43.3 ± 2.1^{a}	44.3 ± 3.9^{A}	15.4 ± 1.0^{b}	18.3 ± 3.2^{B}	20.0 ± 4.6^{b}	19.3 ± 2.9^{B}	–	100
Cu	13.3 ± 2.1^{a}	14.6 ± 3.4^{A}	2.9 ± 1.2^{c}	4.9 ± 3.3^{B}	5.9 ± 3.2^{b}	5.6 ± 2.3^{B}	135–270	100
Fe ($\times 10^3$)	19.5 ± 2.2^{a}	20.4 ± 3.1^{A}	13.9 ± 1.0^{a}	16.0 ± 2.9^{A}	16.9 ± 3.2^{a}	15.9 ± 2.9^{A}	–	–
Mn	314.5 ± 44.2^{a}	333.1 ± 55.7^{A}	223.2 ± 19.5^{a}	261.5 ± 58.4^{A}	273.9 ± 54.8^{a}	265.8 ± 52.1^{A}	–	2000
Mo	0.3 ± 0.0^{a}	0.3 ± 0.1^{A}	0.3 ± 0.0^{a}	$0.2 \pm 0.0^{A*}$	0.3 ± 0.1^{a}	0.4 ± 0.1^{A}	–	–
Ni	20.7 ± 2.5^{a}	21.4 ± 3.8^{A}	14.2 ± 0.9^{a}	16.9 ± 3.4^{A}	18.8 ± 4.5^{a}	16.7 ± 3.4^{A}	75–150	50
Pb	9.3 ± 1.5^{a}	9.7 ± 1.8^{A}	5.8 ± 0.5^{a}	6.8 ± 1.9^{A}	8.2 ± 2.0^{a}	7.4 ± 1.5^{A}	250–500	100
Sb	0.3 ± 0.2^{a}	0.2 ± 0.1^{A}	0.2 ± 0.0^{a}	0.1 ± 0.0^{A}	0.2 ± 0.1^{a}	0.2 ± 0.1^{A}	–	2
Zn	50.5 ± 7.3^{a}	50.3 ± 8.5^{A}	32.1 ± 1.6^{a}	35.3 ± 6.5^{A}	45.2 ± 11.8^{a}	38.3 ± 6.1^{A}	300–600	300

Mean values of metal(loid)s followed by different letters (Lowercase for pre-monsoon, Uppercase for post-monsoon) in rows are significantly different (one-way ANOVA; Tukey's test, $p \le 0.05$) in soils under the three land uses.

* Difference between pre-monsoon and post-monsoon values of metal(loid)s statistically significant (Independent Student's *t*-test, $p \le 0.5$).

[x] Awashthi (2000)

[y] European Union (2009).

Table 3
Genotoxic potential analysis of soils under the three land uses in pre and post-monsoon samples using *Allium cepa* root chromosomal aberration assay.

Land uses	Pre-monsoon			Post-monsoon		
	Average Total Dividing Cells	MI (%)	AC (%)	Average Total Dividing Cells	MI (%)	AC (%)
Agricultural	508	13.4 ± 1.0*	11.6 ± 1.6*	469	13.6 ± 1.0*	11.1 ± 0.9*
Riverbank	494	10.3 ± 1.2*	7.4 ± 1.5	478	12.0 ± 1.1*	9.3 ± 1.1*
Roadside	472	11.0 ± 1.5*	10.3 ± 1.0*	463	12.8 ± 0.5*	10.2 ± 0.9*

Values of negative control- MI – 6.08%; AC – 5.73%.
MI – Mitotic Index; AC – Aberrant cells.
* Difference between soil sample and negative control values of parameters statistically significant (Independent Student's *t*-test, $p \leq 0.5$).

observed in soils under the three land uses in the present study were lower than the contents reported in most studies done in India and other parts of world (Supplementary Table 4). The main factors responsible for such low levels of metal(loid)s can be the sandy texture and low SOM of soils because in such conditions there is low retainment of metal(loid)s in soil and higher leaching to lower layers (Kavianpoor et al., 2012).

Among the three land uses, most metal(loid)s were found to be higher in agricultural soils in comparison to riverbank and roadside soils. The higher metal(loid) contents in agricultural soils in the present study can be attributed to anthropogenic effects such as extensive agriculture using agrochemicals and polluted irrigation water. Various agrochemicals such as NPK fertilizers, fungicides, pesticides etc. used in these areas are potent source of metal(loid)s such as As, Cd, Cr, Cu, Pb etc. (Milinovic et al., 2008; Savci, 2012; Kumar et al., 2015). The river water and groundwater which are main irrigation sources of the study area are severely contaminated by polluted industrial (leather tanning, dying, chrome plating etc.) and urban discharges from upstream areas such as Ludhiana, Jalandhar, Kapurthala (J. Kaur et al., 2014). Therefore, in addition to natural factors (parent rock material, polluted sediment deposition by rivers etc.) which are common for riverbank and roadside soils also, anthropogenic activities have significantly contributed metal(loid)s into agricultural soils in the study area. The statistically significant positive correlation observed between all the metal(loid)s (except Mo) indicated their common sources of origin.

The average contents of As and Mn observed in agricultural soil samples in both seasons (Table 2) in the present study were higher than the levels of these metal(loid)s observed by Kumar et al. (2015) in Bathinda region of south Punjab which has high reported incidences of cancer. The high levels of Fe and Mn observed in the soil samples in present study contributes to As contents in soils, since the oxides, hydroxides and oxyhydroxides of Fe have affinity for As, thus increasing its adsorption in soil (Adegoke et al., 2013). Mn also causes immobilization of As in soil by oxidizing As (III) to As (V) (Deschamps et al., 2003). The statistically significant correlation observed between As, Fe and Mn (Supplementary Table 3) proves the affinity of Fe and Mn for As in soil. Overall, the higher levels of As observed in the study area (central to west part of Punjab) in comparison to soils of Bathinda can be a significant cause of concern for population of study area. The other metal(loid)s i.e. Co, Cr, Cu, Mn, Mo, Ni and Zn were found to be at low levels.

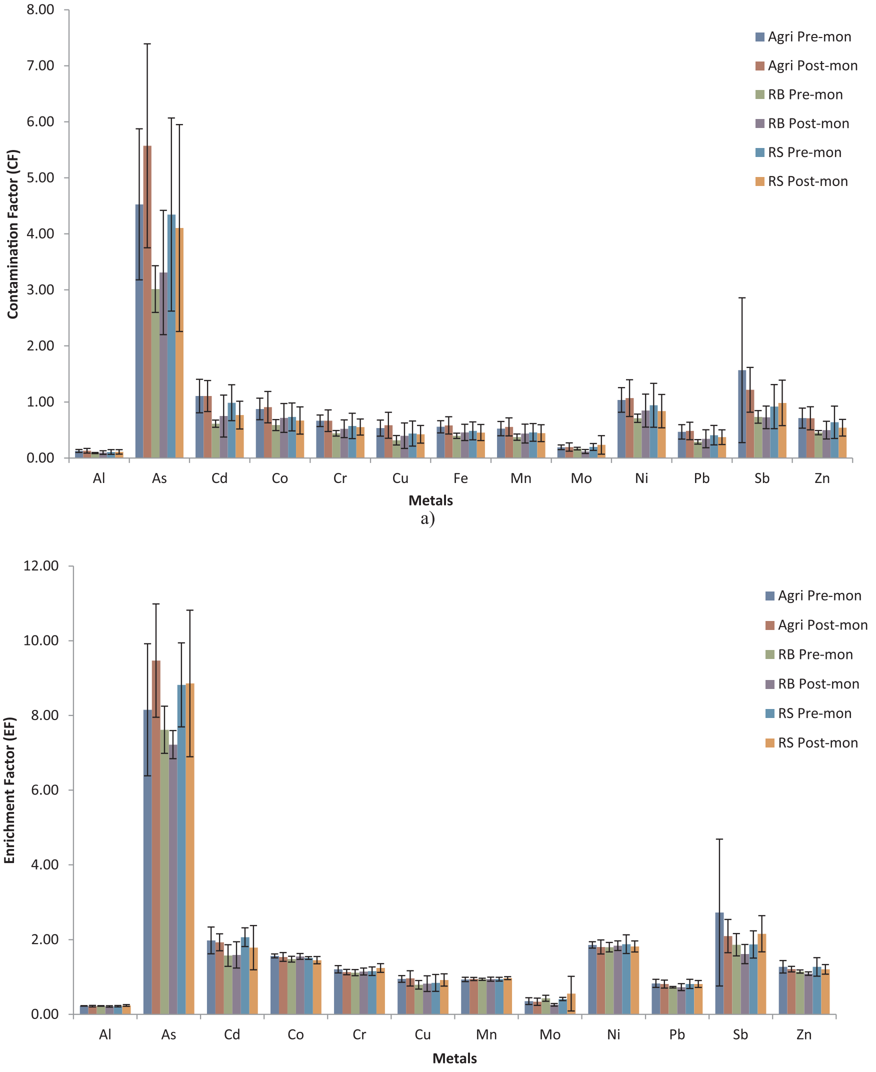
3.2.1. Seasonal analysis: total metal(loid) contents

3.2.1.1. agricultural Soils. In comparison to the pre-monsoon agricultural soil samples the levels of metal(loid)s significantly increased in post-monsoon samples from all villages except Village No. 5 (Supplementary Table 6). The increase can be attributed to rise in silt, clay and SOM levels due to precipitation and flooding effects. The silt and clay particles helps in retaining the metal(loid)s in soil, whereas high sand contents causes decrease in metal(loid) levels due to leaching (Troeh and Thompson, 2005; Brady and Weil, 2008; M. Kaur et al., 2014). This was also evident by the statistically significant positive correlation observed between all the studied metal(loid)s, silt and clay, whereas statistically significant negative correlation was observed between sand and studied metal(loid)s in samples of both seasons (Supplementary Table 3). The enrichment of metal(loid)s in post-monsoon soil samples indicated that precipitation and adsorption processes are more effective than washout process by rainwater. The adsorption of metal(loid) ions by hydrated insoluble metal(loid) complexes of Al, Fe and Mn, which could form in post-monsoon samples, further promotes retaining of metal(loid)s in soil (Pathak et al., 2015).

But in case of Village No. 5 (downstream Harike wetland), the decrease in metal(loid) contents can be attributed to decrease in silt, clay and SOM levels in post-monsoon samples of Village No. 5 (Supplementary Table 2). It was observed that very less water flow downstream Harike wetland, from where the maximum water is transported to southern parts of Punjab by Indira Gandhi Canal for irrigation purposes. Therefore, the intensity of floods is less in areas downstream Harike and the artificial flood barriers made in Village No. 5 also prevents the flood water to enter in agricultural fields. Therefore, the sedimentation of soils in Village No 5 due to floods is very low, which reduces the chances of metal(loid) inputs due to flooding. The case of Village No 5 thus, provides a very significant example of changes in natural soil processes by human activities in riverine floodplain areas like the study area.

3.2.1.2. Riverbank soils. Statistically significant variations were observed in metal(loid)s contents in post-monsoon samples in comparison to pre-monsoon samples in all villages which might be attributed to flooding in monsoon samples (Supplementary Table 6). The sources of metal(loid)s in riverbank soils are both natural factors such as sediment deposition by rivers and anthropogenic activities (Duodu et al., 2016). The anthropogenic activities (industrial, urban and agricultural) pollute the river water with metal(loid)s and riverbank soils are first recipients of these contaminants (Venkatramanan et al., 2014). The constant deposition of these contaminants in riverbank soils due to flooding causes enrichment of the metal(loid) contents in post-monsoon riverbank soils, which was observed in the present study.

3.2.1.3. Roadside soils. The main anthropogenic sources of metal(loid)s in roadside soils are traffic related activities such as vehicular emissions, weathering of crash barriers, abrasion of vehicular bodies etc. (Ahmed et al., 2016; Liu et al., 2016; Aryal et al., 2017) which are least affected by monsoonal precipitation. In the present study, most of the roads in studied villages were away from the flood prone areas. Therefore, the roadside soil samples were least affected due to flooding activities in monsoonal season. But, statistically significant decrease was observed in the metal(loid) contents of post-monsoon roadside soil samples in comparison to pre-monsoon samples in most villages which can be attributed to leaching of metal(loid)s to lower layers of soils profile by precipitation and absorption of metal(loid)s by the natural vegetation which grows during the monsoon season.



a)

b)

Fig. 2. (a-b). Metal(loid)s contamination assessment (mean ± standard deviation) in soils under the three land uses in pre and post-monsoon samples. a)Contamination Factor (CF). b) Enrichment Factor (EF). *Agr Pre-mon - Agricultural Pre-monsoon sample; Agr Post-mon - Agricultural Post-monsoon sample; RB Pre-mon - Riverbank Pre-monsoon sample; RB Post-mon - Riverbank Post-monsoon sample; RS Pre-mon - Roadside Pre-monsoon sample; RS Post-mon - Roadside Post-monsoon sample.

Although an overall low metal(loid) contents in the soils have been observed in this study, but such low metal(loid) contents could also pose several ecological risks in combined form. Therefore, various indices were calculated to analyze the potential ecological risks from these metal(loid)s in the following sections.

3.3. Level of anthropogenic metal(loid) contamination in soils

In order to assess the pollution caused by the analyzed metal(loid)s in the present study, pollution assessment factors i.e. the Contamination factor (CF) and Enrichment factor of the individual metal(loid)s were determined (Fig. 2).

It was observed that the average CF values for Al, Co, Cr, Cu, Fe, Mn, Pb and Zn were < 1 which indicated low pollution in soils under the three land uses in samples of both seasons (Fig. 2a). The CF values of agricultural soil samples for Cd, Ni and Sb were in between 1 and 3, which suggested that these metal(loid)s posed moderate contamination in the soils analyzed. The CF values observed in case of As were between 3 and 6 which indicated considerable anthropogenic contamination of soils with As.

The EF values were found to be < 2 for Al, Cd, Co, Cr, Cu, Mn, Mo, Ni, Pb and Zn (Fig. 2b) in most soil samples under the three land uses, which suggested minimal enrichment of these metal(loid)s in the studied soil samples. The EF values for Cd (pre-monsoon roadside samples) and Sb (agricultural soil samples) were found to range in between 2 and 5, which indicated moderate enrichment. Similar to CF, As had significant enrichment in the studied soil samples with the EF values the range of 5–20. Thus, the anthropogenic enrichment of As in soils may pose a significant risk to human health and the environment (plant and animals) in the study area in both seasons. In comparison to the values observed for samples of two seasons, CF and EF values were higher for post-monsoon samples for most of the metal(loid)s (especially As in agricultural soils) which can be attributed to increase in metal(loid) concentrations in post-monsoon soil samples.

3.4. Potential ecological risk impact

The Potential Ecological Risk Index (Er) for individual metal(loid)s in soils is presented in Fig. 3. The observed Er values for Cd, Co, Cr, Cu, Mn, Pb and Zn suggested that these metal(loid)s posed low ecological risk (< 40) in the analyzed soils. However, the Er value for As (between 40 and 80) suggested that it could pose a moderate potential ecological

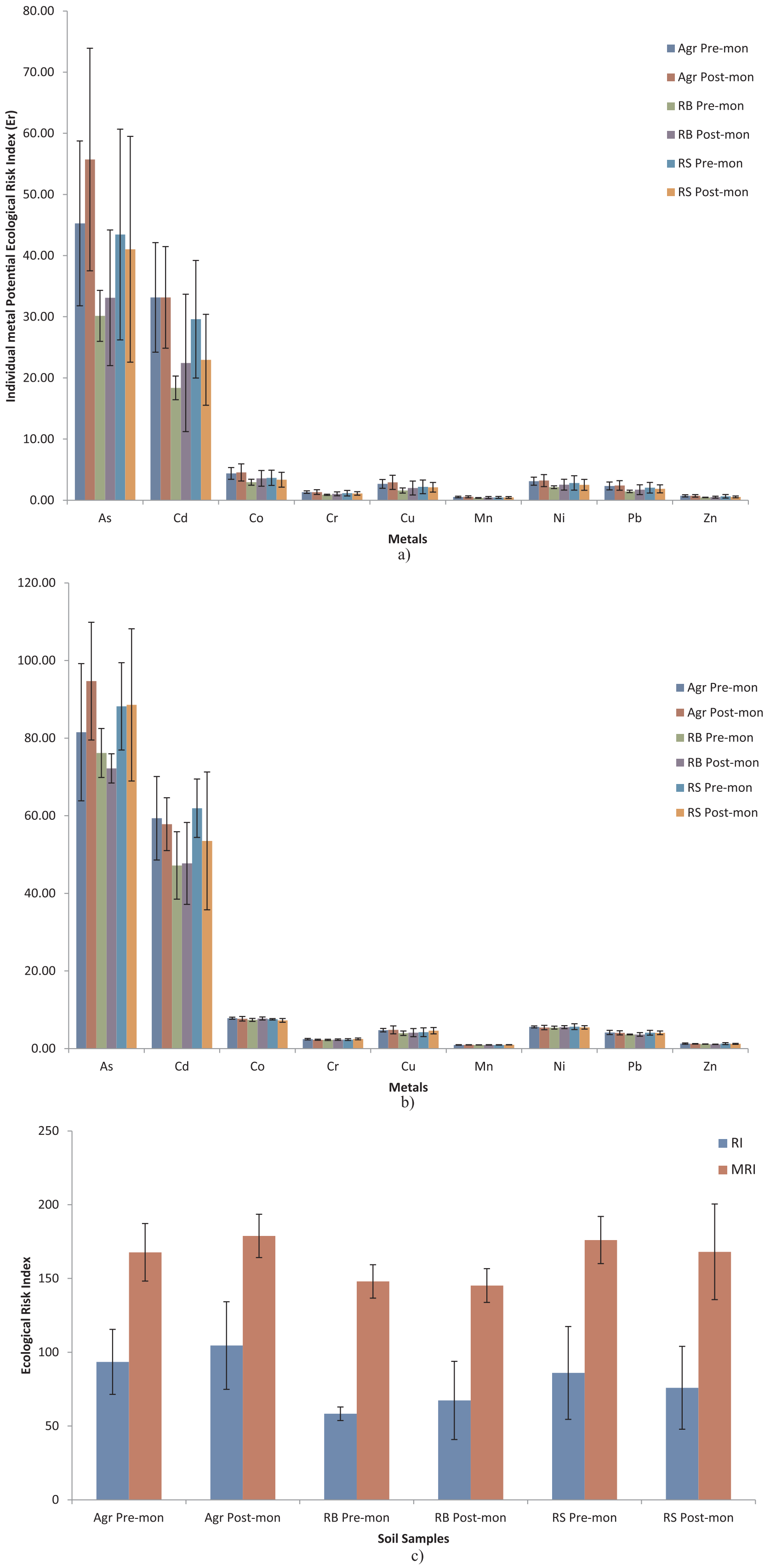


Fig. 3. (a-c). Ecological Risk Assessment (mean ± standard deviation) of metal(loid)s in soils under the three land uses in pre and post-monsoon samples. a) Individual metal(loid) Potential Ecological Risk Index (Er). b) Individual metal(loid) modified Potential Ecological Risk Index (mEr). c) Ecological Risk Index (RI) and Modified Ecological Risk Index (MRI).

risk in soil samples. The combined Ecological Risk Index (RI) of metal (loid)s based upon CF (Fig. 3c) was found to be < 150 in the soils under each land use which indicated that metal(loid)s in these soils posed a low ecological risk in combined form.

The Modified Potential Ecological Risk Index (mEr) of individual metal(loid)s, which is based upon EF is represented in Fig. 3b. It was observed that the mEr values for Co, Cr, Cu, Mn, Ni, Pb and Zn were < 40 in soil samples under the three land uses, thus indicating low potential ecological risks posed by these metal(loid)s in soils. But the mEr values for Cd (ranging in between 40 and 80) indicated its moderate ecological risks in soils. The mEr values of As observed in agricultural and roadside soil samples were found to be > 80, indicating the considerable ecological risk posed by As in the studied soils. Also, the combined Modified Risk Index (MRI) values of metal (loid)s were found to be > 150 for agricultural and roadside soil samples (Fig. 3c) suggesting the moderate ecological risk posed by these metal(loid)s in studied soils (Duodu et al., 2016).

It can be observed from Fig. 3 that the values of MRI were higher than the RI values for each metal(loid), which indicated that the MRI assessed the ecological risks posed by metal(loid)s more efficiently than RI. It was mainly due to the terrestrial element considered (Fe in the present study) for normalization in calculating EF. It represented even the slight variation in sedimentary composition of metal(loid)s (Brady et al., 2015; Duodu et al., 2016). Therefore, MRI can be a better alternative to RI which is traditionally used to determine the potential ecological impact of metal(loid)s in soils.

3.5. *Genotoxicity using Allium cepa genotoxicity assay*

The observed average results of the *Allium cepa* genotoxicity assay i.e. Mitotic Index (MI) and percent Aberrant Cells (AC) of the six villages in the present study are given in Table 3. It was observed that the average MI and percent AC values observed for agricultural soil samples was higher than those observed for riverbank and roadside soil samples in both seasons. The overall higher levels of metal(loid)s in agricultural soils in comparison to riverbank and roadside soils may be the reason for higher level of MI and AC in agricultural soil samples (M. Kaur et al., 2014). Positive correlation of metal(loid)s (significantly positive with many metal(loid)s) with MI and AC suggests that they are responsible for the increasing chromosomal aberrations in cells (Supplementary Table 3). Metal(loid)s such as As, Cd, and Pb may cause direct damage to DNA by binding to nucleotides resulting in base structure modifications, DNA strand breaks, destruction of DNA-protein crosslinks, interference in antioxidative enzyme activity and inhibition of DNA repair enzymes (Rank, 2003; Leme and Marin-Morales, 2009). The percent AC values observed in the soil samples were higher than the percent AC observed in negative control system of tap water (6.1%). The percent AC observed in the studied samples were found to be significantly higher ($p \leq 0.5$) in comparison to the negative control showed the higher genotoxic potential of studied samples.

Thus it can be deduced from the present study that the soils of riverine floodplains of Punjab located on the banks of rivers Beas and Sutlej were contaminated with metal(loid)s and the levels of various physico-chemical parameters and metal(loid)s in the soils were significantly affected by changes in the seasons. Also the metal(loid)s in the soils posed low to moderate contamination and can cause genotoxic abnormalities in plants and thus, ultimately could affect the humans adversely. Hence, this study can give valuable information regarding metal(loid) contamination risks in riverine floodplain soils in different seasons. Further research must be carried out to analyze the effects of variation in weather patterns due to climate change on metal(loid) speciation and their biological effects in riverine floodplain soils because these soils would be the first to get affected. The results from such research can be considered while forming guidelines for the protection of human and environmental health.

4. Conclusions

The present study revealed that among the studied riverine floodplain soils under three land uses (agricultural, roadside and riverbank) comparatively higher levels of soil nutrients and metal(loid) contents were found in agricultural soil samples which can be attributed to extensive application of agrochemicals and use of polluted irrigation water. Heavy metal contamination of irrigation sources and soils of Punjab due to industrial and agricultural pressures had been reported in previous studies also (J. Kaur et al., 2014; Kumar et al., 2015; Bhatti et al., 2018). The levels of majority of metal(loid)s increased during post-monsoon season which could be due to increase in SOM, silt and clay contents in post-monsoon samples due to flooding and sedimentation from adjoining rivers. The CF and EF showed that As, Cd and Sb caused moderate to considerable contamination in studied soils in both seasons. The Er and mEr also indicated that As and Cd posed moderate to considerable ecological risks individually. The RI analysis showed that the metal(loid)s in studied soil samples posed very low risk in combined form, but the MRI analysis indicated that the metal(loid)s in agricultural and roadside soils posed moderate ecological risks. *A. cepa* assay indicated that the soil samples can induce genotoxic effects in plant systems and metal(loid)s were responsible for increase in genotoxicity of soils. Therefore, it can be concluded that the metal(loid) contents in the studied riverine floodplain soils significantly varied in different seasons and can pose ecological and genotoxic risks (especially As, Cd and Sb).

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2018.08.032.

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