

Assessment of metal(loid) contamination and genotoxicity of agricultural soils

Vaneet Kumar (✉ veenumatri@gmail.com)

Guru Nanak Dev University <https://orcid.org/0000-0002-2586-0731>

Sandip Singh

Guru Nanak Dev University

Avinash Nagpal

Guru Nanak Dev University

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Abstract

Soil, a connecting link between biotic and abiotic components of terrestrial ecosystem, receives different kinds of pollutants through various point and nonpoint sources. Among different sources of soil pollution, contaminated irrigation water is one of the most prominent sources affecting soils throughout the globe. The irrigation water (both surface and groundwater) are increasingly getting polluted with contaminants such as metal(loid)s due to various anthropogenic activities. The present study was conducted to analyze metal(loid) contents in agricultural soil samples (N = 24) collected from fields along the banks of rivers Beas and Sutlej flowing through Punjab state of India, using Wavelength Dispersive X-Ray Florescent (WDXRF) Spectroscopy. The soil samples were also analysed for their genotoxic potential using *Allium cepa* root chromosomal aberration assay. The rivers Beas and Sutlej are contaminated with municipal and industrial effluents in different parts of Punjab. The soil samples analyzed were found to have higher contents of Arsenic, Cobalt and Chromium when compared with reference values given by various international agencies. Pollution assessment using different indices like Index of geo-accumulation, Enrichment factor and Contamination factor revealed that the soil samples were highly polluted with cobalt and arsenic. The *Allium cepa* assay revealed that maximum genotoxicity was found in soil samples having higher contents of As and Co. Pearson's correlation analysis revealed strong positive correlation between the different metal(loid)s which indicated common sources of these metal(loid)s. Therefore, efforts must be taken to reduce the levels of these metal(loid)s in these agricultural soils.

Introduction

A polluted aquatic ecosystem is a critical threat to the environment as it can have an immediate effect on the sustenance of life. Most severe sources of pollution are related to the disposal of untreated and partially treated industrial wastes that contain hazardous toxic substances (such as metal(loid)s) most of which are not completely degradable and potentially harmful. Due to continued disposal of industrial wastes, many hydrological ecosystems in developing countries are stressed beyond repair (Singh et al., 2017; Rigo et al., 2020).

Soil can concentrate contaminants it receives from different sources, and they (contaminants) move down stream and accumulate in catchment area, sediments and biological tissues (Juarez-Santacruz et al., 2013; Bhatti et al., 2018). Among various contaminants identified, presence of heavy metals is a major concern because these are water soluble and can easily enter the food chains. They have been reported to combine with proteins, nucleic acids and other biomolecules, thus impairing their functions (Gastaldo et al., 2007; Sharma et al., 2019).

On a global basis, agriculture is the single largest user of fresh water and because of the presence of various metal(loid)s and other toxic chemicals in irrigation water, it contributes significantly to the accumulation of metal(loid)s in soil. Excessive accumulation of metal(loid)s in agricultural soils not only result in soil contamination, but also affect food quality and human health (Zhang et al., 2019; Bhatti et al., 2020). Because of their non-biodegradable nature, long biological half-lives and potential to accumulate in different body parts, metal(loid)s are harmful for human beings (Mudgal et al., 2010). Generally, plants require some metal(loid)s in trace amounts but their presence in large quantities severely affect physiology and biochemical functions (Nagajyoti et al., 2010). Some of the heavy metals like Pb, Cd, Cr, and Hg are non-essential for plants and can adversely affect plant growth even at lower concentration (Khan et al., 2015).

Researchers around the globe are using different analytical methods to evaluate the elemental composition of environmental samples (Bielecka et al., 2014; Soodan et al., 2014a). X-Ray Fluorescence (XRF) is one of the analytical methods, which is fast, accurate, non-destructive and is used to determine the chemical composition of solids and liquids (Yusuf et al., 2014). There are two main variants, Energy (EDXRF) and Wavelength Dispersive XRF spectrometer (WDXRF) systems, with the ability to detect a wide range of elements. EDXRF goes from Sodium to Uranium while WDXRF can detect metals ranging from Beryllium to Uranium. These can analyze concentrations as low as sub ppm to 100 % (Funtua, 1996).

Hazardous and risk assessment of soil samples is usually carried out by analysis of various physico-chemical parameters. However, these parameters are not sufficient for biological risk assessment because physico-chemical analysis alone is not able to provide information on effect of chemical compounds and does not consider the interaction between contaminants, soil matrix and biota (Bhatti et al., 2017; Hu et al., 2019). Therefore, the use of bioassays is equally important to estimate ecological risk in soil or other matrices. Among different bioassays used, plant bioassays are widely used for initial screening methods to determine the genotoxic potential of chemicals, drugs or pollutants. *Allium cepa* root chromosomal aberration assay is one of the plant bioassays which has been validated by International Programme on Chemical Safety (IPCS) under auspices of World Health Organization (WHO) and United Nations Environment Programme (UNEP) for determination of genotoxicity of various agent including soil samples (Cabrera and Rodriguez, 1999; Hammann et al., 2020).

Punjab is a north-western state of India located in Indo-Gangetic alluvial plains extending from 29°30' N to 32°32' N latitude and 73°52' E to 76°56' E longitude. Beas and Sutlej are important tributaries of Indus River system and traverse most of the part of Punjab plains. The water of these two rivers is used for irrigation in Punjab. Both these rivers culminate at Harike wetland, a Ramsar site in district Taran-Taran of Punjab plains. After their culmination at this Ramsar site, the joint stream of Beas and Sutlej rivers is known as Sutlej. During their flow in Punjab and even before entering Punjab, these rivers receive industrial and municipal wastes of various districts such as Kangra, Pathankot, Amritsar, Jalandhar and Ludhiana. Major industries in these districts are related to pharmaceuticals, paper, chemical, textile and sugar manufacturing and thermal power plants, all these industrial establishments are discharging their wastes directly or indirectly into these rivers which contaminate their water. The increasing pollution in these rivers also poses significant risk of contamination of agricultural soils of Punjab with various contaminants including metal(loid)s. Thus, the present study was conducted to assess the contents of metal(loid)s in agricultural soils of six villages situated on the banks of rivers Beas and Sutlej, and pollution status using different indices like Index of geo-accumulation (I_{geo}), Enrichment Factor (EF) and Contamination Factor (C_f). The soil samples were also analysed for their genotoxic potential using *Allium cepa* root chromosomal aberration assay.

Materials And Methods

Study area

The study area consisted of six villages situated on the banks of rivers Beas and Sutlej in Punjab state of India. Among six villages, four villages are situated on banks of river Beas (upstream to Harike wetland) and two were situated on banks of river Sutlej (downstream to Harike wetland). The soil of the study area is composed of sediments of Shiwalik and Himalyan hills brought down and laid by the rivers Beas and Sutlej. The water of these two rivers is used for irrigation in the study area.

Sample collection

Six villages on banks of rivers Beas and Sutlej selected for the present study are shown in Fig. 1. shows the sampling villages with sample codes and geographical coordinates of soil samples. Soil samples were collected in the month of October, 2012. From each village, four agrarian fields (two under rice and two under sugarcane cultivation) were selected for sampling. Twenty-four composite surface soil samples were collected from horizon 'A' of the soil (i.e., 5-15 cm depth). Horizon 'A' was particularly chosen because it has been reported to be heavily contaminated with heavy metals and roots of these crops do not penetrate beyond this horizon (Gowd et al., 2010). Samples were collected by using a plastic spatula and transferred to a clean polythene bag to avoid all possible adulterations.

Elemental Analysis

Elemental analysis of soil samples was performed using Wavelength Dispersive X-Ray Fluorescence Spectroscopy (WDXRF) (S8 TIGER, Make Bruker, Germany). The soil samples were air dried by placing soil on steel mesh containing blotting paper on it. Each air-dried soil sample (9 gm) was ground along with binder tablets (9 tablets/gm of soil sample) to make homogenized fine powder of having particle size less than 50 μm (Lu et al., 2010). Pellets having diameter of 34 mm and thickness 4 mm were made from the ground soil samples by applying a pressure of 15 tons using a hydraulic press. These pellets were then subjected to X-rays for a 30 min run time. Geo-quant, a geological sample dedicated software was used for sample analysis. To authenticate the quality of chemical analysis data and examine the accuracy of the data, soil reference material, GBW07406 was analyzed.

Pollution Assessment

Pollution assessment of different soil samples was carried out on the basis of results of elemental analysis and calculating different indices like Index of geo-accumulation (I_{geo}), Enrichment Factor (EF) and Contamination Factor (C_f).

Index of geo-accumulation

The index of geoaccumulation (I_{geo}) compares the content of any element studied in soil sample to its pre-industrial concentration to enable the assessment of contamination of that particular soil (Muller, 1969). The index of geoaccumulation can be calculated by using equation

$$I_{geo} = \frac{\log_2 C_n}{1.5 B_n}$$

where, C_n is the content of element in the soil sample studied, B_n is the concentration of element in the earth's crust (Taylor and McLennan, 1995) and 1.5 is the constant to allow natural fluctuations in the contents of elements studied. Soils were classified into different classes on the basis of index of geoaccumulation (I_{geo}) as per Supplementary Table 1.

Enrichment factor

The enrichment factor enables us to assess the enrichment or depletion of element of interest in soil sample by normalizing the content of element studied with a reference element. A reference element must be relatively inert with respect to chemical weathering and which has no significant anthropogenic source and shows low occurrence and variability (Sutherland, 2000). Zirconium (Zr), is the element which has been widely used for assessment of depletion of reactive heavy metals in geological samples because of its high chemical stability during weathering processes (Cobela-Garcia and Prego, 2003). In nature zirconium exists in oxide or silicate forms and belongs to lithophile elements which accumulate in sediments and have very little circulation in geochemical cycles (Wang et al., 2013). Keeping these in mind, the crustal zirconium (Zr) value of 190 mg/kg (Taylor and McLennan, 1995) was used as reference element. The value of enrichment factor was calculated by using modified formula given by Loska et al., (2003):

$$EF = \frac{C_n(\text{sample})/C_{ref}(\text{sample})}{B_n(\text{background})/B_{ref}(\text{background})}$$

where, $C_n(\text{sample})$ is content of an element in soil sample, $C_{ref}(\text{sample})$ is the content of the reference element (Zr) in soil sample, $B_n(\text{background})$ of element in the earth crust (Taylor and McLennan, 1995) and $B_{ref}(\text{background})$ is the content of the reference element (Zr) in the earth crust (Taylor and McLennan, 1995). Soils were classified into different classes on the basis of enrichment factor (EF) as per Supplementary Table 2.

Contamination factor

The contamination factor, as suggested by Hakanson (1980), provides an assessment of contamination level of a soil sample with particular element as compared to its reference value in preindustrial level and is calculated as:

$$C_f = \frac{C_{0-1}^i}{C_n^i}$$

where, C_{0-1}^i is the average content of a particular element from at least five sampling sites and C_n^i is the content of particular metal in the earth's crust. Soils were classified into different classes on the basis of contamination factor (C_f) as per Supplementary Table 3.

Estimation of genotoxic potential

Allium cepa root chromosomal aberration assay was performed to evaluate genotoxic potential of collected soil samples. *In situ* conditions were simulated by setting up pot cultures. For this assay, healthy uniform sized onions were bought from local market. The onion bulbs were allowed to root directly in soil samples contained in small pots. The acid washed sand was used as negative control. After emergence of roots, the onion root tips were washed thoroughly with water, root tips measuring 0.5-1 cm were cut and fixed in Farmer's fluid (3:1:: Ethanol:Glacial Acetic Acid) and kept till further use (15-30 days). The fixed root tips were hydrolyzed in 1 N HCl at 60°C for 1 min and transferred to a watch glass that contained a mixture of aceto-orcein and 1 N HCl in the ratio of 9:1 for half an hour. The root tips were warmed at regular intervals of 5 min while in the stain. The root tips were then carefully transferred to clean glass slide and squashed in 45% glacial acetic acid under a cover slip. The slides were screened for different types of chromosomal aberrations. The whole experiment was set up in triplicate. From each set up 300, and a total of 900 dividing cells were scored for each sample. Percent aberrant cells were calculated as:

$$\text{Percent aberrant cells} = \frac{\text{No. of aberrant cells}}{\text{Total no. of dividing cells}} \times 100$$

Statistical Analysis

The descriptive and other statistics of metal(loid) concentrations in the agricultural soil samples was done using IBM SPSS (Statistical Package for the Social Sciences) software version 16.0 (New York, USA) and PAST (Paleontological Statistics) software version 3.06 (Hammer et al. 2001). Pearson's correlation analysis was done to determine the correlation between metal(loid)s and genotoxicity parameter of the soil samples.

Results And Discussion

Table 1 summarizes the contents of various heavy metal(loid)s in agricultural soil samples collected from agricultural fields on the banks of river Beas and Sutlej analyzed by WDXRF. The results observed for genotoxicity assessment of the soil samples are represented in Table 2 and Table 3 represents the Pearson's correlation analysis of heavy metals and genotoxicity parameters. On the basis of results of elemental analysis, different indices *viz.*, I_{geo} , EF and CF, the soil samples were classified into different categories of contamination and the results were represented as box plots in Figs. 2, 3 and 4, respectively.

Barium

Barium is a group 2 element that shares a number of chemical characteristics with calcium (Ca), strontium (Sr), and lead (Pb). Barium is used in the production of soaps, explosives, fire extinguishers, drilling fluids, insecticides etc. (Ippolito, 2006; Menzie et al., 2008). Exposure of humans to high quantities of Ba is reported to cause hypokalaemia, acute hypertension, vomiting, diarrhea and cardiac arrhythmia (Dallas and Williams, 2001). Mobilization of Ba in soil is governed by different physico-chemical characteristics of soil. Ba is known to displace other sorbed alkaline earth metals from oxides of MnO_2 and TiO_2 . Barium levels in the present study ranged from 399 to 558 mg/kg, having an arithmetic mean of 478.6 mg/kg. In the present study content of Ba in all the soil samples exceed the reference value (Gs, C. S. Q., 2007) of 300 mg/kg. However, the contamination factor analysis classifies these soil samples under Class 1, indicating low pollution level for Barium (Fig. 4).

Cobalt

Cobalt content in all the soil samples was found to be more than the reference value i.e. 10.6 mg/kg given by CNEMC (1990). Cobalt levels ranged from 47 to 132 mg/kg, with an average of 85.92 mg/kg. The values of enrichment factor showed that the soil samples under study are significantly enriched with Co and giving a significant pollution signal. The contamination factor analysis showed that these soil samples are highly contaminated with cobalt (Fig 4). According to (Stiborova et al., 1988), application of cobalt at lower concentrations improved the root system by helping the plant to absorb water and ultimately the uptake of several nutrients dissolved in this water resulting in better growth of plant. Cobalt in the form of Vitamin B₁₂ (hydroxycobalamin) is an essential element. Heggveit et al. (1970) reported that individuals with a habit of heavy alcohol consumption coupled with dietary deficiencies when exposed to cobalt were susceptible of cardiomyopathy.

Chromium

The content of Cr observed in different soil samples ranged from 78 to 147 mg/kg with an average content of 95.42 mg/kg. According to Wedepohl (1995), the safe value for Cr is 100 mg/kg. Twenty-nine percent of soil samples in the present study exceeded this normal value. Pollution assessment also revealed that the soil samples are unpolluted to moderately polluted with chromium. Chromium is mainly added to the ecosystem through various anthropogenic activities as it is used in the manufacturing of steel and other alloys, chrome plating and pigment production. Chromium exists in eight oxidation states

ranging from -2 to +6. Cr⁺³ and Cr⁺⁶ are common occurring states and Cr⁺⁶ is most toxic to biological systems. Adsorption of Cr⁺⁶ decreases with increase in pH, while that of Cr⁺³ increases with increase in pH of soil sample (Machender et al., 2011). It has been reported that in most of the industrial effluents, Cr is present in less toxic state (Cr⁺³) but because of varying environmental conditions Cr⁺³ is oxidized to toxic state (Cr⁺⁶) (Gowd and Govil, 2008).

Copper

In the present study, content of copper was found to range from 10-27mg/kg with a mean value of 17.92 mg/kg, which is less than the normal threshold range (135-270 mg/kg) prescribed for soil samples (Awasthi, 2000). Copper was one of the first metals used by ancient civilizations dated back to approximately 5000 BC (Stern et al., 2007), including Indus valley civilization. The discovery that copper is mixed with Tin to produce Bronze, marked the end of Stone Age and beginning of Bronze Age. Cu tends to accumulate in the surface horizon as a result of various anthropogenic activities including use of Cu based agrochemicals in agro-based industries (Kabata-Pendias, 2004). High concentrations of copper can inhibit both photochemical and biochemical reactions of photosynthesis (Kupper et al., 1998). Copper is an important component of metalloenzymes and functions as electron donor and acceptor. High dose of copper in human diet is reported to induce adverse health effects including Alzheimer's and Wilson's disease (Llanos and Mercer, 2002).

Nickel

Nickel content was found to range from 20 to 43 mg/kg with an average value of 31.75 mg/kg. The values of Ni are also lower than the reference value given by Awasthi (2000). The pollution assessment by different indices also shows that the soil samples are practically unpolluted with nickel (Figs. 2 - 4). Nickel is a silver-white metal found in different oxidation states varying from -1 to +4. Amongst all the oxidation states, +2 oxidation state [Ni(II)] is the most common in biological systems (Denkhaus and Salnikow, 2002). It finds its entry into atmosphere through various natural sources including dusts from volcanic emissions and the weathering of rocks and soils. Nickel is used in production of stainless steel, nickel alloys, and nickel cast iron. Nickel is reported to cause lung and nasal cancer in workers exposed to it (Seilkop and Oller, 2003). Nickel in soil is usually found in organically bound form. Its mobility increases in acidic and neutral conditions (Kabata-Pendias and Pendias, 1999).

Arsenic

Arsenic is one of the most common contaminants found in soil and waters which finds its entry into the ecosystem from various natural and anthropogenic sources (Goldhaber et al., 2003). The natural sources include various metallic sulfide ores, coal deposits, tectonic movements etc., while the anthropogenic sources include paints, tanning agents of leather, metal alloys, and wood preservative agents (Welch et al., 2000). Despite the use of arsenic in variety of industrial establishments, its release into the atmosphere was more by natural processes (>60%) than the anthropogenic sources (>40%) (Ayres and Ayres, 1999). The concentrations of arsenic in all the soil samples were found to be higher than the reference value given by Canadian soil quality guidelines (C.So.Q.Gs. 2007). The content of arsenic ranged from 23 to 33 mg/kg with an average value of 27.33 mg/kg. The pollution assessment also indicated that the soil samples were highly contaminated with arsenic (Figs 2 - 4).

Lead

The average content of lead in different agricultural soil samples was 22.63 mg/kg and ranged from 19 to 27 mg/kg. A baseline value of 25 mg/kg was estimated for surface soil on global scale and level above this limit indicates the anthropogenic influence (Gowd et al., 2010). Lead (Pb) is a naturally occurring metal, which occurs ubiquitously in both organic (tetraethyl lead) and inorganic (lead acetate, lead chloride *etc.*) forms in the environment (Shalan et al., 2005; Jabeen et al., 2010). It is one of the major toxicants in the environment because of its potential hazards to living organisms and is being used by many industries including mining and refining (Marques et al., 2006; Ahmad et al., 2014). Intoxication by lead (Pb) induces a broad range of physiological, biochemical, genetic and behavioural changes in living organisms especially animals which include disorders of nervous system, cardiovascular system, excretory system and reproductive system (Mudgal et al., 2010). In the present study, only two samples showed values slightly higher than this baseline value.

Rubidium

The average content of rubidium in different soil samples was found to be 114.60 mg/kg with a minimum and maximum values of 100 and 135 mg/kg, respectively. Rubidium (Rb) is located between potassium (K) and cesium (Cs) in the periodic table and is a rarely studied but abundant alkali metal. Rb is analogous to K⁺ and can compete with K⁺ for enzymatic sites in fish and other organisms (Campbell et al., 2005). Results of index of geo-accumulation used for pollution assessment revealed that the soil samples were practically unpolluted with rubidium.

Strontium

The mean content of strontium in different soil samples was found to be 89.58 mg/kg and ranged from 67 to 162 mg/kg. The mobility of strontium is very swift during weathering, particularly in oxidizing acidic environment. Strontium is indicative of calcareous rocks, because of its strong association with calcium (Gowdet et al., 2010). In nature, Strontium occurs in four stable isotopes (⁸⁴Sr, ⁸⁶Sr, ⁸⁷Sr, and ⁸⁸Sr). Three of these (⁸⁴Sr, ⁸⁶Sr, and ⁸⁸Sr) are non-radiogenic while, ⁸⁷Sr is radiogenic and is formed by the radioactive decay of ⁸⁷Rb, with a half-life of approximately 4.88×10¹⁰ years (Faure and Mensing, 2005). Many scientists around the globe opined that because of large atomic mass, strontium contents change a little while passing through weathered rock to soil and finally to food chain (Ruggeberg et al., 2008).

Vanadium

In the present study, content of vanadium (V) ranged from 51 to 90 mg/kg with an average of 68.33 mg/kg. Normal threshold value for vanadium in soil is 100 mg/kg (Larocque and Rasmussen, 1998). Vanadium, a member of group VB of the periodic table is named after the Norse goddess Vanadis, the goddess

of beauty and fertility (Morinville et al., 1998). Vanadium finds its entry into the environment through leaching of rocks, combustion of coal or petroleum products, contamination from the use of phosphate fertilizers (Vachirapatama et al., 2002). Tosukhowong et al. (1999) reported a positive correlation between vanadium content in soil and concentration of V in patient's urine, who were suffering from distal renal tubular acidosis in Thailand.

Yttrium

Yttrium is categorized as a rare earth element (REE) that occurs in moderate amounts in the environment i.e. approximately equal in amounts to those of Cr, Co and Zn (Zhang et al., 2015). According to Wedepohl (1995), the Earth's crust contains 24 mg/kg of Yttrium, which is about twice more than lead. The content of Yttrium ranged from 21 to 32 mg/kg and results of index of geo-accumulation showed that the soil samples are practically unpolluted with yttrium. Its biological significance has not yet been clearly established, but there are reports available showing disruptive and protective effects of Y on plants and blue green algae, respectively (Maksimović et al., 2014).

Zinc

In the present study, average content of Zn was found to be 62.67 and ranged from 45 to 101 mg/kg. Threshold value for zinc is 200 mg/kg (BIS, 1991). Zinc being a trace metal is essential for the growth of human, animal and plants if present in low concentrations (Nagajyoti et al., 2010; Tsonko and Fernando, 2012). It is reported to develop potential phytotoxicity in leaf tissues at concentrations above 0.2 mg g⁻¹ dry matter (Bonnet et al., 2000). Sources of pollution ranged from industries, liquid manure, composted manure and agrochemicals including fertilizers and pesticides (Gowd et al., 2010). The values of contamination factor showed that the soil samples are having low contamination of zinc.

Zirconium

Zirconium (Zr), popularly known as the gemstone *zircon*, is a greyish-white metal having atomic number 40, density of 6.506 g/cm³, with five stable isotopes and three unstable isotopes (Allin, 2010). It is quite abundant in the Earth's crust with total percent amounts greater than the combined total of all available copper, nickel, lead, tin, zinc and mercury deposits, making it the 12th most common element in nature (Shahid et al., 2013). It's concentrations in the present study ranged from 147-241 mg/kg (Table 1). The results of pollution assessment factors showed that the soil samples were moderately polluted with zirconium (Figs 2 - 4).

Genotoxic Potential

The meristematic cells of *Allium cepa* roots were analyzed to record the mitotic anomalies induced in root tip cells following *in-situ* treatment with different soil samples collected from the fields on the bank of rivers Beas and Sutlej, Punjab. Different types of chromosomal aberrations induced in root tip cells *Allium cepa* were differentiated into physiological and clastogenic aberrations which arise because of abnormalities in spindle fibers and direct action on chromosome, respectively. The spectrum of physiological aberrations includes C-mitosis, delayed anaphase/s, stickiness, laggard/s, vagrant/s, abnormal anaphase/s and abnormal metaphase/s while that of clastogenic aberrations includes chromatin bridge/s, chromosomal break/s and ring chromosome/s. Fig. 5 shows different kinds of chromosomal aberrations induced in *Allium cepa* and suggests the presence of certain cytotoxic/genotoxic substances present in the soil samples. Total chromosomal aberration frequency including both physiological and clastogenic aberrations for different samples ranged from 10.55% to 16% (Table 2). All the soil samples except V1, B1 and B2 were found to have significant genotoxic potential as compared to control. The level of significance was checked at p≤0.05 and 0.01 level indicated by * and **. From the spectrum of physiological and clastogenic aberrations, the most abundant were delayed anaphases and chromatin bridges respectively. Delayed anaphase is characterized by two anaphasic groups of chromosomes lying close to each other near equatorial plate (Soodan, et al., 2014b), whereas, chromatin bridges are formed due to unequal exchanges of chromatin material which results in the formation of dicentric chromosome and subsequently pulled equally to both poles at anaphase (Sax and Sax, 1968). In case of most soil samples (except B4 and K4), frequency of cells with delayed anaphases was highest, followed by c-mitosis. C-mitosis is a term coined by Levan (Levan, 1938) to describe the effects of various physical and chemical agents which act in a fashion similar to that of colchicine. After delayed anaphase and c-mitosis, cells with stickiness were frequent among various physiological aberrations. Kong and Ma (1999) gave a hypothesis that stickiness can be caused because of incomplete separation of daughter chromosomes which were cross linked by chromoproteins. Sample K1 showed maximum (16%) percentage of total chromosomal aberrations, in the same sample cobalt is 10 times higher than the reference value given by CNEMC (1990). Sample K1 was followed by N4 (15.22%) and B3 and B4 (both 15.11%), respectively which also have higher content of cobalt as compared to reference value indicating contamination of soil with cobalt because of continuous usage of fertilizers containing cobalt and may be a potential cause of genotoxicity in these soil samples. Results of the present study are in consistence with some previous studies around the globe which evaluated the genotoxic potential of agricultural soil samples (Souza et al., 2013; Soodan et al., 2014b; Bhatti et al., 2018).

Correlation analysis of heavy metals

The correlation analysis among different metal(loid)s and percent aberrant cells showed statistically significant positive correlation between most metal(loid)s which points towards the common sources of these metal(loid)s in the studied agricultural soils. These sources include both natural and anthropogenic sources (Chandrasekaran et al., 2015; Bhatti et al., 2018). Overall, it was observed that agricultural soils analyzed in the present study were contaminated with metal(loid)s due to different anthropogenic activities and posed significant genotoxic risks. Therefore, immediate steps must be taken to reduce the levels of metal(loid)s in the soils in order to ensure healthy agricultural practices.

Conclusions

The present study was conducted to analyze the contents of different metal(loid)s in agricultural soil samples collected from six villages situated on the banks of rivers Beas and Sutlej in Punjab, India. The soils were under rice and sugarcane cultivation. Among different metal(loid)s analyzed, contents of As, Co and Cr were found to be above the maximum permissible limits. The factors/indices (Index of geo-accumulation, Enrichment factor and Contamination factor), calculated to determine the metal(loid) contamination indicated that the studied soils were highly contaminated with metal(loid)s (especially As and Co) which could be attributed to different anthropogenic sources. *Allium cepa* root chromosomal aberration assay revealed that the soils samples may pose significant genotoxic risks to the exposed living beings which might be due to high levels of metal(loid)s in the soils. Statistically significant positive correlation was observed among the different metal(loid)s in the soil samples which points towards the common sources (natural and anthropogenic) of these metal(loid)s. Overall it was observed that the agricultural soils in the study area were contaminated with metal(loid)s and steps must be taken to reduce their levels.

Declarations

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Tables

Table 1. Heavy metal(loid) content (mg/kg) in soil samples (n=24) collected from agricultural fields on the banks of rivers Beas and Sutlej

Metal(loid)	Range	Average ± SD	1 st Quartile	Median	3 rd Quartile	Skewness	Kurtosis	Reference value
Ba	399 – 558	478.6 ± 41.24	450	466.5	506.75	0.40	-0.45	300 ^a
Co	47 - 132	85.92 ± 22.97	66.75	89.50	100.75	0.15	-0.93	10.6 ^b
Cr	78 – 147	95.42 ± 27.88	86	93	108.50	1.25	0.95	64 ^a , 100 ^c
Cu	10 – 27	17.92 ± 5.53	13	18	21.75	0.39	-1.60	135-270 ^d
Ni	20 – 43	31.75 ± 7.81	23.75	33.50	38.50	-0.14	-1.60	75-150 ^d
As	23 – 33	27.33 ± 2.60	26	27	28.25	0.58	0.37	12 ^a
Pb	19 – 27	22.63 ± 1.78	22.00	23	23.25	0.32	0.93	250-500 ^d
Rb	100 – 135	114.60 ± 8.02	109.75	113	118.75	0.72	0.58	–
Sr	67 – 162	89.58 ± 28.99	71	77	88.75	1.70	1.60	333 ^c
V	51 – 90	68.33 ± 13.83	54	69.5	78.50	0.17	-1.41	98 ^c
Y	21 – 32	26.75 ± 2.90	24	27	28.25	0.03	-0.65	–
Zn	45 – 101	62.67 ± 12.02	55	62	70	1.35	3.35	300-600 ^d
Zr	147 -241	191.80 ± 23.78	174	190.5	207.25	0.32	-0.31	–

^aGs,C.S.Q. (2007); ^bCNEMC (1990); ^cWedephol (1995); ^dAwasthi (2000).

Table 2. Genotoxic potential of soil samples employing *Allium cepa* root chromosomal aberration assay

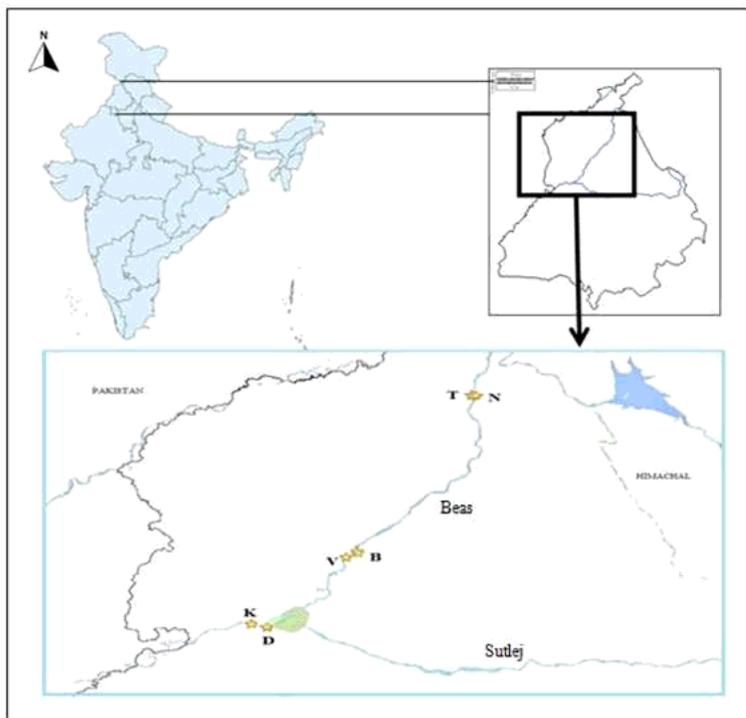
S. No.	Village Code	Physiological aberrations (%)	Clastogenic aberrations (%)	Total aberrations (%)
1.	N	10.00-12.11	2.77-3.11	13.11-15.22
2.	T	9.11-10.33	2.22-3.22	12.33-12.77
3.	V	8.77-10.88	1.77- 2.44	10.55-12.88
4.	B	8.88-12.22	2.11-3.88	11.00-15.11
5.	K	11.55-12.88	2.44-3.44	14.11-16.00
6.	D	10.00-11.33	2.33-3.44	13.22-14.77

Table 3. Pearson correlation matrix of contents of heavy metals in soil samples from agricultural fields under study and percent aberrant cells

	As	Ba	Co	Cr	Cu	Ni	Pb	Rb	Sr	V	Y	Zn
Ba	-0.206											
Co	0.831*	-0.537*										
Cr	-0.363	0.115	-0.406*									
Cu	-0.720*	0.581*	-0.839*	0.499*								
Ni	-0.696*	0.417*	-0.743*	0.582*	0.887*							
Pb	-0.065	0.765*	-0.499*	0.219	0.527*	0.354						
Rb	-0.258	0.921*	-0.550*	0.161	0.570*	0.396	0.800*					
Sr	-0.171	0.514*	-0.480*	0.237	0.342	0.431*	0.549*	0.434*				
V	-0.734*	0.632*	-0.894*	0.547*	0.969*	0.892*	0.547*	0.598*	0.469*			
Y	-0.713*	0.498*	-0.720*	0.501*	0.841*	0.682*	0.341	0.524*	0.108	0.843*		
Zn	-0.564*	0.556*	-0.666*	0.130	0.763*	0.543*	0.402	0.473*	0.167	0.733*	0.735*	
Zr	-0.726*	-0.033	-0.477*	0.383	0.443*	0.424*	-0.238	-0.026	-0.103	0.479*	0.665*	0.277
PAC	-0.027	-0.082	0.044	-0.144	-0.281	-0.108	-0.268	-0.128	-0.002	-0.242	-0.410*	-0.346

*Correlation is significant at the 0.05 level (2-tailed).

Figures



Village code	Village Description	Geographical coordinates
N	Naushera Pattan	N 31°59'23.6807", E 75°33'31.1157"
T	Tandem	N 31°59'42.5044", E 75°32'41.1548"
V	Verowal	N 31°23'40.4479", E 75°10'10.1140"
B	Baguana	N 31°24'43.7580", E 75°13'1657"
K	Kutianvala	N 31°9'10.1993", E 74°53'15.2981"
D	Deneke	N 31°8'10.3169", E 74°56'4.6234"

Figure 1

Map showing the location of villages from where agricultural soil samples were collected

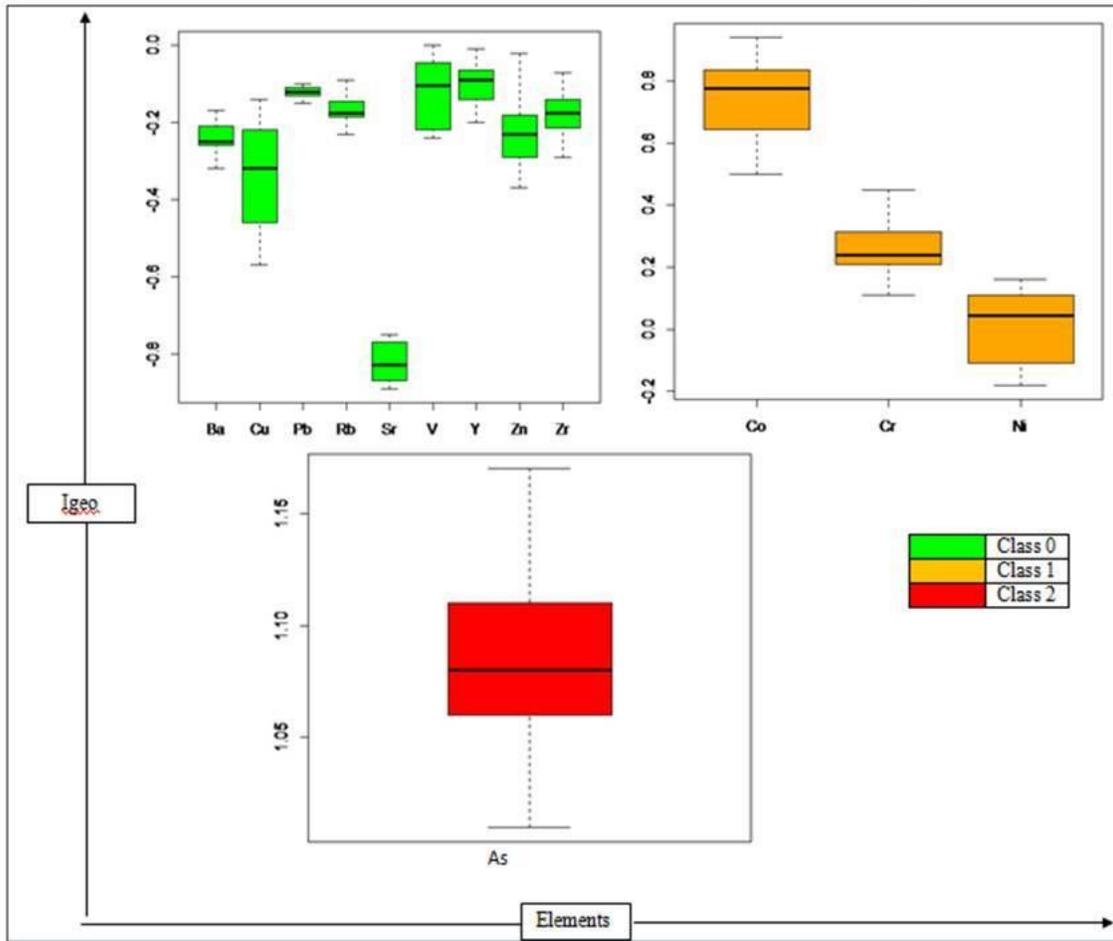


Figure 2

Box plot of Index of geo-accumulation (Igeo) of various elements. Note: Class 0, 1 and 2 as described in Supplementary Table 1.

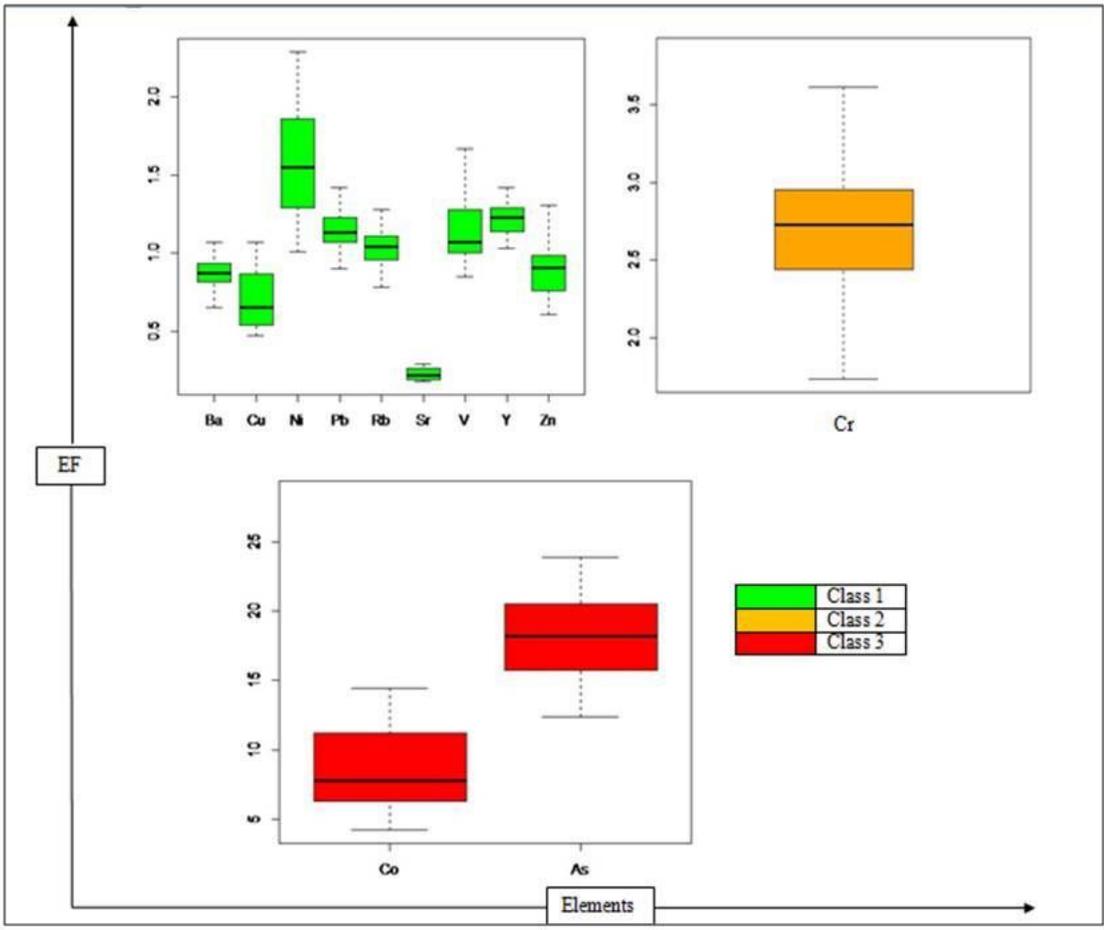


Figure 3

Box plot of Enrichment Factors (EF) of various elements. Note: Class 1, 2 and 3, as described in Supplementary Table 2.

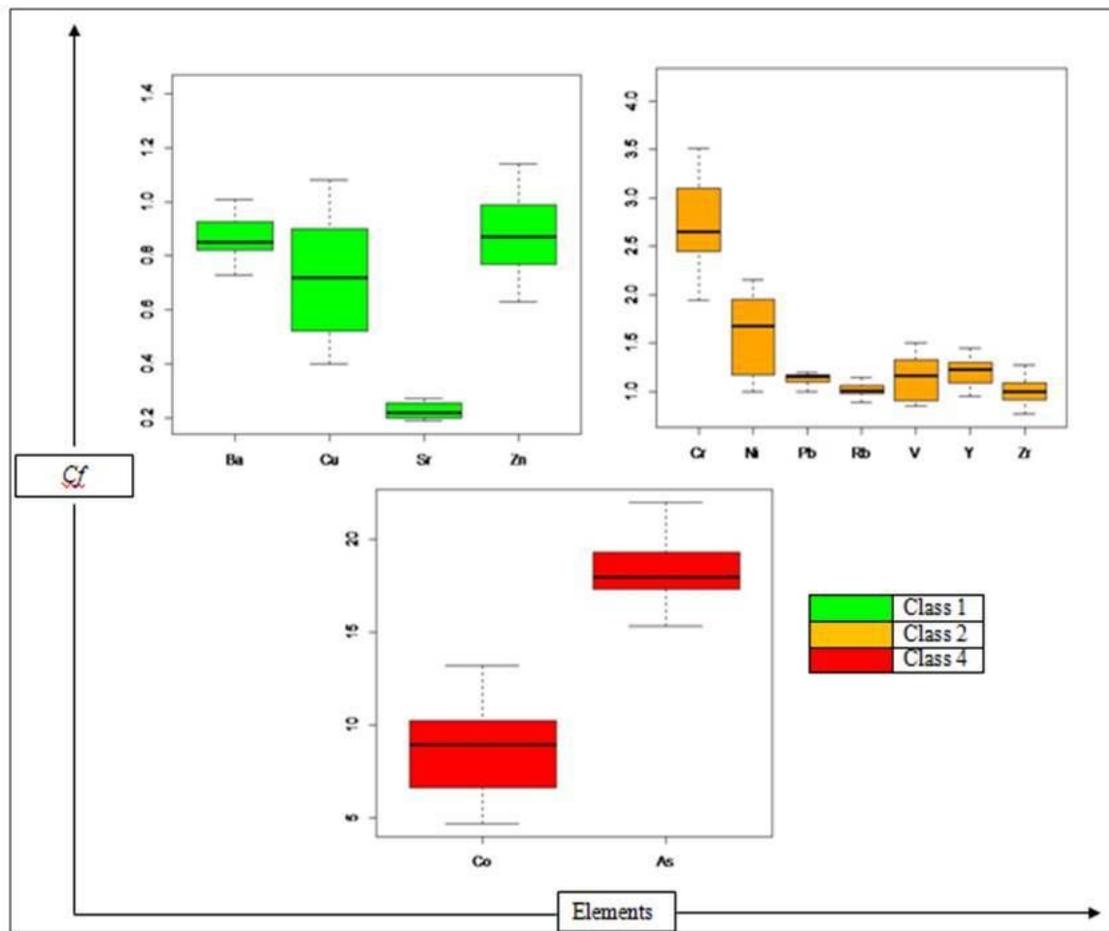


Figure 4

Box plot of Contamination Factors (Cf) of various elements. Note: Class 1, 2 and 4 as described in Supplementary Table 3.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryTable1Igeo.docx](#)
- [SupplementaryTable2EF.docx](#)
- [SupplementaryTable3CF.docx](#)