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

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Research Article

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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 non-point 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 (EDXRFl) and Wavelength Dispersive XRF spectrometer (WDXRF) systems, with the ability to detect a wide range of elements. EDXRFl 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

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

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):

$$EF = \frac{C_{ref}(sample)}{B_{ref}(background)}$$

where, $C_{n}(sample)$ is content of an element in soil sample, $C_{ref} (sample)$ is the content of the reference element (Zr) in soil sample, $B_n$ (background content) of element in the earth crust (Taylor and Mclennan, 1995) and $B_{ref} (background)$ 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:

$$C_f = \frac{C_{i,n}}{C_{n}}$$

where, $C_{i,n}$ is the average content of a particular element from at least five sampling sites and $C_{n}$ 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 B$_{12}$ (hydroxycynocobalamine) is an essential element. Heggtveit 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 an average 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-Pendas, 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-Pendas and Pendas, 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.S.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 (Gowd et al., 2010). In nature, Strontium occurs in four stable isotopes (\(^{84}\)Sr, \(^{86}\)Sr, \(^{87}\)Sr and \(^{88}\)Sr). Three of these (\(^{84}\)Sr, \(^{86}\)Sr, and \(^{88}\)Sr) are non-radioenic while, \(^{87}\)Sr is radiogenic and is formed by the radioactive decay of \(^{87}\)Rb, with a half-life of approximately 4.88×10\(^{10}\) 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/cm\(^3\), 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 12\(^{th}\) 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 metalloid/s and percent aberrant cells showed statistically significant positive correlation between most metalloid/s which points towards the common sources of these metalloid/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 metalloid/s due to different anthropogenic activities and posed significant genotoxic risks. Therefore, immediate steps must be taken to reduce the levels of metalloid/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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**Compliance with ethical standards**

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**Competing interests** The authors declare that they have no competing interests.

**References**

Ahmad, A., Toor, R. H., Aftab, S., & Shakoori, A. R. (2014). Anti-proliferative and genotoxic effect of Arsenic and Lead on human brain cancer cell line. Pakistan J. Zool., 46(4),1069-1076.

Allin, C. W. (Ed.). (2010). Encyclopedia of Global Resources: South Korea-Zirconium; Appendixes; Indexes (Vol. 4). Salem Press Inc.

Awashthi, S. K. (2000). Prevention of Food Adulteration Act no 37 of 1954. Central and State Rules as Amended for 1999. Ashoka Law House, New Delhi.

Ayres, R. U., & Ayres, L. (1999). Accounting for Resources, 2: The Life Cycle of Materials. Edward Elgar Publishing.

Bhatti, S. S., Sambyal, V., Singh, J., & Nagpal, A. K. (2017). Analysis of soil characteristics of different land uses and metal bioaccumulation in wheat grown around rivers: possible human health risk assessment. Environment, Development and Sustainability, 19(2), 571-588.

Bhatti, S. S., Kumar, V, Kumar, A., Gouzos, J., Kirby, J., Singh, J., Sambyal, V. & Nagpal, A. K. (2018). Potential ecological risks of metal (loid) s in riverine floodplain soils. Ecotoxicology and Environmental Safety, 164, 722-731.

Bhatti, S. S., Kumar, V, Kumar, A., Kirby, J. K., Gouzos, J., Correll, R., Singh, J., Sambyal, V. & Nagpal, A. K. (2020). Potential carcinogenic and non-carcinogenic health hazards of metal(loid)s in food grains. Environmental Science and Pollution Research, 27,17032–17042 (2020). https://doi.org/10.1007/s11356-020-08238-w

Bielecka, K., Kurtek, W., Banas, D., Kubala-Kukus, A., Braziewicza, J., Majewska, U., Pajek, M., Wudarczyk-Moçołko, J. & Stabrawa, I. (2014). X-ray diffraction and elemental analysis of medical and environmental samples. Acta Physica Polonica A., 125, 911-918.

Bonnet, M., Camares, O., & Veisseire, P. (2000). Effect of zinc and influence of *Acremonium foliolum* growth parameters, chlorophyll a fluorescence and antioxidant enzyme activities of ryegrass (*Lolium perenne* cv Apollo). J. Exp. Bot., 51(346), 945-953.

Cabrera, G. L. & Rodriguez, D. M. G. (1999). Genotoxicity of soil from farmland irrigated with wastewater using three plant bioassays. Mutation Research, 426, 211-214.

Campbell, L. M., Fisk, A. T., Wang, X., Kock, G. & Muir, D. C. G. (2005). Evidence for biomagnification of rubidium in freshwater and marine food webs. Can. J. Fish. Aquat. Sci., 62, 1161–1167.

Chandrasekaran, A., Ravisankar, R., Harikrishnan, N., Satapathy, K. K., Prasad, M. V. R., & Kanagasabapathy, K. V. (2015). Multivariate statistical analysis of heavy metal concentration in soils of Yelagiri Hills, Tamilnadu, India–Spectroscopical approach. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 137, 589-600.
China National Environmental Monitoring Center (CNEMC) (1990). The backgrounds of soil environment in China. China Environmental Science Press, Beijing (in Chinese).

Cobela-Garcia, A., & Prego, R. (2003). Heavy metal sedimentary record in a Glacian Ria (NW Spain): background values and recent contamination. Mar. Pollut. Bull., 46, 1253−1262.

Dallas, C. E., & Williams, P. L. (2001). Barium: rationale for a new reference dose. J. Toxicol. Environ. Health, 4, 395−429.

Denkhaus, E., & Salnikow, K. (2002). Nickel essentiality, toxicity, and carcinogenicity. Crit. Rev. Oncol./Hematol., 42: 35-56.

Faure, G., & Mensing T. M. (2005). Isotopes: principles and applications. Wiley, Hoboken.

Funtua. (1996). Application of the transmission emission method in EDXRF for the determination of trace element in geological and biological materials. J. Trace Microprobe Tech., 17, 293-297.

Gastaldo, J., Viau, M., Bencokova, Z., Joubert, A., Charvet, A., Balosso, J., & Foray, N. (2007). Lead contamination results in late and slowly repairable DNA double-strand breaks and impacts upon the ATM-dependent signaling pathways. Toxicology Letters, 173, 201-214.

Goldhaber, M. B., Lee, R. C., Hatch, J. R., Pashin, J. C., & Treworgy, J. (2003). Role of large scale fluid-flow in subsurface arsenic enrichment. In Arsenic in ground water. Springer, Bostn, MA, pp. 127-164.

Gowd, S. S. & Govil, P. K. (2008). Distribution of heavy metals in surface water of Ranipet industrial area in Tamil Nadu, India. Environ. Monit. Assess., 136, 197−207.

Gowd, S. S., Reddy, M. R., & Govil, P. K. (2010). Assessment of heavy metal contamination in soil at Jajmau (Kanpur) and Unnao industrial areas of the Ganga Plain, Uttar Pradesh, India. Journal of Hazardous Material, 174, 113-121.

Gs, C. S. Q. (2007). Canadian Soil Quality Guidelines for the Protection of Environmental and Human Health. Summary Tables.

Hakanson, L. (1980). An ecological risk index for aquatic pollution control. A sedimentological approach. Water Res., 14, 975-1001.

Hammann, A., Ybañez, L. M., Isla, M. I., & Hilal, M. (2020). Potential agricultural use of a sub-product (olive cake) from olive oil industries composting with soil. Journal of Pharmacy & Pharmacognosy Research, 8(1), 43-52.

Hammer, Ø., Harper, D. A., & Ryan, P. D. (2001). PAST: Paleontological statistics software package for education and data analysis. Palaeontol. Electron., 4(1), 9.

Heggtev, H. A., Grice, H. C., & Wiberg, G. S. (1970). Cobalt cardiomyopathy. Experimental basis for the human lesion. Path. Microbiol., 35, 110−113.

Hu, J., Lin, B., Yuan, M., Lao, Z., Wu, K., Zeng, Y., Liang, Z., Li, H., Li, Y., Zhu, D., & Liu, J. (2019). Trace metal pollution and ecological risk assessment in agricultural soil in Dexing Pb/Zn mining area, China. Environmental Geochemistry and Health, 41(2), 967-980.

Ippolito, J. (2006). Biosolids affect soil barium in a dryland wheat agro-ecosystem. J. Environ. Qual., 35, 23-33.

Jabeen, S., Shah, M. T., Khan, S., & Hayat, M. Q. (2010). Determination of major and trace elements in ten important folk therapeutic plants of Haripur basin, Pakistan. Journal of Medicinal Plants Research, 4, 559-566.

Juarez-Santacruz, L., Garcia-Nieto, E., Costilla-Salazar, R., Garcia-Gallegos, E., Coronel-Olivares, C., Gomez-Camarillo, M., & Gaytan-Oyarzun, J. (2013). Assessment of the genotoxic potential of sediments contaminated with POPs and agricultural soils using Vicia faba micronucleus assay. Soil and Sediment Contamination, 22, 288-300.

Kabata-Pendas, A. (2004). Soil-plant transfer of heavy metals—an environmental issue. Geoderma, 122, 143-149.

Kabata-Pendas, A., and Pendias, H. (1999). Biogeochemistry of trace elements, 2nd edn. PWN, Warsaw (In Polish).

Khan, A., Khan, S., Khan, M. A., Qamar, Z., & Waqas, M. (2015). The uptake and bioaccumulation of heavy metals by food plants, their effects on plants nutrients, and associated health risk: a review. Environmental Science and Pollution Research, 22(18), 13772-13799.

Kong, M. S., & Ma, T. H. (1998). Genetic toxicity of contaminated soil and shallow well water detected by plant bioassays. Mutation Research, 426(2), 221 - 226.

Kupper, H., Kupper, F., & Spiller, M. (1998). In situ detection of heavy metal substituted chlorophylls in water plants. Photosynth. Res., 58, 125-133.

Kupper, H., Kupper, F., & Spiller, M. (1998). In situ detection of heavy metal substituted chlorophylls in water plants. Photosynth. Res., 58, 125-133.

Levan, A. (1938). The effect of colchicine on root mitosis in Allium. Hereditas, 24, 471-486.

Llanos, R. M., & Mercer, J. F. (2002). The molecular basis of copper homeostasis and copper-related disorders. DNA Cell Biol., 21, 259−270.

Loska, K., Wiechula, D., & Korus, I. (2004). Metal contamination of farming soils affected by industry. Environment International, 30(2), 159-165.
Lu, X., Wanga, L., Li, L. Y., Lei, K., Huang, L., & Kang, D. (2010). Multivariate statistical analysis of heavy metals in street dust of Baoji, NW China. J. Hazardous Mat., 173, 744-749.

Machender, G., Dhakate, R., Prasanna, L., & Govil, P. K. (2011). Assessment of heavy metal contamination in soils around Balanagar industrial area, Hyderabad, India. Environ. Earth Sci., 63, 945-953.

Maksimovic, I., Kastori, R., Putnik-Delic, M., & Borišev, M. (2014). Effect of yttrium on photosynthesis and water relations in young maize plants. Journal of Rare Earths, 32(4), 372-378.

Marques, C. C., Nunes, A. C., Pinheiro, T., Lopes, P. A., Santos, M. C., Viegas, C. A. M., Ramalhinho, M. G., & Mathias, M. L. (2006). An assessment of time dependent effects of lead exposure in Algerian mice (Mus spretus) using different methodological approaches. Biological Trace Element Research, 109, 75-89.

Menzie, C., Southworth, B., Stephenson, G., and Feisthauer, N. (2008). The importance of understanding the chemical form of a metal in the environment: The case of barium sulfate (barite). Hum. Ecol. Risk Assess., 14, 974-991.

Morinville, A., Maysinger, D., & Shaver, A. (1998). From Vanadis to Atropos: vanadium compounds as pharmacological tools in cell death signaling. Trends Pharmacol. Sci., 19, 452–460.

Mudgal, V., Madaan, N., Mudgal, A., Singh, R. B., & Mishra, S. (2010). Effect of toxic metals on human health. The Open Nutraceuticals Journal, 3, 94-99.

Muller, G. (1969). Index of geoaccumulation in sediments of the Rhine River. GeoJournal, 2, 108-118.

Nagajyoti, P. C., Lee, K. D., & Sreekanth, T. V. M. (2010). Heavy metals, occurrence and toxicity for plants: a review. Environmental Chemistry Letters, 8(3), 199-216.

Rigo, A. A., Cezaro, A. M. D., Muenchen, D. K., Martinazzo, J., Manzoli, A., Steffens, J., & Steffens, C. (2020). Heavy metals detection in river water with cantilever nanobiosensor. Journal of Environmental and Health Science, Part B, 55(3), 239-249.

Ruggeberg, A., Fietzke, J., Liebetrau, V., Eisenhauer, A., Dullo, W., & Freiwald, A. (2008). Stable strontium isotopes (d88Sr/86Sr) in cold-water corals – a new proxy for reconstruction of intermediate ocean water temperatures. Earth Planet Sci. Lett., 269, 570–575.

Sax, K., & Sax, H. J. (1968). Possible hazards of some food additives, beverages and insecticides. Idengakuzasshi, 43, 89-94.

Seilkop, S. K., & Oller A. R. (2003). Respiratory cancer risks associated with low-level nickel exposure: an integrated assessment based on animal, epidemiological, and mechanistic data. Regul. Toxicol. Pharmacol., 37: 173-190.

Shahid, M., Ferrand, E., Schreck, E., & Dumat, C. (2013). Behavior and impact of zirconium in the soil–plant system: plant uptake and phytotoxicity. In Reviews of Environmental Contamination and Toxicology Volume 221. Springer, New York, NY, pp. 107-127.

Shalan, M. G., Mostafa, M. S., Hassouna, M. M., Hassan El-Nabi, S. E., & El-Rafaie, A. (2005). Amelioration of lead toxicity on rat liver with vitamin C and silymarin supplements. Toxicology, 206, 1-15.

Sharma, S., Nagpal, A. K., & Kaur, I. (2019). Appraisal of heavy metal contents in groundwater and associated health hazards posed to human population of Ropar wetland, Punjab, India and its environs. Chemosphere, 227, 179-190.

Singh, N., Kaur, M., & Katnoria, J. K. (2017). Spatial and temporal heavy metal distribution and surface water characterization of Kanjli Wetland (a Ramsar site), India using different indices. Bulletin of Environmental Contamination and Toxicology, 99(6), 735-742.

Soodan, R. K., Pakade, Y. B., Nagpal, A., & Katnoria, J. K. (2014a). Analytical techniques for estimation of heavy metals in soil ecosystem: a tabulated review. Talanta, 125, 405-410.

Soodan, R. K., Katnoria, J. K., & Nagpal, A. (2014b). Allium cepa root chromosomal aberration assay: An efficient test system for evaluating genotoxicity of agricultural soil. International Journal of Science and Research, 3, 245-250.

Souza, T. S., Hencklein, F. A., Angelis, D. F., & Fontanetti, C. S. (2013). Clastogenicity of landfarming soil treated with sugar cane vinasse. Environmental Monitoring and Assessment, 185, 1627-1636.

Stiborova, M., Ditrichova, M., & Brezinova, A. (1988). Mechanism of action of Cu^2+, Co^2+ and Zn^2+ on ribulose 1-5 biphosphate carboxylase from barley (Hordeum vulgare L.). Photosyntheticia, 22, 161-167.

Sutherland, R. A. (2000). Bed sediment-associated trace metals in an urban stream, Oahu, Hawaii. Environ. Geol. 39, 611–627.

Taylor, S. R. & McLennan, S. M. (1995). The geochemical evolution of the continental crust. Rev. Geophys., 33, 241-265.
Tosukhowong, P., Tungsanga, K., Eiam-Ong, S., & Sitprija, V. (1999). Environmental distal renal tubular acidosis in Thailand: an enigma. American Journal of Kidney Diseases, 33(6), 1180-1186.

Tsonko, T., & Fernando, J. C. L. (2012). Zinc in plants - An overview. Emir. J. Food Agric., 24 (4), 322-333.

Vachirapatama, N., Dicinoski, G., Townsend, A. T., & Haddad, P. R. (2002). Determination of vanadium as PAR-hydrogen peroxide complex in fertilisers by ion-interaction RP-HPLC. Journal of Chromatography A., 956, 221-227.

Wang, W., Zhou, M. F., Yan, D. P., Li, L., & Malpas, J. (2013). Detrital zircon record of Neoproterozoic active-margin sedimentation in the eastern Jiangnan Orogen, South China. Precambrian Research, 235, 1-19.

Wedepohl, K. H. (1995). The composition of the continental crust. Geochim. Cosmochim. Acta., 59, 1217-1232.

Welch, A. H., Westjohn, D. B., Helsel, D. R., & Wanty, R. B. (2000). Arsenic in ground water of the United States: Occurrence and geochemistry. Ground water, 38(4), 589.

Yusuf A. Z., Halima S. A., Zakir A., Abdullahi M., & Sani K. (2014). X-ray fluorescence: An accessible and effective technique for evaluation of hazards and economic potential associated with important mineral deposits in Nigeria. Journal of Geology and Mining Research. 6, 13-17.

Zhang, D., Wang, X. M., Li, T. F., Luo, X. Q., Wu, W., Nori, F., & You, J. Q. (2015). Cavity quantum electrodynamics with ferromagnetic magnons in a small yttrium-iron-garnet sphere. npj Quantum Information, 1(1), 1-6.

Zhang, H., Mao, Z., Huang, K., Wang, X., Cheng, L., Zeng, L., Zhou, Y., & Jing, T., 2019. Multiple exposure pathways and health risk assessment of heavy metal (loid) s for children living in fourth-tier cities in Hubei Province. Environment International, 129, 517-524.

### 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 | 1st Quartile | Median  | 3rd Quartile | Skewness | Kurtosis | Reference value |
|------------|--------|--------------|--------------|---------|-------------|----------|----------|----------------|
| Ba         | 399 – 558 | 478.6 ± 41.24 | 450         | 466.5   | 506.75      | 0.40     | -0.45    | 300<sup>a</sup> |
| Co         | 47 - 132  | 85.92 ± 22.97 | 66.75       | 89.50   | 100.75      | 0.15     | -0.93    | 10.6<sup>b</sup> |
| Cr         | 78 – 147  | 95.42 ± 27.88 | 86          | 93      | 108.50      | 1.25     | 0.95     | 64<sup>c</sup>, 100<sup>d</sup> |
| Cu         | 10 – 27   | 17.92 ± 5.53  | 13          | 18      | 21.75       | 0.39     | -1.60    | 135-270<sup>d</sup> |
| Ni         | 20 – 43   | 31.75 ± 7.81  | 23.75       | 33.50   | 38.50       | -0.14    | -1.60    | 75-150<sup>d</sup> |
| As         | 23 – 33   | 27.33 ± 2.60  | 26          | 27      | 28.25       | 0.58     | 0.37     | 12<sup>a</sup> |
| Pb         | 19 – 27   | 22.63 ± 1.78  | 22.00       | 23      | 23.25       | 0.32     | 0.93     | 250-500<sup>d</sup> |
| 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<sup>c</sup> |
| V          | 51 – 90   | 68.33 ± 13.83 | 54          | 69.5    | 78.50       | 0.17     | -1.41    | 98<sup>c</sup> |
| 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<sup>d</sup> |
| Zr         | 147-241   | 191.80 ± 23.78 | 174         | 190.5   | 207.25      | 0.32     | -0.31    | – |

<sup>a</sup>Gs,C.S.Q. (2007); <sup>b</sup>CNEMC (1990); <sup>c</sup>Wedephol (1995); <sup>d</sup>Awasthi (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).