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ASSESSMENT OF HEAVY METALS AND PESTICIDES CONTAMINATION OF WASTE WATER AND SOIL SAMPLES FROM DISTRICT FATEHGARH SAHIB, PUNJAB, INDIA

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ABSTRACT

Unawareness among the farmers and unregulated industrialization inundated the water with harmful residues of pesticides and heavy metals salts which resulted in deterioration of quality of water in the state of Punjab, India. In the present paper, we report the quality and quantity of said pollutants in waste water and agricultural soil samples from district Fatehgarh Sahib, Punjab. HPLC analysis was carried out to analyze and quantify the concentration of pesticides (triazophos, simazine, fipronil, atrazine, carbendazim, cartap, chloropyrifos and paraquat) and heavy metals (lead, manganese, uranium, mercury, ferrous, barium and cadmium) contamination. Pesticides simazine (0.161-0.221 ppm), fipronil (0.0338-0.068 ppm), paraquat (0.0079-0.012 ppm), chloropyrifos (0.0065-0.0089) and carbendazim (0.0076-0.0090 ppm) were observed in higher concentration in soil samples as compared to water sample. Whereas, heavy metals like lead (31-69 µg/L), manganese (112-132 µg/L), uranium (49-67 µg/L), mercury (83-113 µg/L), ferrous (55-88 µg/L), barium (24-49 µg/L) and cadmium (53-141 µg/L) were found to be more than the permissible limit in both water and soil samples.

KEY WORDS : Heavy metals, Pesticides, Waste water

INTRODUCTION

Indiscriminate and excessive use of pesticides, fertilizers and unplanned industrialization results in excessive release of heavy metals salts and pesticides residues in water (Mittal *et al.*, 2014; Iqbal, 2018), which makes the water toxic and unsuitable for drinking and other life activities (Zhao *et al.*, 2013). This contaminated water further inundated the soil ecosystem with these toxic substances which finally reached to human bodies through plant-based foods. Biomagnification of these pollutants at every tropic level of food chain and their bioaccumulation in various vital organs resulted in severe adverse effect on human health. In humans, accumulated pesticides residues and heavy metals salts disturbed or vanish the activity of various enzymes, damage the DNA and cell wall proteins and the cumulative effect of these activity resulted in irritability, damage to kidney, brain, liver, and certain types of cancer. Not only this, heavy metal salts and pesticides residues have potential inhibitory effect on soil microflora and microfauna (Mittal *et al.*, 2014; Chen *et al.*, 2015).

Fresh water is essential for survival and existence of all living forms and it is estimated that out of total water present on this earth, only 0.036% is accessible to human beings. Beside untreated and improper treated sewage, unregulated industrialization and agriculture activities polluted the water with toxic pesticides residues and heavy metals ions. Sustainable development of a country needs conservation of water for future generations, and for achieving that goal water treatment plants and activities must be designed for removal of these harmful pesticides and heavy metals ions. Before the treatment of wastewater, it is utterly essential to study the quality and quantity of various pollutants like heavy metal, pesticide and microbial contaminations.

Punjab is an agriculturally enriched state in north India and the effluents coming out from unfettered and inappropriately designed industries has gradually pierced the surrounding ecosystem with noxious substances. The aim of the present study is to analyze and quantify the heavy metals salts and pesticides contaminations of waste water and nearby field soil of village Chunni Kalan, Fatehgarh Sahib as there is no available data that could reveal the factual level of heavy metal and pesticide profile in this region. This study will create primary data about these toxic pollutants which is essential for efficient and economical waste management programmes and choice of treatment methods in a planned and controlled manner by government or non-government organizations.

MATERIALS AND METHODS

Sample collection

Fatehgarh Sahib is situated between 30 degree-382 North and 76 degree-272 East and is 50 kilometers towards the west of Chandigarh (capital of Punjab). For the study of heavy metals and pesticides contamination in waste water and nearby field soils of village Chunni Kalan, three samples were collected (R1, S1 and S2) as shown in Figure 1. R1 sample (municipality waste water and surface runoff water of village) was a water sample, whereas sample S1 (1 meter from waste water source) and S2 (100 meters from waste water source) were soil samples. Soil samples were collected from 5-15 cm below the surface and stored in aerated polythene bags.

Chemicals

All chemicals used in the present study were of analytical grade (AR) and purchased from Merck (India) and Sigma-Aldrich (India). Autoclaved double distilled water was used for all experiments. The pesticide standards of pestanal grade (95–99% purity) and respective heavy metal salts were purchased from Sigma-Aldrich. The individual stock standard solutions of pesticides and heavy metal salts at the concentration of 1000 mg/L were prepared by dissolving the respective chemicals in methanol and acetonitrile. All stock standard solutions were stored in the dark at -4 °C for further use.

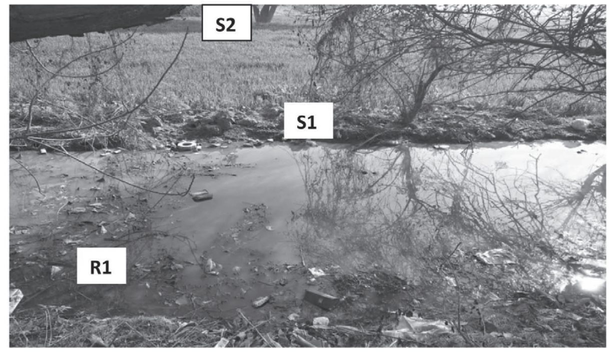

Fig. 1. View of different sites (R1, S1 and S2) described in present study from where samples were collected for analysis of pesticides and heavy metals contamination.

HPLC analysis of heavy metals and pesticides

In accordance with international standards for soil analysis (Radojevic and Bashkin, 1999), the soil samples were dried at a temperature of 40 °C to get a constant weight. 2 g of the soil sample were homogenized in 10 mL cold PBS (0.02M, pH 7.4) at 4 °C for 5-10 mins or until uniformly even out. The homogenate was centrifuged at 10,000 rpm at 4 °C for 12 mins. The supernatant was collected and stored at -20 °C. The residues of eight selected pesticides (triazophos, simazine, fipronil, atrazine, carbendazim, cartap, chloropyrifos and paraquat dichloride) and seven heavy metals (barium, cadmium, lead, manganese, uranium, mercury, ferrous) were extracted from soil samples by using methanol/acetonitrile as solvent. Solid phase extraction (SPE) was carried out for clean-up of the samples. The pesticide residues and heavy metal analyses were carried out by using high performance liquid chromatograph (UltiMate 3000) attached with binary gradient pump (HPG-3200A) controlled by Chromeleon (6.8) software. Mobile phase which is comprised of acetonitrile and water (80:20 v/v in case of pesticides and 90:10 v/v for heavy metals) is used with a flow rate of 0.5 mL/min for pesticides and 0.75 mL/min for heavy metals. For the calibration curve, 1000 µg/mL of the standard was diluted to give solutions of 0.01, 0.05, 0.1, 0.2, 0.4 and 0.6 µg/mL concentrations with the acetonitrile/water mixture. Standards of pesticides and heavy metals were injected into the instrument, and the peak areas and the retention times of the standards were used to interpret the data. The quantified residues were the means from three replicates of each sample and all the data were analyzed using simple descriptive statistics. From the calibration curve and linear regression equation, the standard deviation (SD), slope, intercept and standard error (SE) were calculated. Limit of Detection (LOD) and Limit of Quantification (LOQ) were calculated based on the method of SD of the response and the slope.

RESULTS AND DISCUSSION

Pesticides contamination

The soil microbes play very important role in the soil ecosystem and are essential for nutrient cycling and decomposition (Khan *et al.*, 2010). Therefore, presence of excessive pesticides could adversely affect the soil ecosystem. The present study area comes under Malwa region of Punjab, which is already facing the problem of pesticides-pollution. It was claimed in a report that the Malwa region (15% of total area of Punjab) is using 75% of total pesticides consumption of Punjab (Mittal *et al.*, 2014).

Enzyme dehydrogenase activity is regarded as an indicator of healthy microbial activities of soil (Sidhu *et al.*, 2019) and it has been revealed that presence of triazophos (3-(O, O-diethyl)-1-phenyl thiophosphoryl-1, 2, 4-triazol), an organophosphorous broad-spectrum insecticide, in the soil is responsible for decreased dehydrogenase and urease activity in comparison to the controls (Kalyani *et al.*, 2015). Triazophos contamination was present in the concentration of 0.0084, 0.0089 and 0.0128 ppm in samples R1, S1 & S2, respectively, whereas contamination of fipronil (5-amino-1-[2, 6-dichloro-4-(trifluoromethyl) phenyl]-4-[(trifluoromethyl) sulfinyl]-1H-pyrazole-3-carbonitrile) was found to be 0.0338, 0.0529 and 0.0686 ppm in samples R1, S1 & S2 respectively (Figure 2, Table 1). Fipronil, triazophos and their mixture were found to have adverse effect on miRNA expression of zebrafish and might be related to such adverse effect in humans (Wang *et al.*, 2010).

Table 1. Concentration of pesticides (ppm) at sites R1, S1 and S2.

Pesticides	R1	S1	S2
Triazophos	0.0084	0.0089	0.0128
Simazine	0.1612	0.2212	0.1755
Fipronil	0.0338	0.0529	0.0686
Atrazine	0.0045	0.0071	0.0044
Carbendazim	0.0076	0.0089	0.0090
Cartap	0.0058	0.0071	0.0072
Chloropyrifos	0.0065	0.0084	0.0089
Paraquat	0.0083	0.0079	0.0127

Simazine (6-chloro-N, N′-diethyl-1, 3, 5-triazine-2, 4-diamine) belongs to triazine family of pesticides which includes other herbicides such as atrazine. Simazine toxicity towards reproductive and immune system of human has been well demonstrated in some studies (Park and Bae, 2012; Ren *et al.*, 2013). In addition, atrazine whose concentration in samples R1, S1 & S2 was determined as 0.0045, 0.0071, 0.0044 ppm respectively, has been reported to have toxicological effects on certain antioxidant mechanisms and neuroendocrine systems of human (Abarikwu *et al.*, 2010; Hovey *et al.*, 2011). Cartap (S, S-[2-(dimethylamino)-1, 3-propanediyl] dicarbamothioate), is a stomach poison, and was found to be in concentration of 0.0058, 0.0071, 0.0072 ppm in sample R1, S1 and S2, respectively. Earlier, it was considered to be a safe pesticides upto certain concentration, but some recent studies have shown that it can cause diaphragmatic contracture and death in rabbits (Liao *et al.*, 2003; Boorugu and Chrispal, 2012). Carbendazim, a broad-spectrum fungicide, side effects showed arrest of spermatogenesis and siderosis in liver and kidneys in wistar rats (Veerappan *et al.*, 2012) and was quantified in samples R1, S1 and S2 in the concentration of 0.00762, 0.00892, 0.00195 ppm, respectively in the present study.

Chlorpyrifos (*O,O*-diethyl-*O*-(3,5,6-trichloro-2-pyridinyl) phosphorothionate) belongs to family of organophosphorothionates pesticides, one of the most widely used insecticides due to its broad spectrum activity against various agriculture pests. In present study, it was found to be in concentration of 0.0065, 0.0084, 0.0089 ppm in the samples R1, S1 & S2, respectively (Figure 2 and Table 1). Its toxicity is due to the inhibition of a neuro-enzyme acetylcholinesterase, which results in alteration of certain molecular signalling pathways and hence responsible for harmful functional changes at tissue

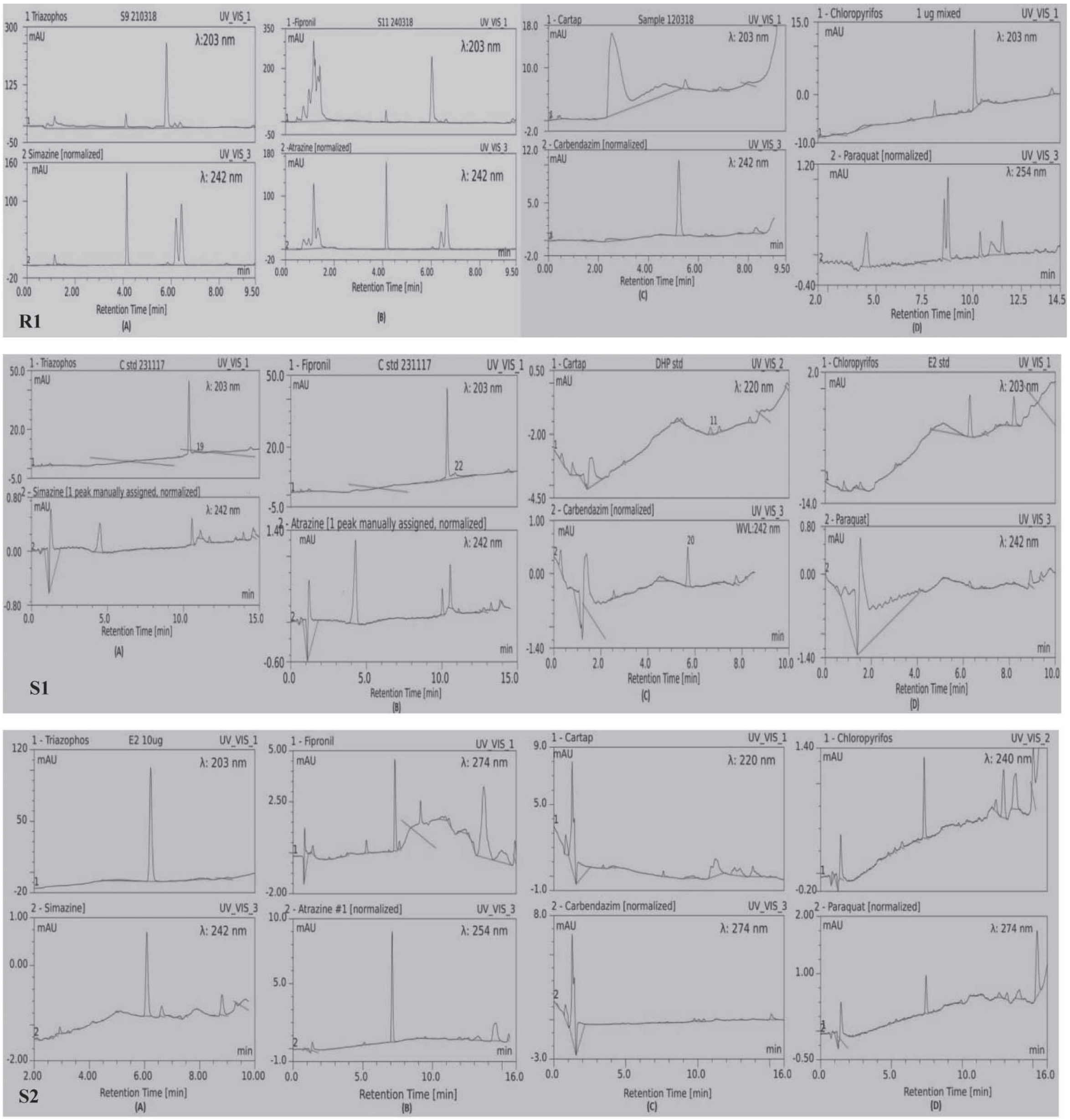

Fig. 2. HPLC Chromatogram of Pesticides residues in samples R1, S1 and S2. (A) Triazophos and Simazine; (B) Fipronil and Atrazine; (C) Carbendazim and Cartap; (D) Chloropyrifos and Paraquat dichloride.

or organ levels (Pope *et al.*, 2005). Chlorpyrifos contamination in our samples was found to be more than Malout and Mansa region, but less than the Abohar and Bathinda area of Punjab (Mittal *et al.*, 2014). Paraquat (1, 1'-dimethyl-4,4' -bipyridylium dichloride) is a non-selective herbicide and used for weed control. Paraquat causes toxicity in cells due to increased production of superoxides (Smith, 1986). Soil sample S2, which is collected from the middle area of the field showed the highest concentration of paraquat, i.e. 0.0127 ppm as compared to other samples (Table 1).

Heavy metals contamination

As per BIS guidelines (BIS, 2004), maximum permissible limit of lead in drinking water is 0.01 mg/L and above this desirable limit, lead poisoning results in many health hazard to humans which includes abdominal discomfort, irritation, anemia etc. Physical and mental disability, low development rate in rat was also reported due to lead toxicity (Sharma and Datta, 2017). In our study, Pb contamination was found to be highest in sample S2, a soil sample (69 µg/L). Previously, level of Pb in the blood of animals was reported to be higher than the tolerable limit in Punjab state (Yeotikar *et al.*, 2018). High level of Pb toxicity was also reported from soil of Ropar district (14.11-21.30 mg/Kg) of Punjab state (Sharma *et al.*, 2018; Iqbal, 2018).

Cadmium comes into environment because of various anthropogenic activities mainly industrial activities. As per BIS guidelines, maximum allowable limit for cadmium is 0.003 mg/L. In the present study, cadmium concentration was found to be highest in sample S2 (141 µg/L). In a previous study, the contents of heavy metals cadmium and lead were found to be well above the maximum permissible limits in sugarcane and sorghum plants in Punjab (Bhatti *et al.*, 2016). Cadmium can either be incorporated through the lungs or gastrointestinal tract and accumulate in various organs such as liver and kidneys. This accumulation may cause lung edema, shortness of breath and cadmium-induced pneumonitis (Seidal *et al.*, 1993).

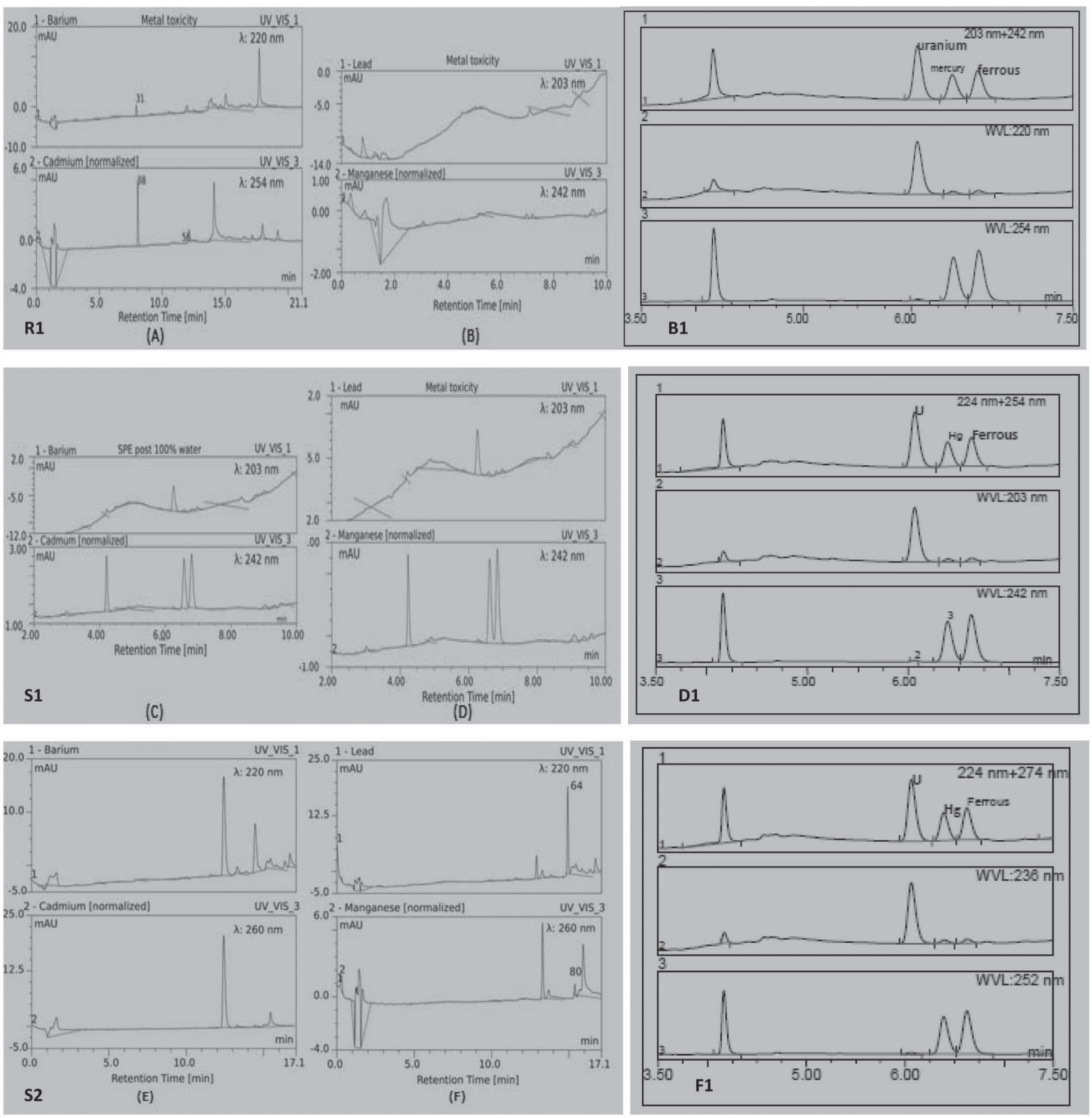

Fig. 3. HPLC Chromatogram of Heavy metals in samples R1, S1 and S2. A & B, C & D and E & F chromatogram shows barium, cadmium, lead & manganese peaks in respective samples, whereas B1, D1 and F1 shows chromatogram of uranium, mercury and ferrous in samples R1, S1 and S2, respectively.

In a previous study from Punjab, cadmium contamination was found to be highest in water samples of Ferozpur district (2.9 mg/L) followed by Faridkot (0.022 mg/L) district (Sharma and Datta, 2017). Manganese is absorbed mainly in intestine and accumulation of this heavy metal takes place in erythrocytes, liver, kidneys, and pancreas (Mahoney and Small, 1968). Manganese was found to be highest in sample S2 (132 µg/L) in the present study as compared to other samples as shown in Table 2.

Barium has a preferential site of accumulation in bones and also found to be accumulated predominantly in lungs, aorta, kidneys, and spleen (Schroeder *et al.*, 1972). In sample S2, barium was found to be highest (49 µg/L) in concentration as compared to sample S1 (45 µg/L) and R1 (24 µg/L). Heavy metal mercury toxicity to humans is well documented and results in mental defects, impairment of speech, vision and hearing in human. As per BIS guidelines, allowable mercury limit in drinking water is 0.001 mg/L. We have recorded highest concentration of mercury in R1 sample (113 µg/L) followed by sample S1 (91 µg/L) and S2 (83 µg/L) as shown in Table 2. Previously, high mercury toxicity was reported from Muktasar and Moga district (270 µg/L) of Punjab (Sharma and Datta, 2017). The desirable and permissible limit of ferrous in drinking water is 0.3-1.0 mg/L. Higher concentration of iron in drinking water leads bitter taste of water and makes it unsuitable for drinking purposes. Steel industries wastes and other anthropogenic activities are responsible for increased iron contamination in water and soil (Sharma and Datta, 2017). In water sample (R1), ferrous concentration was quantified to be the highest (88 µg/L) than soil samples S1 (74 µg/L) and S2 (55 µg/L).

From the last decade there has been more than twelve media reports showing high toxicity of uranium in Punjab (Iqbal, 2018). As per WHO guidelines, the acceptable uranium contamination in drinking water is 15 µg/L (15 ppb), whereas criteria set by Atomic Energy Regulatory Board (AERB) is 60 µg/L (60 ppb). As per Government of India records, if we consider WHO criteria for contamination of uranium, then approximately 35% of Punjab water is unsuitable for drinking purposes (Iqbal, 2018). Uranium was found to be higher in our water sample R1 (67 µg/L) in comparison to soil samples S1 (56 µg/L) and S2 (49 µg/L). Effect of uranium toxicity in human is not well recognized but its effect on kidney has been reported in some reports (Iqbal, 2018; Virk, 2017). Further, as radioactivity in uranium starts above the concentration of 100 µg/L (Virk, 2017), it becomes hazardous and life threatening to all life forms.

Table 2. Heavy metals concentration (µg/L) at sites R1, S1 and S2.

Heavy Metals	R1	S1	S 2
Barium	24	45	49
Cadmium	53	132	141
Lead	31	57	69
Manganese	112	127	132
Uranium	67	56	49
Mercury	113	91	83
Ferrous	88	74	55

CONCLUSION

Due to current agricultural practices and unregulated industrialization, the presence of harmful pesticides residues and heavy metals salts were observed at a significantly higher level in our water and soil samples. This continuous and still uncontrolled evolving problem of water and soil crisis may results in bioaccumulation of these toxic chemicals in human and other life forms through food chain which may further resulted in increased morbidity and mortality cases. This project is completed with the objectives to get the primary information on concentration of various pesticides and heavy metals contamination in water and soil samples, which may be useful for different government and non-government environment protection agencies to reenergize the health of water and soil by removing these harmful contaminations from the environment.

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