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ENVIRONMENTAL TOXICOLOGY AND RISKS ASSOCIATED WITH HUMAN HEALTH

Risk assessment of heavy metal(loid)s via *Spinacia oleracea* ingestion after sewage water irrigation practices in Vehari District

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Abstract

The use of sewage water as an irrigation source can be beneficial in agricultural practices, however, it may result in human health risks due to the consumption of heavy metal(loid)–contaminated food. This study evaluated the suitability of using sewage water (SW), freshwater (FW), and groundwater (GW) for vegetable irrigation in District Vehari. Spinach (*Spinacia oleracea*) plants were grown in pots irrigated with FW, GW, and SW in different proportions and combinations. The results indicated the substantial lesser buildup of heavy metal(loid)s (As (− 0.8%), Cd (− 38%), Cr (− 6.2%), Cu (− 20%), Fe (− 9.2%), Mn (− 13%), Ni (− 16%), Pb (− 19%), and Zn (−15%)) in soil after *S. oleracea* cultivation compared to unirrigated soil possibly due to high metal(loid) uptake by *S. oleracea*. Irrigation with all types of waters resulted in metal(loid) accumulation in *S. oleracea* predominantly in roots. The combinations of FW, GW, and SW resulted in high metal(loid) accumulation (As, Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn) in the edible *S. oleracea* leaves than their alone application. Owing to high metal(loid) buildup, plants showed a linear trend in physiological imbalance in terms of reduced pigment content, induction of peroxidation, and oxidation of lipids. The severe oxidative stress was observed in *S. oleracea* plants under FW and GW irrigation due to high metal(loid) accumulation. The risk indices showed possible carcinogenic risk (CR > 0.0001) and non-carcinogenic risk (HI > 1) from the consumption of metal(loid)-contaminated *S. oleracea* leaves. Results revealed unsuitability of all waters and their combinations for *S. oleracea* irrigation. Moreover, this study does not encourage the use of mixed water for vegetable irrigation in Vehari District. Therefore, it is of utmost importance to monitor the quality of irrigation waters to ensure food safety and prevent chronic health risks to the exposed population.

Keywords Sewage water irrigation · Heavy metal(loid)s · *Spinacia oleracea* · Oxidative stress · Physiological imbalance · Health risk

Introduction

Food safety is a matter of great concern for public health. Increasing demand for food safety has diverted researchers' attention to evaluate the food-borne diseases and health risks associated with ingestion of contaminated foodstuffs. Heavy metal(loid)s are non-biodegradable and can magnify in the food chain with potentially adverse effects on both human and environment health (Antoniadis et al. 2019; Natasha et al. 2018). Once heavy metal(loid)s enter the soil, they can be assimilated by plants and bioaccumulate in the food chain due to their high persistence and bioaccumulation nature (Natasha et al. 2020c).

Sewage water irrigation for crop cultivation without any prior treatment is being widely practiced in Pakistan due to inadequacy of the freshwater supplies (Khan et al. 2019). In many developing countries, including Pakistan, India, and Bangladesh, farmers have begun to irrigate their crops with industrial effluents due to unavailability of any other freshwater source (Hussain et al. 2019; Hussain and Qureshi 2020; Karimi et al. 2020). It is estimated that about 7% of the global area is under sewage water irrigation in more than 50 countries (Iqbal et al. 2019). However, the unreported area is even much more which has not been declared at a global/regional level.

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Due to increasing population, a high volume of sewage water is being generated every day and their treatment services are not available, particularly in developing countries. The annual municipal water withdrawal in Pakistan was 9.65 × 10^9 m^3, in 2008–2012 (FAO 2020). Due to inadequate water resources, farmers rely on the use of sewage water for crop cultivation. In Pakistan, about 99% of sewage water is directly used for crop cultivation and also discharged into different water bodies and only 1% is treated in big cities (Karachi, Lahore, Faisalabad) (WB-SCEA 2006). The use of sewage water is considered highly economical as it reduces the cost of fertilizer, groundwater/freshwater billing, and handling of generated waste (Khalid et al. 2018b; Sarwar et al. 2019). However, soil irrigated with sewage water exhibits a high risk of metal(loid) accumulation and buildup in soil (Ahmad et al. 2019; Chaoua et al. 2019). These effluents from domestic, commercial, and industrial units contain alarming levels of toxic pollutants particularly toxic heavy metal(loid)s (Hejna et al. 2020; Jahany and Rezapour 2020).

Spinach (*Spinacia oleracea* L.) is a quick-maturing, cool-season leafy green vegetable. *Spinacia oleracea* is considered as one of the most nutrient-enriched foods. Owing to higher nutrient contents, heavy metal(loid)s may also adjoin the food chain in a direct proportionate manner. Previously, many of the studies have reported the metal(loid) acquisition in edible parts of the *S. oleracea* plants (Natasha et al. 2019a, 2019b). To study the kinetics of metal(loid) toxicity and human health consequences, *S. oleracea* plant remained under the due consideration of researchers owing to its small growth period and worldwide consumption. The disruption of metabolic and physiological processes in *S. oleracea* plants has been widely reported in the recent years with a special focus on metal(loid) accumulation and its resulting food chain contamination (Rydzyński et al. 2019; Zubair et al. 2019). However, such scenarios are not previously reported in *S. oleracea* plants under sewage water irrigation.

Keeping in view the multi-farious environmental problems of sewage water irrigation, the study was planned to (1) evaluate the heavy metal(loid) contamination of *S. oleracea* plants grown under sewage water, groundwater, and freshwater-irrigated soils; (2) determine the physiological and biochemical alteration in *S. oleracea* plants; and (3) evaluate human exposure risk associated with food chain contamination of heavy metal(loid)s routing from irrigation sources.

Research methodology

Description of the study area

District Vehari is located at 29.9719° N, 72.4258° E and is 443 ft above the mean sea level. About 57 m^3 waste is generated every day with a yearly count of 20,796 m^3 in 2018 which is being used for irrigating agricultural lands (ESSA 2018; PMDFC 2011). Before irrigation, the sewage water of the district Vehari is being collected into 17 sewage water disposal points. Three sewage water disposal points, containing the highest levels of heavy metal(loid)s, were selected for the pot experiments at COMSATS University Islamabad, Vehari campus. These disposal points included People Colony Vehari (30.038294, 72.334675), 69 W.B Mailsi (29.961742, 72.177539), and Lat Bhattian Burewala (30.144423, 72.623732) from three tehsils of Vehari District. Our previous studies showed that these three SW collection and disposal sites in District Vehari contain maximum concentrations of heavy metal(loid)s (Khalid et al. 2017; Sarwar et al. 2019).

Experimental setup and plant growth

The experiment was conducted during spring 2019 in a wire-house under the following conditions: temperature 12–39 °C and humidity 21–100%. Pots were filled with 7 kg of soil collected from the agricultural area of the campus, which had not been irrigated by wastewater previously. *Spinacia oleracea* seeds were sown on February 16, 2019. Ten seeds were sown in each pot. After seedling growth to 5–6 cm, six healthy plants were maintained in each pot and the others were eradicated. All of the eradicated plants were incorporated into the soil. This vegetable was selected as it is widely cultivated in the studied area, have high consumption and nutritional value, has small growth period, and is efficient in absorbing and accumulating metals in the edible leaves.

For the irrigation purpose, sewage water, freshwater, and groundwater were applied in different proportions (Table 1). Plants were irrigated on a daily basis and a total of 11 L of water was used in each pot. Water samples were collected in gallons six times from sewage water disposal points during the whole research period. The gallons were manually shaken every time before irrigation. The plants were sprayed with pesticide (chlorpyrifos) three times to avoid the economic threshold level of pests.

After maturation, *S. oleracea* plants were harvested on April 8, 2019 and separated into roots and shoots. Out of six, three plants were dried and used for metal(loid) analysis. The remaining were dipped in liquid nitrogen and stored at − 30 °C for physiological analysis.

Before sowing and soon after the harvest of the experiment, the soil samples were collected to analyze for residual heavy metal(loid) and nutrient contents due to sewage water irrigation (Supplementary Table 1).

Determination of heavy metal(loid) content

Water samples (50 mL) were acidified (using HNO_3 as pH stabilizer) soon after collection, filtered, and analyzed for

Table 1 Treatment plan for pot experiment

Sr. #	Symbols	Irrigation water source
T1	SW-1	Sewage water-1
T2	SW-2	Sewage water-2
T3	SW-3	Sewage water-3
T4	FW+SW-1	Fresh water + sewage water 1
T5	FW+SW-2	Fresh water + sewage water 2
T6	FW+SW-3	Fresh water + sewage water 3
T7	GW+SW-1	Ground water + sewage water 1
T8	GW+SW-2	Ground water + sewage water 2
T9	GW+SW-3	Ground water + sewage water 3
T10	GW	Ground water
T11	FW	Fresh water
T12	GW+FW	Ground water + fresh water

heavy metal(loid)s using PerkinElmer Atomic Absorption Spectrophotometer (AAS) PinAAcle 900F.

The dried soil and plant samples were crushed and ground in a domestic grinder. The samples were wet digested (HNO_3 and $HClO_4$, 2:1) on a hot plate to achieve clear samples. The samples were filtered, diluted, and analyzed for heavy metal(loid) content on AAS. The heavy metal(loid)s analyzed in this study were As, Cd, Cu, Cr, Fe, Mn, Ni, Pb, and Zn. Arsenic content in the samples was determined using Hydride Vapor Generation technique (HG-AAS).

Physiological analysis

Each plant sample (1 g of fresh weight root/shoot) was extracted with 10 mL of hydro-acetone solution (1:4 v/v) under liquid nitrogen. The supernatant collected after centrifugation at 10,000 ×*g* for 10 min was stored at − 30 °C in a refrigerator.

Pigment contents in the supernatant were determined by recording the mixture's absorbance at 645 and 663 nm wavelength using a spectrophotometer (Lichtenthaler 1987; Nascimento et al. 2019).

The H_2O_2 contents were measured by recording the absorbance at 390 nm using a spectrophotometer. Before absorbance, the mixture was prepared from plant extract (root/shoot), 1 M potassium iodide and 10 mM potassium phosphate buffer (Islam et al. 2008).

Lipid peroxidation assay was carried out by determining the concentration of TBARS (thiobarbituric acid reactive substances). Plant extract was incubated using 20% trichloroacetic acid (0.75 mL) and 0.01% butylhydroxytoluene (0.75 mL) at 95 °C in a water bath. The incubation of samples was performed in the absence and presence of 0.65% thiobarbituric acid. The absorbance of the supernatant was recorded at 532 nm (Hodges et al. 1999).

Exposure risk assessment

The average values of total heavy metal(loid) concentrations in the edible parts (shoot) of *S. oleracea* were used to calculate the estimated daily intake (EDI), hazard quotient (HQ), hazard index (HI), and cancer risks (CR), and were calculated using Eqs. (1), (2), (3), and (4), respectively (Shah et al. 2019)

$$EDI = \frac{C \times IR \times Cf \times ED \times EF}{BW \times AT} \tag{1}$$

$$HQ = \frac{EDI}{RfD} \tag{2}$$

$$HI = \sum_{n=1}^{i} (HQ)n \tag{3}$$

$$CR = EDI \times CSF. \tag{4}$$

Note that all the parameters used in equations for risk assessment are previously described by Sarwar et al. (2019).

Statistical analysis

The experimental design was completely randomized with 12 treatments and six replications. All data were checked for homogeneity of variance and normality. The collected data were evaluated with ANOVA and the means were compared by the Tukey test ($p < 0.05$), using statistical software (Statistix 8.1). A principal component analysis (PCA) using XLSTAT software was applied to all collected data as a single set.

Results and discussion

Concentration of heavy metal(loid)s in irrigation water

All the water samples had high Cd contents (0.001–0.08 mg L^{-1}) (Supplementary Fig. 1). Some water samples had high concentrations of Pb (0.1 mg L^{-1}), Ni (1.49 mg L^{-1}), Fe (5.2 mg L^{-1}), and Cu (1.39 mg L^{-1}). However, concentrations of As (0.0003–0.05 mg L^{-1}), Zn (0.01–2.6 mg L^{-1}), and Mn (0.03–0.2 mg L^{-1}) were low in all irrigation waters. The sewage water samples contained high levels of Cd (0.03 mg L^{-1}), Ni (0.4 mg L^{-1}), and Cu (0.3 mg L^{-1}). The FW and GW were primarily contaminated by Fe (5.2 mg L^{-1}) and Pb (0.1 mg L^{-1}), respectively. From this study, it is evident that sewage water contains less metal(loid) content than FW and GW because water passes through different mediums before being discharged as waste. For example, metals from drinking water are absorbed by the organisms. For example, adults can absorb 35–50% of Pb through drinking water (Tchounwou et al. 2012). Moreover, it is also possible that heavy metal(loid)s may get diluted due to addition of other solutes/solvents (e.g., oils, soaps, kitchen waste) at household levels.

Heavy metal(loid) contamination of soil

Before *S. oleracea* cultivation, the values of As, Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn in soil were 2.7, 3.6, 198, 102, 5912, 535, 83, 50, and 119 mg kg^{-1}, respectively (Supplementary Table 1). Among these, the concentrations of Cd, Cr, Cu, Fe, Mn, and Ni were higher than their permissible limits (FAO 1992; WWF-Pakistan 2007). After *S. oleracea* cultivation, the concentration of As, Pb, and Zn in soil remained lower than their respective permissible limits, while the concentrations of Cd, Cr, Cu, Fe, Mn, and Ni were higher than their permissible limits (Supplementary Table 1).

Overall, the concentration of heavy metal(loid)s decreased in the soil after *S. oleracea* cultivation (Fig. 1). This decrease varied greatly for different heavy metal(loid)s and irrigation treatments. Overall, the maximum decrease was observed for Cd (mean 38% for all the irrigation treatments) followed by Cu (20%), Pb (19%), Ni (16%), Zn (15%), Mn (13%), Fe (9%), Cr (6%), and As (1%). In case of irrigation treatments, the maximum decrease (mean 72% for all the heavy metal(loid)s) was observed for FW+SW-3 followed by SW-2 (23%), FW (19%), GW+SW-3 (18%), and SW-1 (17%).

The possible reason for this decrease in the concentration of heavy metal(loid)s can be their solubilization after irrigation and *S. oleracea* cultivation. Cadmium has comparatively high mobility in soil and can be easily taken up by plants (Shahid et al. 2017). This can be a possible reason for its highest decrease in the soil after *S. oleracea* cultivation. In case of irrigation water types, the variation in composition (physicochemical characteristics such as TDS, pH, EC etc.) may have also affected heavy metal(loid) mobility and phytouptake as the concentration of metal(loid)s in plants increased with reduction in soil pH values (Javed 2011). Generally, the change in the equilibrium of the complex dynamic reactions taking place simultaneously in the soil may affect the soil pH after sewage water application (Khalid et al. 2018b). However, further studies are needed to fully elucidate these speculations.

Heavy metal(loid) accumulation in *S. oleracea* plants

The uptake of heavy metal(loid)s by *S. oleracea* varied for different irrigation treatments. In *S. oleracea* leaves (edible part), heavy metal(loid) concentration was found to be maximum in lone as well as co-application of GW and FW

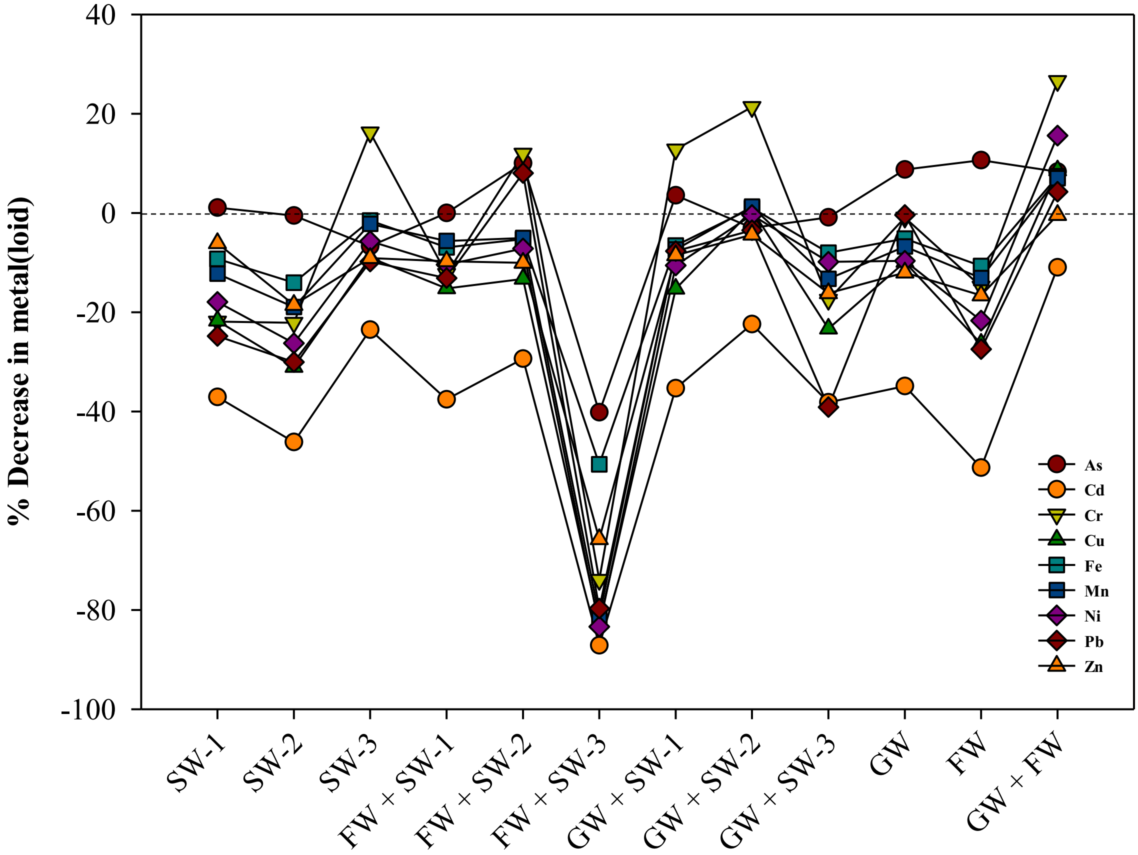

Fig. 1 Percent decrease or increase in soil metal(loid) content after *Spinacia oleracea* cultivation under different irrigation sources

(Fig. 2A, Supplementary Table 2A). The concentrations of As (3.5 mg kg^{-1}), Cd (9.3 mg kg^{-1}), Mn (278 mg kg^{-1}), and Pb (85 mg kg^{-1}) were significantly higher in the leaves of GW irrigated plants than the other treatments. The Cr (2049 mg kg^{-1}), Ni (36 mg kg^{-1}), and Zn (186 mg kg^{-1}) concentrations were higher in plants treated with the combination of FW and GW. The maximum concentration of 697 mg kg^{-1} and 5104 mg kg^{-1} of Cu and Fe was found correspondingly for FW and FW+SW-1 irrigated plant leaves. It was observed that all heavy metal(loid) concentrations were found higher in FW and GW irrigated plants than other treatments. The possible reasons could be the high metal(loid) concentration in FW and GW. Moreover, SW irrigation adds up organic matter in the soil which may immobilize the heavy metal(loid)s in the soil and may result in low uptake of metal(loids)s in SW-treated plants as compared to FW- and GW-irrigated plants (Khalid et al. 2018b; Lu et al. 2005).

In roots of the *S. oleracea* plants, concentrations of Cr (3624 mg kg^{-1}), Cu (209 mg kg^{-1}), Fe (10,941 mg kg^{-1}), Mn (194 mg kg^{-1}), Ni (459 mg kg^{-1}), Pb (315 mg kg^{-1}),

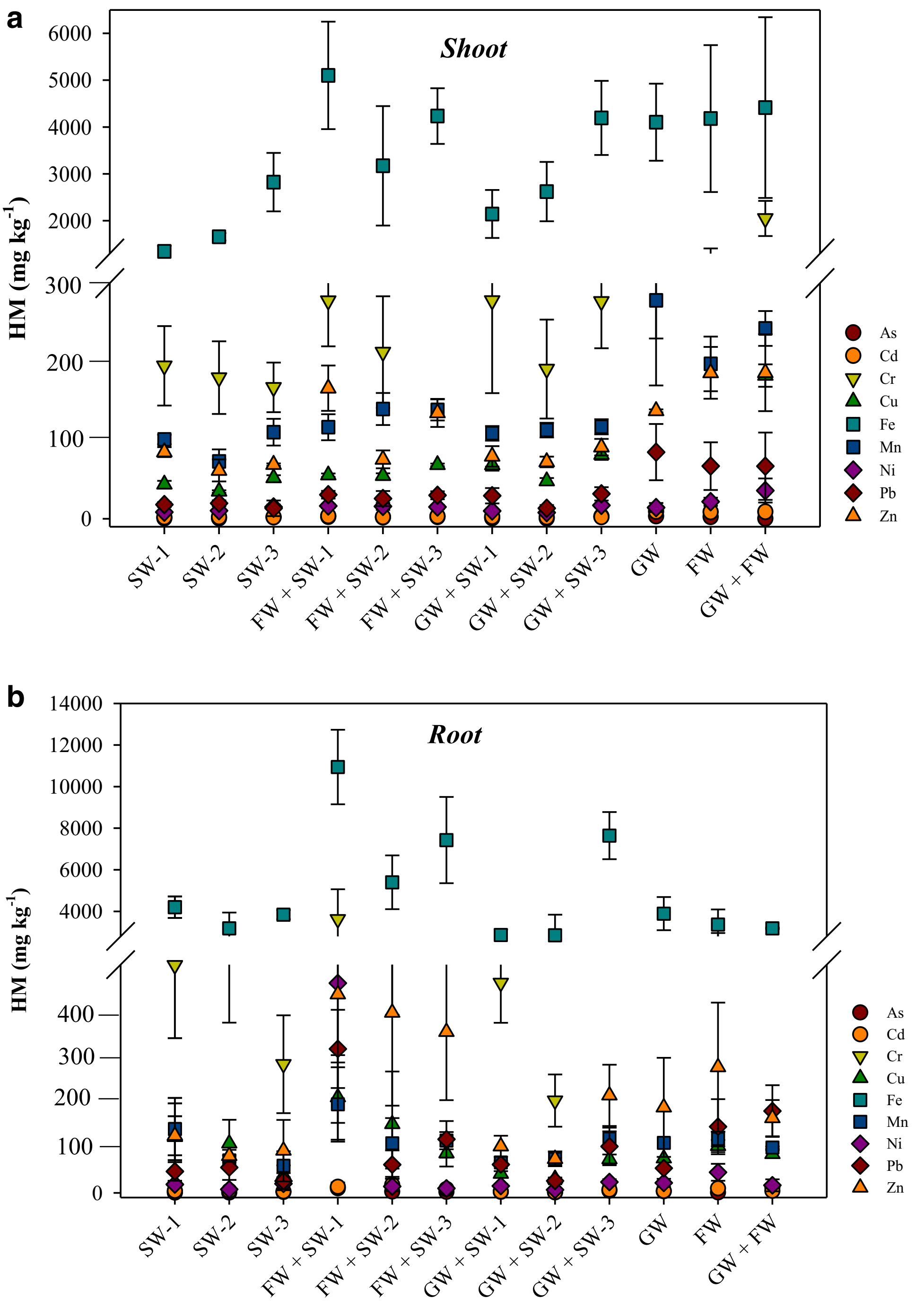

Fig. 2 Heavy metal(loid) accumulation in shoot (2A) and roots (2B) of *S. oleracea* under different irrigation sources. Values are the average of three replicates

and Zn (435 mg kg^{-1}) were found significantly higher in FW+SW-1–irrigated plants compared to other irrigation treatments (Fig. 2B, Supplementary Table 2B). However, FW+GW– and FW+SW-2–irrigated plants showed significantly high concentration of As (12 mg kg^{-1}) and Cd (18 mg kg^{-1}), respectively. This shows that plant roots had taken up varied concentrations of different types of metal(loid)s under different irrigation waters (FW, GW, and SW). This can be due to their varied composition and contamination of irrigation waters.

It was observed that plants irrigated with GW, FW, and their combination had high uptake and accumulation of heavy metal(loid)s as compared to alone SW irrigation. This could be, in all probability, due to the high contamination of GW and FW of the study area. Both GW and FW of the study area contain high levels of heavy metal(loid)s. This corroborates by several previous studies that reported high levels of metal(loid)s in GW of Vehari (Fatima et al. 2018; Khalid et al. 2018a; Shah et al. 2019). Similarly, the FW resources (rivers and canals) of Pakistan originates from Himalaya which have also been reported to contain high levels of heavy metal(loid)s (Muhammad et al. 2010). Moreover, the organic matter in SW bound the heavy metal(loid)s in the soil and reduce the bioavailability of these heavy metal(loid)s and hinder their uptake by plants (Gonzaga et al. 2020).

It was noticed that most of the metal(loid)s were ended up in roots, whereas less were translocated towards shoots. High sequestration in root tissues with the limited root-shoot transfer is a well-known phenomenon for most of the heavy metal(loid)s (Cardwell et al. 2002; Pourrut et al. 2011). Under such conditions, the vegetables serving leaves/shoot as an edible part (such as *S. oleracea*) may show less risk when cultivated under metal(loid) contaminated soil.

Heavy metal(loid)-induced physiological and biochemical alterations in *S. oleracea*

The variations in physiological and biochemical attributes of *S. oleracea* differed greatly for different irrigation waters (Figs. 3A, B and 4). Overall, a clear trend was observed in metal(loid) accumulation and oxidative stress in *S. oleracea*. Plants under FW and GW irrigation showed high H_2O_2 content in leaves and roots compared to SW-irrigated plants (Fig. 3A). This was possibly attributed to high metal(loid) accumulation in FW- and GW-irrigated plants. The production of H_2O_2 was more pronounced in mixed-water irrigated plants as compared to SW-alone irrigated plants, due to high metal(loid) concentration (Fig. 3A). Previously, some studies have elaborated a linear trend in metal(loid) accumulation and H_2O_2 production in *S. oleracea* (Pinto et al. 2017). Moreover, roots showed relatively higher H_2O_2 content as compared to leaves probably due to high metal(loid) accumulation in roots than leaves.

Due to elevated levels of ROS, plants exhibited oxidative stress in terms of peroxidation of lipids. The plants irrigated with FW and GW showed severe membrane deterioration and high TBARS contents (Fig. 3B). The trend in ROS production and TBARS content in *S. oleracea* leaves was almost the same showing high lipid peroxidation in mixed-water treated plants as compared to plants irrigated with SW alone. Such an interaction between ROS production and lipid peroxidation has been reported previously in vegetables (Al Mahmud et al. 2019; Natasha et al. 2020a).

Almost a similar trend was observed in plant pigment contents. Owing to the high metal(loid) accumulation in FW- and GW-irrigated *S. oleracea* plants, the pigment contents were found lowest in the leaves as compared to other plants (Fig. 4). The plants irrigated with SW alone had high chlorophyll contents as compared to their combinations with GW and FW. Decrease in plant pigment contents has been observed in *Typha latifolia* and *Aegilops columnaris* after wastewater irrigation (Noori et al. 2014, Xu et al. 2010). This decrease in pigment contents can be due to heavy metal(loid)–induced oxidative stress through high production of ROS (Natasha et al. 2020b).

Exposure risk assessment

In this study, exposure risk assessment from contaminated *S. oleracea* leaves was evaluated using *EDI*, *HQ*, *CR*, and *HI* (Supplementary Table 3A, B, C, D). It was observed that *EDI* values remained high for Fe (0.53), Cr (0.25), Mn (0.034), and Zn (0.022). The EDI value in plants varies corresponding to the metal(loid) accumulation in leaves. Among all metal(loid)s, the minimum EDI values were observed for As (0.00002). Among treatments, the EDI values remained higher for FW+GW–irrigated plants (e.g., 0.2498 for Cr) (Supplementary Table 3A).

The non-carcinogenic assessment was carried out in terms of HQ (Fig. 5A, Supplementary Table 3B). It was noticed that HQ remained < 1 for all the plants except for As (FW+SW-1 and GW+SW-3), Cd (FW, GW, and FW+GW), and Cu (FW and GW). The highest HQ value of Cu (2.1) was observed in FW-irrigated plants. The HQ value < 1 is considered safe.

The CR values below 0.0001 are considered safe. In this study, slightly high *CR* values were observed for As (0.0006), Cd (0.007), Cr (0.12), and Ni (0.004) (Fig. 5B, Supplementary Table 3C). However, no *CR* was observed in plants by Pb contamination. Among all the treatments, the maximum *CR* was observed for FW+GW–irrigated plants.

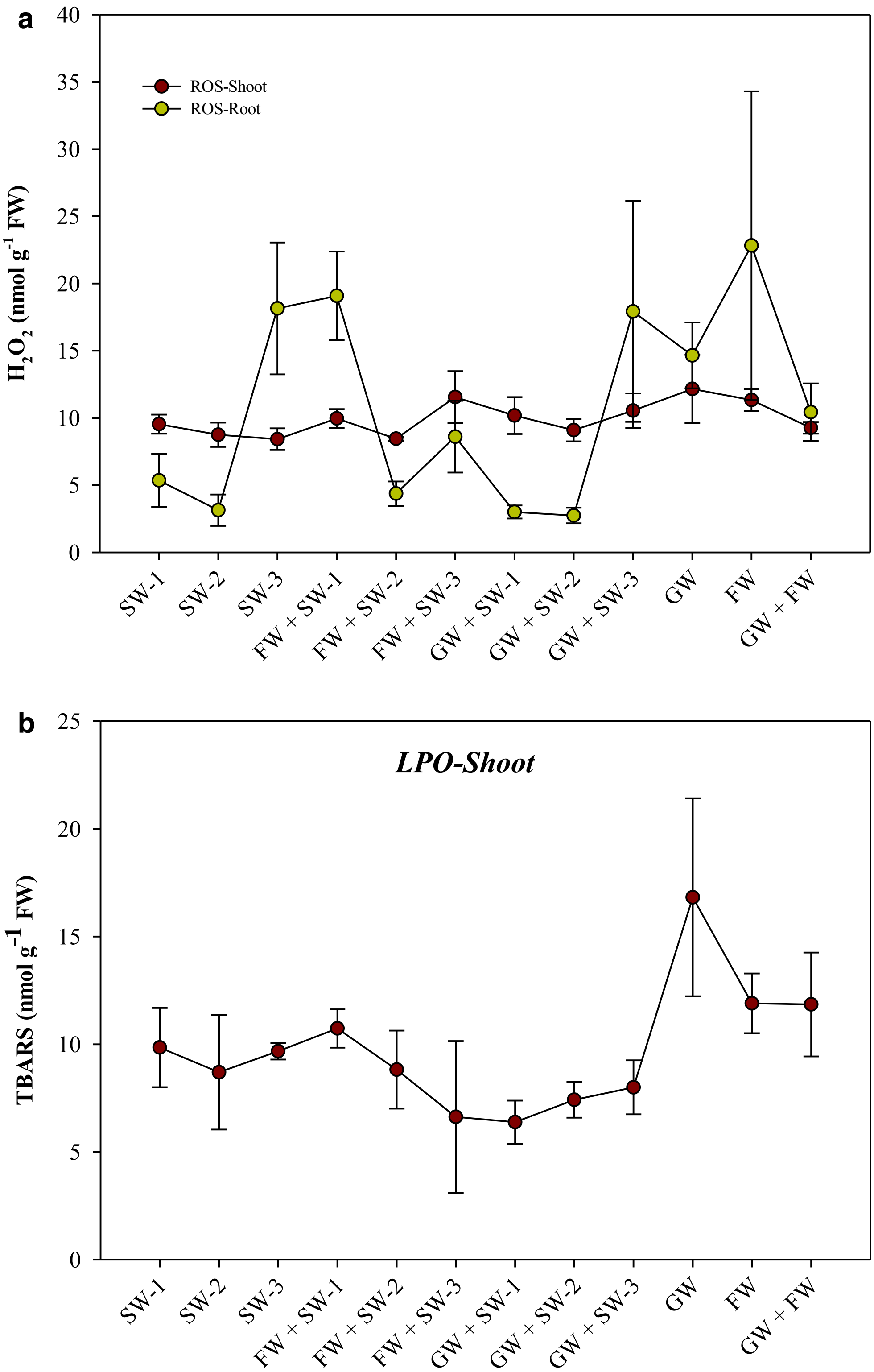

Fig. 3 Influence of heavy metal(loid) buildup on H_2O_2 contents (mol g^{-1} FW) (3A) and TBARS contents (nmol g^{-1} FW) (3B) in roots and leaves of *S. oleracea* under different irrigation sources. Values indicate mean ± S.E. of six replications

The integrated risk, *HI*, is the estimation of total non-carcinogenic risk induced by the accumulation of more than one metal(loid) in the plants. In this study, all of the treatments showed HI > 1 indicating a possible risk of consuming contaminated *S. oleracea* leaves. In the integrated risk, the major contributing metal(loid)s were As, Pb, Cu, Ni, and Zn (Supplementary Table 3D).

From the risk assessment parameters, it is evident that almost all the irrigation waters especially GW and SW were not fully ideal for *S. oleracea* cultivation due to uptake and

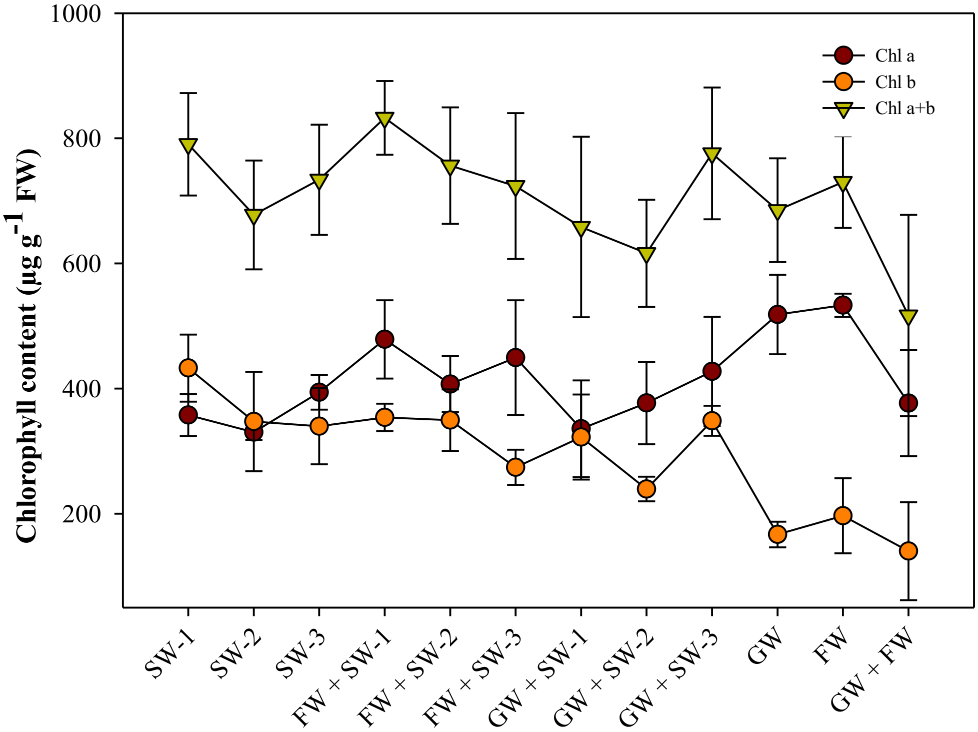

Fig. 4 Changes in pigment content after heavy metal(loid) buildup in *S. oleracea* under different irrigation sources. Values indicate mean ± S.E. of six replications

accumulation of metal(loid)s in edible parts. Therefore, prior treatment of these waters is necessary before *S. oleracea* cultivation.

Multi-variate and principal component analysis

The PCA is used to find the linear combination of a set of variables that have maximum variances (Murtaza et al. 2019). The PCA is considered one of the most popular source apportion modeling methods in environmental analytical chemistry (Slavković et al. 2004). In this study, PCA analysis of all the data of soil and plants individually and the combined PCA of heavy metal(loid)s in soil and plants was carried out. The PCA generally groups different factors/parameters based on their covariance or correlation (Shahid et al. 2018).

The PCA graph of soil showed that despite their varied concentrations in soil w.r.t. treatments, all the heavy metal(loid)s were grouped together in the PCA graph

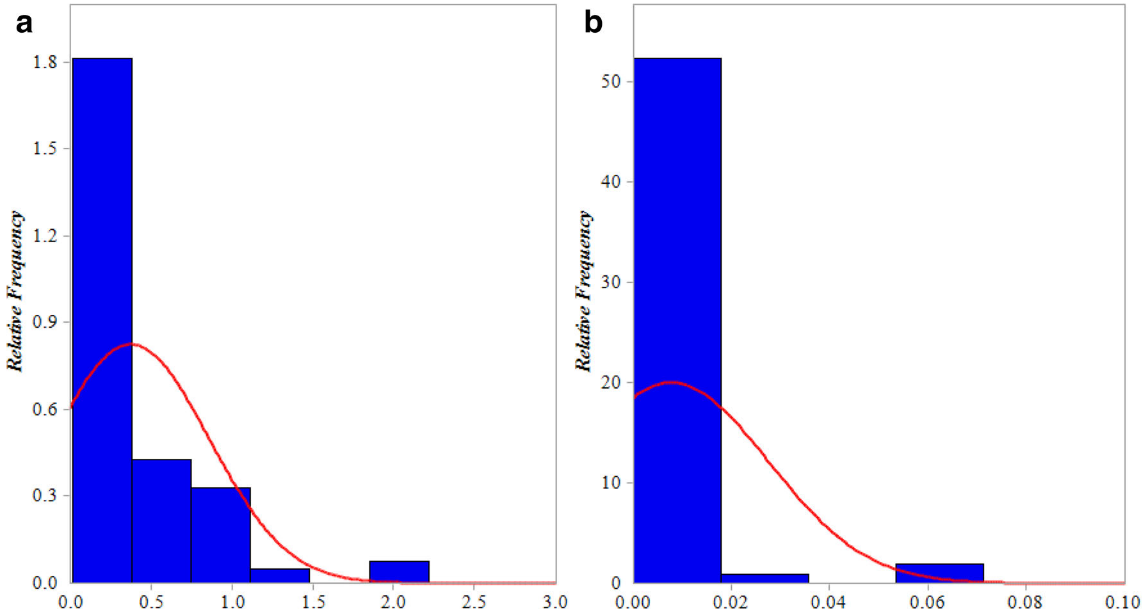

Fig. 5 Hazard quotient (5A) and cancer risk (5B) of trace elements for *S. oleracea* under different irrigation sources

(Supplementary Fig. 2A). This shows possible covariance in their concentrations in soil. Moreover, all the physicochemical parameters were grouped together. Similar grouping of metal(loid)s and other soil parameters were also observed by Shehzad et al. (2019) in the contaminated soils of Rawalpindi.

In the PCA graph of plants, it was noticed that most of the heavy metal(loid)s in the shoot were grouped together along with the physiological parameters (ROS, LPO, pigment contents) (Supplementary Fig. 2B). Likewise, the metal(loid) concentrations in root and shoot were also grouped accordingly on the left side of the graph. This indicated the ability of plants to uptake and transport/sequester the metal(loid)s in different plant parts and their possible impact on physiological attributes of plants. Such grouping of metal(loid)s along with stress indicators were also observed by Natasha et al. (2020b) when cultivating spinach plants under As and Pb contamination.

The combined PCA graph for soil and plant showed that the heavy metal(loid) concentrations in soil were grouped together (Fig. 6). Likewise, the metal(loid) concentrations in the root and shoot were grouped accordingly. This showed that the buildup of heavy metal(loid)s in soil and their accumulation in plants varied from each other. The correlation of heavy metal(loid)s in soil and *S. oleracea* shoot showed that there was no linear correlation of heavy metal(loid) transfer from soil to plants (Supplementary Fig. 3).

Conclusions

Results of the present study revealed that irrigation with sewage water, groundwater, and fresh water increased metal(loid) phytouptake and thereby decreased their concentration in soil. Sole and/or integrated irrigation of groundwater and freshwater resulted in relatively higher metal(loid) accumulation in plants as compared to the sewage water. All these treatments lead to severe toxicity in terms of chlorophyll degradation, the formation of peroxides, and membrane deterioration under metal(loid)-stressed conditions. Furthermore, the health risk indices showed severe carcinogenic risk (*CR*) for metal(loid)-contaminated *S. oleracea* leaves under all irrigation water sources. The non-carcinogenic risk (*HQ*) evaluated for single metal was under the safe limits, however, the integration of metal(loid)s showed possible non-carcinogenic risk (*HI*) from the intake of multi-metal(loid)–contaminated *S. oleracea* leaves. The plants receiving FW and GW alone or in combination with SW showed high exposure risk, possibly due to high metal(loid) contents. This pilot study shows

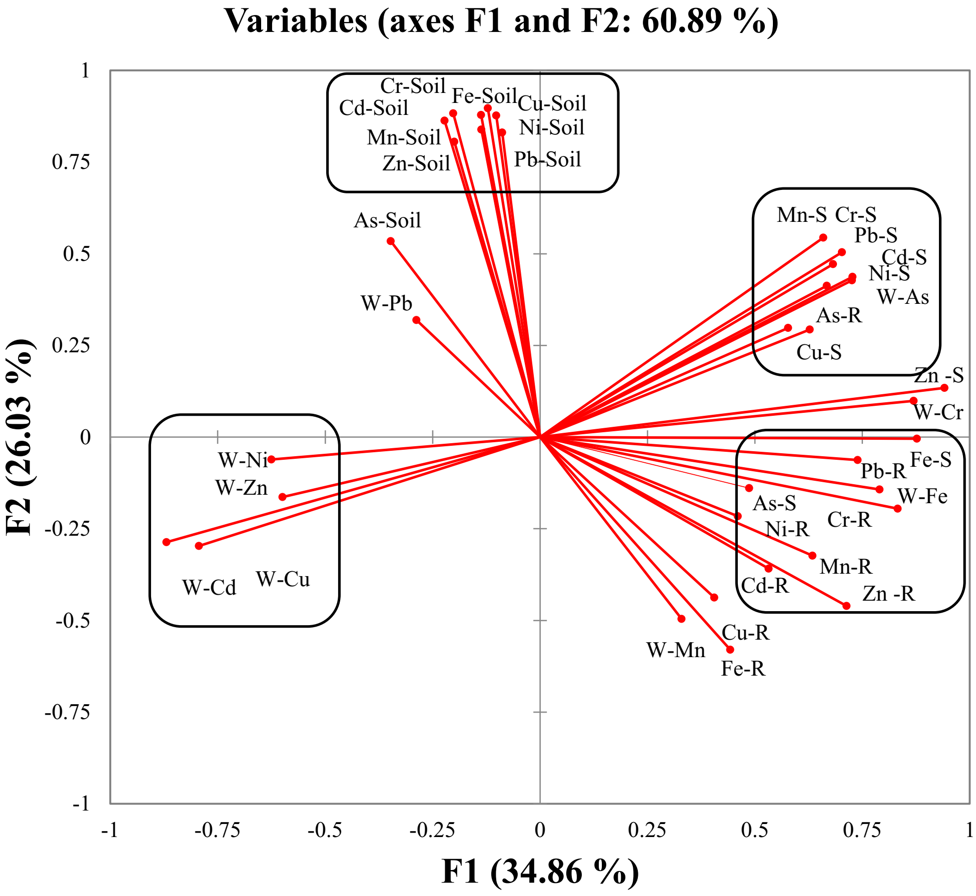

Fig. 6 Comparison of heavy metal(loid) content in irrigation water, soil, and plant tissues using PCA

a dire need to monitor irrigation water quality for food crop to minimize the human exposure risk.

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