Trace Metal Accumulation in *Trigonella foenum-graecum* Irrigated with Wastewater and Human Health Risk of Metal Access Through the Consumption

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Abstract

The aim of the present research was to determine the trace metal accumulations in *Trigonella foenum-graecum* irrigated with three different water regimes (ground water, canal water and sugar mill water). Also, transfer factors, pollution load indices, and health risk indices were assessed to evaluate metal transport and accumulation through the food chain. The analysis was conducted by Atomic Absorption Spectrophotometer (Shimadzu model AA-6300) to evaluate the concentration of metals in water, soil and vegetables. Trace metal concentrations in water samples ranged from 0.84 to 1.67, 0.42 to 0.72, 0.45 to 0.85, 2.51 to 9.99, 1.21 to 1.92, 1.82 to 9.98 and 0.64 to 0.91 mg/kg for Cd, Cr, Cu, Fe, Ni, Zn and Mn, respectively. The mean metal concentrations in soil samples were determined as 0.25, 0.86, 0.96, 3.37, 0.4, 0.44 and 2.31 mg/kg for Cd, Cr, Cu, Fe, Ni, Zn and Mn, respectively. Trace metal accumulations in *T. foenum-graecum* samples gathered from where soil samples were taken are as follows: The contents of Cd, Cr, Cu, Fe, Ni, Zn and Mn ranged from 0.48 to 1.06, 0.11 to 0.35, 0.15 to 0.29, 1.43 to 8.39, 0.39 to 0.54, 2.1 to 3.05 and 0.42 to 0.47 mg/kg, respectively. Statistical analyses showed that the treatments have non-significant effect ($p > 0.05$) on concentrations of metals in *T. foenum-graecum* samples collected from three sites for Ni, Cr, Cu, Zn and Mn and significant effect on Fe and Cd.

Keywords Trace metal · Vegetable · Wastewater · Health risk · Biomonitoring

Trace metals are natural and non-biodegradable ingredients in the outer layer of earth, and they cannot be besmirched or damaged (Ugulu 2015a). They have long-term residual effects on various food products which require immediate remedial strategies (Liu et al. 2005; Khan et al. 2019a). In biological systems, these metals react with the proteins and stop the indispensable reactions (Biddle 1982; Ugulu et al. 2012). Toxic metals and metalloids cause serious problems including carcinogenesis. Thus, increasing the level of awareness of the health risks associated with the environmental chemicals related to the intake of trace metals in vegetables is one of the most important topics of recent times (Dogan et al. 2010; Ahmad et al. 2019). The concentration of

metals and metalloids in vegetables depends on soil texture as well as nature and type of plants (Dogan et al. 2014a; Unver et al. 2015).

Anthropogenic activities disturb the soil structure and contents through pollutants released from the environment into the soil (Ugulu 2015b, c; Yorek et al. 2016). About 25 cm depth of soil is severely affected by these toxic compounds and trace metals. Trace metal accumulation in the soil as well as in crop plants is mainly due to irrigation of crops with wastewater (Ahmad et al. 2018). Unnecessary deposition of trace metals in soil due to wastewater irrigation not only cause soil pollution but also disturb the safety and nutritional value of food (Ugulu et al. 2016; Khan et al. 2018a). Uptake of trace metals occurs in vegetables and these metals accumulate in their different eatable and inedible parts. The high quantity of these toxic compounds causes health problems in both human beings and animals (Muchuweti et al. 2006; Durkan et al. 2011; Dogan et al. 2014b).

Vegetables are not only a valuable source of nutrients but also provides vital food components such as vitamins,

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proteins, carbohydrates and calcium which have a prominent impact on human health. Leafy vegetables such as fenugreek, which grows in polluted soils, store a significant amount of trace metals compared to those that grow in unpolluted soils. The reason is that they absorb trace metals through their roots (Muhammad et al. 2008; Ugulu et al. 2009a). Different parts of vegetables accumulate a large amount of trace metals from the polluted land in which these vegetables are grown; therefore, these toxic metals can produce public health complications (Khan et al. 2018b, c).

Wastewater irrigation has a significant effect on metal uptake by vegetables and *Trigonella* species are among these vegetables in Pakistan (Muchuweti et al. 2006; Nadeem et al. 2019). *Trigonella foenum-graecum*, from the family Fabaceae, is extensively cultivated in most regions of the world. *T. foenum-graecum* (fenugreek) is known to have several pharmacological effects such as hypoglycemia, hypocholesterolemia, antioxidation, laxation, fungicide and appetite stimulation (Ugulu et al. 2009b; Ugulu and Baslar 2010; Dogan and Ugulu 2013). There are several reports concerning the anti-inflammatory, antipyretic and antinociceptive effects of the plant *T. foenum-graecum*. This plant is also known to contain alkaloids, flavonoids, salicylate, and nicotinic acid (Ahmadiani et al. 2001; Ugulu 2012).

The present study was performed to evaluate accumulation and translocation of trace metals in water, soil, and vegetables, to determine the health risk index by leafy vegetables irrigated with sugar mill water and to assess the health hazard effects caused by trace metals in fenugreek samples.

Materials and Methods

This study was conducted under field conditions in Khushab District, Punjab Province, Pakistan. The climate of Khushab is extremely hot in summer and moderate cold in winter. The maximum temperature in summer is about 50°C and the minimum temperature recorded in winter is 12°C.

Seeds of *Trigonella foenum-graecum* were grown at the end of October 2016 in 60 small plastic pots. Four replicates, 15 pots were set in each replication about 2.5 kg of soil was put in each pot. Ten seeds of vegetable were sown in each plastic pot. Large volumes of ground water, canal water and sugar mill water samples were collected from the sources and 1 L of the water samples were applied in experimental pots for irrigation purpose. Pots were irrigated twice a week. Mill water was obtained from Kooh e Noor Sugar Mill, Khushab. Different treatments used for this experiment were: T-I: Ground water irrigation (GWI), T-II: Canal water irrigation (CWI), T-III: Mill water irrigation (MWI). After maturation of vegetables four plants were left in each pot other were eradicated. Urea fertilizer were applied @ 210 kg/ha to each pot for the better development

of vegetables. Soil samples were taken out from each pot with auger at the depth of 5 cm. Vegetable leaves were harvested at the end of April 2017. The vegetable samples were dried in open environment and ground in a domestic grinder. Fine powder of vegetable samples was kept in the oven for 3 days at 75°C temperature. After the samples were completely dried, they removed from the oven and digested by "Wet Digestion Method".

The analysis was conducted by Atomic Absorption Spectrophotometer (Shimadzu model AA-6300) to evaluate the concentration of metals in the water, soil and vegetable samples. The trace metals investigated in this study were Cd, Cu, Cr, Fe, Zn, Ni and Mn.

To avoid any infectivity that may affect laboratory performance, appropriately clean equipment was used. Acid and other chemicals required for digestion and spectrophotometry were bought from a superior company to give surety for correctness and precision. Standards were prepared carefully. Measurement of soil and vegetable samples was done on the basis of dry weight. Analyses were performed three times for each sample. Quality control procedures were strictly followed for the entire sample to ensure the quality of results. Analytical grade calibration standards were used for instrument calibration, purchased from Merck (Germany). Precision and accuracy of results was assured through repetitive samples against National Institute of Standard Technology, Standard Reference Material (NIST-SRM, 1570a for leaves and SRM 2709 for soil). The mean recoveries of the SRM for soil were 105%, 96%, 97%, 92%, 95%, 99% and 90%, for Cd, Cu, Cr, Fe, Zn, Ni and Mn, correspondingly, and the mean recoveries of SRM for vegetable of these metals were 98%, 90%, 94%, 109%, 92%, 97% and 89% correspondingly.

Correlations and variance of data were calculated by SPSS (Statistical Package for Social Sciences). The variance of vegetables and soil was obtained by applying one-way ANOVA. Correlations for vegetables and soil were calculated. Mean significance was at 0.05, 0.001 and 0.01 probability levels given by Steel and Torrie (1980).

Bioconcentration factor was calculated as the ratio of the concentration of metal in plants (on dry weight basis). The following formula was used to evaluate the uptake of trace metals from soil and their bioaccumulation in the vegetable:

$$\text{Bioconcentration factor} = \frac{C_{veg}}{C_{soil}}$$

where C_{veg} is the metal concentrations in plant tissue, mg/kg fresh weight and C_{soil} is the metal concentrations in soil, mg/kg dry weight (Cui et al. 2004).

Specific methods are taken into consideration for finding out consumer-based health risks. One of them is the daily intake of metals.

Daily intake of metals $= C_{metal} \times C_{food\ intake}/B_{average\ weight}$

where C_{metal} represents the concentration of metals ingrains, $C_{food\ intake}$ represents the daily food intake and $B_{average\ weight}$ represents average body weight. The daily food intake was taken as 0.345 mg/kg and the average body weight is taken as 60 kg.

Health risk index (HRI) was measured to find the overall risk of exposure to all trace metals via ingestion of particular food crops. This shows the maltreatment of people which consume contaminated food stuff. It is defined as the ratio of daily intake of metals in food crops to the oral reference dose (USEPA 2002).

$$HRI = \frac{\text{Daily intake of metals}}{\text{Oral reference dose}}$$

An HRI < 1 for any metal in vegetable means that consumer population faces a serious risk of health. However, HRI > 1 does indicate a considerable health risk to the organisms consuming these vegetables (Khan et al. 2019b).

Based on the concentration factor of each metal in the soil, pollution load index (PLI) gives us an estimation of the metal contamination status and the necessary action that should be taken. PLI was estimated using the following equation as given by Liu et al. (2005) for each treatment:

$$PLI = \frac{\text{Determined level of metal in examined soil}}{\text{Reference value of soil metal}}.$$

The background values (mg/kg) of Cd (1.49), Cr (9.07), Cu (8.39), Ni (9.06), Zn (44.19), and Mn (46.75) were taken according to Singh et al. (2010) and Fe (56.90) was taken according to Dosumu et al. (2005).

Results and Discussions

In the present study, trace metal concentrations in water samples ranged from 0.84 to 1.67, 0.42 to 0.72, 0.45 to 0.85, 2.51 to 9.99, 1.21 to 1.92, 1.82 to 9.98 and 0.64 to 0.91 mg/kg for Cd, Cr, Cu, Fe, Ni, Zn and Mn, respectively. Among the three treatments, the mean concentrations of Fe and Zn were higher than other metal accumulations for all treatments (Fig. 1). The results from the variance of the data showed that the treatments have a non-significant effect ($p > 0.05$) on the concentrations of metals in the water samples for Cr, Cd, Cu, Ni and Mn while the significant effect on Fe and Zn in the soil of *T. foenum-graecum* (Table 1).

Maximum permissible limits of the Cd, Cr, Cu, Fe, Ni, Zn and Mn in water were reported as 0.01, 0.5, 0.2, 5, 0.2, 2 and 0.2 mg/L, respectively by WHO, FAO, Standard Guidelines in Europe (Chiroma et al. 2014) and USEPA (2002). Except for Mn, heavy metal accumulation values in the present study were higher than these permissible maximum limits in the water. According to these results, it can be mentioned that there is water pollution in the study area. High levels of metals observed in the

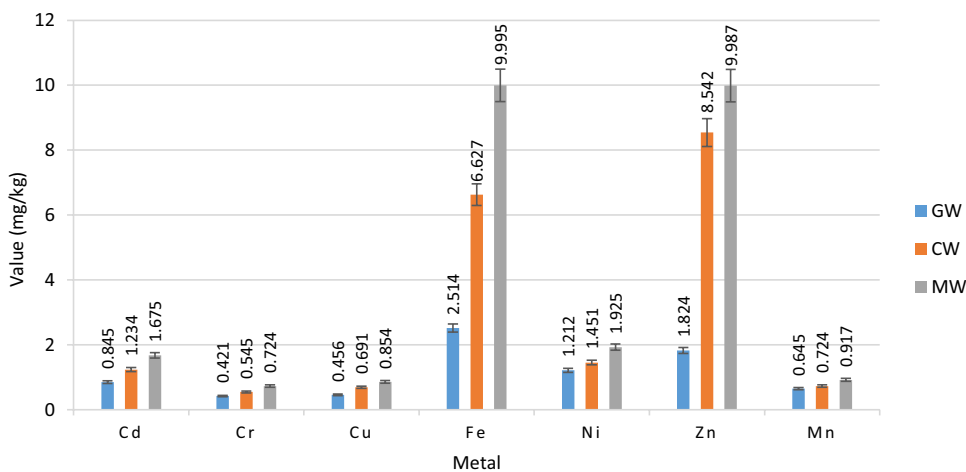**Fig. 1** Trace metal concentrations in irrigation water samples (n = 60)

Table 1 Analysis of variance for trace metals in irrigation water of fenugreek

Source of variation	Degree of freedom	Mean squares						
		Cd	Cr	Cu	Fe	Ni	Zn	Mn
Treatments	4	.215[ns]	.002[ns]	.364[ns]	6.189[*]	.750[ns]	.006[***]	.186[ns]
Error	10	.052	.001	.074	.813	.307	.001	.039

ns Non-significant

*, ***Significant at 0.05 and 0.001 levels

present study could be due to various sources such as soil erosion, urban run offs and aerosol particles. However, non–significant differences were found for mean metal concentrations among the samples of groundwater, canal water and municipal water except for Fe and Zn (Table 1). Khan et al. (2018d) reported the trace metal values in ground water, canal water and industrial water samples collected from Khushab as 1.69–1.76–1.88 mg/L for Cd, 0.54–0.57–0.65 mg/L for Cr, 0.01–0.02–0.03 mg/L for Cu, 0.64–0.72–0.83 mg/L for Fe, 0.08–0.10–0.14 mg/L for Ni, 0.57–0.61–0.66 mg/L for Zn and 0.07–0.08–0.12 mg/L for Mn, respectively. They also stated that concentrations of these metals in water samples were higher than the pre-scribed maximum permissible limits by WWF (2007).

The mean trace metal concentrations in soil samples were determined as 0.25, 0.86, 0.96, 3.37, 0.4, 0.44 and 2.31 mg/kg for Cd, Cr, Cu, Fe, Ni, Zn and Mn, respectively. Among the three treatments, the mean concentrations of Fe and Zn were higher than other metal accumulations for all treatments (Fig. 2). At treatment-I, treatment-II and treatment III, the mean concentrations of metals in soil was in the order: Zn > Fe > Cd > Cu > Mn > Ni > Cr, Zn > Fe > Cd > Mn > Ni > Cr > Cu and Fe > Zn > Cd > Ni > Mn > Cu > Cr, respectively. The results from the variance of the data showed that the treatments have a non-significant effect ($p > 0.05$) on the concentrations of metals in the soil which was used to grow vegetables for Cr, Cd, Cu, Zn and Mn while the significant effect on Fe and Ni in the soil of *T. foenum-graecum* (Table 2).

Maximum permissible limits of the Cd, Cr, Cu, Fe, Ni, Zn and Mn accumulation in soil were reported as 3, 100, 50, 21,000, 50, 200 and 2000 mg/kg, respectively by USEPA (2002). All metal concentrations studied in the present research remained below these limits under all treatment conditions. Despite the high trace metal content of water in the study area, as stated in the present study, all metal concentrations in soil samples were lower than the maximum permissible limits reported by USEPA (2002). This result may be due to periodic differences in water samples as well as the metal uptake of plant samples. Constant uptake of trace metals by crops and infiltration into deeper soil layers could be possible explanations for the relatively low levels of these metals in the soils even at wastewater–irrigated sites as the geological composition of soils affect metal concentrations in agricultural lands (Singh et al. 2010).

When the results of the present study compared to the other similar studies, it was seen that Manzoor et al. (2004) analysed the concentrations of Cr, Fe, Cd, Ni, Zn in the soil irrigated with water having effluents of Hattar Industrial Region and found the mean concentrations of 0.370, 1.082, 0.017, 0.180, and 0.055 mg/kg, respectively. In the present study, the concentrations of these metals were lower. The reason for these results may be the type and number of industrial elements in the study area. Gupta et al. (2013) revealed that trace metal concentrations had a variety depending on the nature of the industry.

Trace metal accumulations in *T. foenum-graecum* samples gathered from where soil samples were taken are as follows:

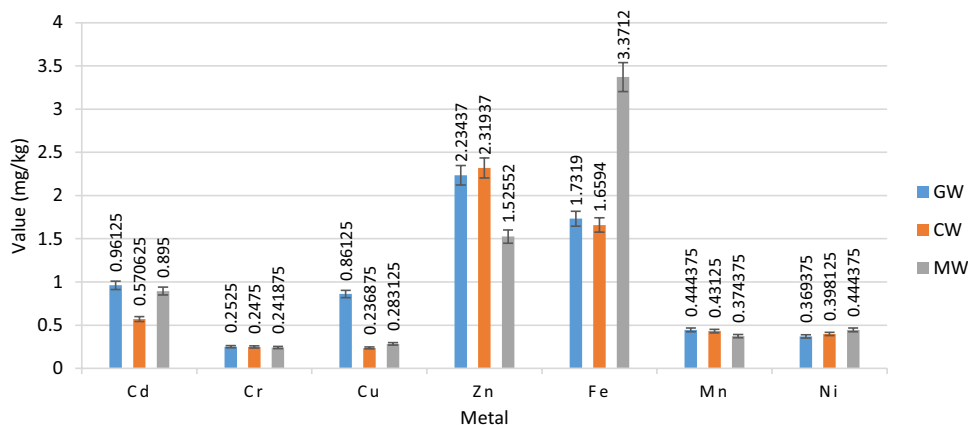**Fig. 2** Trace metal concentrations in soil samples (n = 60)

Table 2 Analysis of variance for trace metals in soil of fenugreek

Source of variation	Degree of freedom	Mean squares						
		Cd	Cr	Cu	Fe	Ni	Zn	Mn
Treatments	4	.175[ns]	.001[ns]	.484[ns]	3.749[*]	.006[***]	.760[ns]	.006[ns]
Error	10	.262	.001	.214	.813	.001	.857	.002

ns Non-significant

*, ***Significant at 0.05 and 0.001 levels

The contents of Cd, Cr, Cu, Fe, Ni, Zn and Mn ranged from 0.35 to 0.44, 0.32 to 0.89, 0.09 to 0.73, 1.93 to 3.02, 1.11 to 1.82, 0.36 to 0.43 and 0.37 to 0.40 mg/kg, respectively. In all treatments, the mean concentrations of metals in *T. foenum-graecum* were in the order: Fe > Zn > Cd > Mn > Ni > Cr > Cu (Fig. 3). Among the three treatments, the mean concentrations of Fe and Zn were higher than the other metals and mean concentrations of Cr and Cu were less (Fig. 2). The results from the variance of the data showed that the treatments have a non-significant effect ($p > 0.05$) on concentrations of metals in *T. foenum-graecum* samples collected from three sites for Ni, Cr, Cu, Zn and Mn and significant effect on Fe and Cd (Table 3).

Maximum permissible limits of the Cd, Cr, Cu, Fe, Ni, Zn and Mn accumulation in plants were reported as 0.1, 5, 73, 425, 67, 100 and 500 mg/kg, respectively by WHO (1996), FAO/WHO (2001), Standard Guidelines in Europe (Chiroma et al. 2014). The range values of metal accumulation in *T. foenum-graecum* samples in the present study were lower than the maximum permissible limits in plant samples except for Cd. Fytianos et al. (2001) found that leafy vegetables absorb more Cd contents as it is stored in leaf and vegetables grown in the industrial region have more Cd. The Cr, Ni and Cd values in the present study were lesser than the values reported by Parveen et al. (2012) as 0.8, 0.4 and 0.4 mg/kg, respectively. This result may be due to their vegetables were cultivated in sewage water. The range values

of different trace metals in vegetables determined by Arora et al. (2008) were 116–378, 12–69, 5.2–16.8 and 22–46 mg/kg for Fe, Mn, Cu and Zn, respectively. According to the results presented by Latif et al. (2008), all vegetables which were grown in sewage water irrigated areas showed high concentrations of Cr, Cd and Ni.

Bioconcentration factor (BCF) was used to assess the uptake of trace metals from soil and their bioaccumulation in vegetables. In the present study, values of BCF for Fe and Zn were the highest in sugar mill water irrigated leafy vegetables. In treatment-I, BCF values for Fe and Zn was higher than Cd and Cu. In treatment-II, BCF values for Zn and Fe was higher than Cr and Cu. In treatment-III, BCF values of Fe and Zn were higher than the values of Cr and Cd (Table 4). The order of BCF values for treatment I: Fe > Zn > Cr > Ni > Mn > Cd > Cu, treatment II: Fe > Zn > Mn > Ni > Cd > Cu > Cr and treatment III: Fe > Zn > Mn > Ni > Cu > Cr > Cd. According to Okereke et al. (2016), green leafy vegetables had the highest accumulation factor for Cr (0.14), Cu (1.07) and Ni (0.38). However, BCF values presented in this study for Cr (1.10), Cu (0.4-1.3) and Ni (0.9) higher than the values of BCF noted by Okereke et al. (2016). A higher value of BCF of metal shows that the potential of a metal to store in vegetables is maximum (Cui et al. 2004).

Analysis of all values of daily intake of metal (DIM) exposed that DIM values for Fe and Zn were higher and Cr value was the lowest value in all treatments. The order

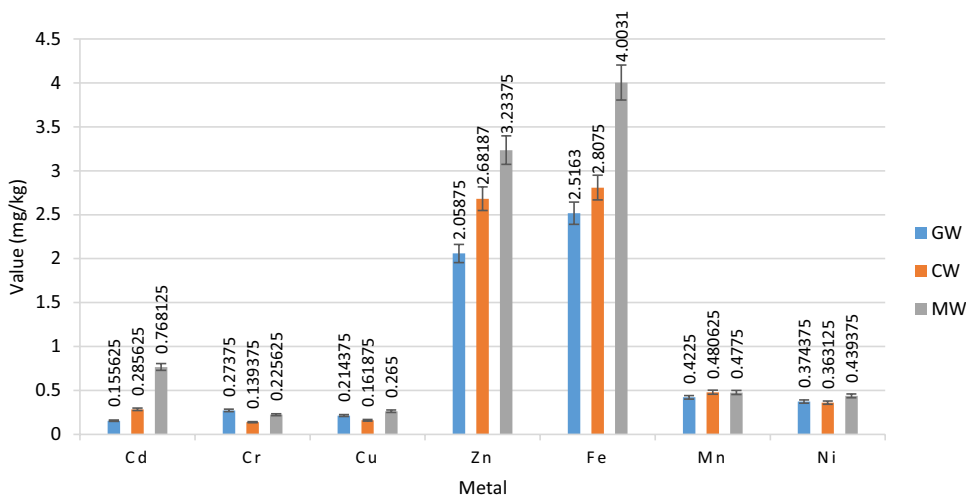**Fig. 3** Trace metal concentrations in fenugreek samples (n = 60)

Table 3 Analysis of variance for trace metals in fenugreek

Source of variation	Degree of freedom	Mean squares						
		Cd	Cr	Cu	Fe	Ni	Zn	Mn
Treatments	4	.087*	.019[ns]	.011[ns]	790.08***	.007[ns]	.682[ns]	.004[ns]
Error	10	.016	.019	.004	24.335	.003	.712	.007

ns Non-significant

*, ***Significant at 0.05 and 0.001 levels

Table 4 Bioconcentration factor, daily intake of metal, pollution load index and health risk index values for fenugreek

Irrigation		Trace metal						
		Cd	Cr	Cu	Fe	Ni	Zn	Mn
BCF	GWI	0.578	1.084	0.2489	1.452	1.0135	1.368	0.900
	CWI	0.8510	0.563	0.683	1.691	0.912	1.1562	1.0617
	MWI	0.858	0.932	0.935	8.009	0.988	1.464	1.365
DIM	GWI	0.00319	0.00157	0.00123	0.01446	0.00215	0.01758	0.00242
	CWI	0.00279	0.00080	0.00093	0.01614	0.00208	0.01542	0.00276
	MWI	0.00441	0.00129	0.00152	0.15526	0.00252	0.01284	0.00274
PLI	GWI	0.6451	0.0278	0.1026	0.0304	0.0407	0.050	0.0095
	CWI	0.3829	0.0272	0.0282	0.0291	0.043	0.052	0.0092
	MWI	0.600	0.0266	0.033	0.0592	0.049	0.0345	0.008
HRI	GWI	3.1948	0.0010	0.0308	0.02066	0.10763	0.05862	0.05925
	CWI	2.7923	0.00053	0.02326	0.02306	0.10439	0.05140	0.06740
	MWI	4.41671	0.00086	0.03809	0.22181	0.12632	0.04281	0.06696

of DIM values for treatment I, treatment II and treatment III were: Zn > Fe > Cd > Mn > Ni > Cu > Cr, Fe > Zn > Cd > Mn > Ni > Cu > Cr and Fe > Zn > Cd > Mn > Ni > Cu > Cr, respectively (Table 4). DIM values for all metals were below the permissible limit given by WHO (1996). DIM value for Fe was (0.006–0.012) lesser than 0.329 mg/kg per day, given by Santos et al. (2004) and 0.248 mg/kg per day, reported by Biego et al. (1998). On the other hand, the highest DIM value in the present study was observed for Fe. Similar to this result, Charan et al. (2014) explained that DIM value of Fe in vegetables grown in wastewater was higher. Ramteke and Gogate (2016) reported the DIM values of Fe, Cr, Mn, Cu, Zn and Cd for vegetables as 82–958, 0.4–7.2, 7–287, 11–26, 10–50, 0.11–0.67 mg/g/day/person, respectively. These values were higher than the present findings.

The pollution load index (PLI) in *T. foenum-graecum* grown with three diverse irrigations was in the following arrangement. The orders of the PLI values in treatment-I, treatment-II and treatment-III were Cd > Cu > Zn > Ni > Fe > Cr > Mn, Cd > Zn > Ni > Fe > Cu > Cr > Mn and Cd > Fe > Ni > Zn > Cu > Cr > Mn, respectively (Table 4). The maximum PLI was observed for Cd and the minimum PLI was observed for Mn at all treatments. Ashfaq et al. (2015) reported the PLI values of Cd, Fe, Mn and Cr as 0.4, 0.01–0.03, 0.01–0.009 and 0.01–0.02, respectively. Also, Ramteke and Gogate (2016) reported the metal pollution index (MPI) for Fe, Cr, Mn, Cu, Zn and Cd as 4.8, 3.1, 11.0, 15.0, 16.2 and 11.0, respectively. These values are higher than the values presented in this research and the reason for this may be various industrial sources of metals, mining, and other geochemical mechanisms (Shardendu et al. 2003). Harikumar et al. (2009) suggested that if the value of PLI is greater than 1 then food is contaminated; if less than 1 then it is not contaminated. In the present study, PLI values for all metals were lesser than 1, it means that these vegetables can be consumed.

According to the analysis results, health risk index (HRI) of Cd was higher than 1 in each treatment which was above the permissible limit. However, HRI value for Cr was the minimum in all treatments. The orders of the HRI values in treatment-I, treatment-II and treatment-III were Cd > Ni > Mn > Zn > Cu > Fe > Cr, Cd > Ni > Mn > Zn > Cu > Fe > Cr and Cd > Fe > Ni > Mn > Zn > Cu > Cr, respectively (Table 4). HRI values for Cd (2.04–2.54) and Cr (0.001–0.0006) in the study of Ashfaq et al. (2015) were higher than the values presented in this study. Likuku and Obuseng (2015) reported the HRI for Cd, Cr, Cu, Ni, and Zn as 1.4, 32.5, 4.2, 1.5 and 1.7 respectively and HRI values of all studied metal were higher than 1 which was opposite to the present study. In the current study, all values were lesser than 1 except for Cd (ATSDR 1993).

The results revealed the positive non-significant correlation of Cd, Cr, Cu, Ni and Zn and negative non-significant correlation of Mn. Fe showed the significant result (Table 5). The positive relationship shows shared presence and advancement of the trace metals in soil, although negative relationship demonstrates their competition to involve the

Table 5 Metal correlation between soil-vegetable

Correlation	
Metals	Soil-vegetable
Cd	.568
Cr	.321
Cu	.077
Fe	.999*
Ni	.864
Zn	.841
Mn	− .608

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

same destinations in soil trade base or cross-section (Khan et al. 2018a, b, c, d).

Pollution by trace metals released from industrial effluent is one of the major challenging issues in many countries. In this direction, Cd, Cr, Cu, Fe, Ni, Zn and Mn accumulation values in *T. foenum-graecum* samples irrigated with sugar mill water were investigated in the present study. The range values of metal accumulation in plant samples in the present study were lower than the maximum permissible limits except for Cd. Bioconcentration factor for Fe and Zn were the highest for sugar mill water irrigated leafy vegetables. Concentrations of metals in vegetables depend upon many factors such as distance from pollution sources, and the quality of irrigation water. On the other hand, the accumulation of metals in plants depends on different factors, including genetic specialties in addition to characteristics of metals and surfaces of vegetables, climatic factors and intake from the soil. Sugar industry effluent contains minerals and toxic metals. So, it is necessary to give proper treatment to sugar industry wastewater before its application on agricultural land. On the other hand, all health risk index values recorded in the present study were lesser than 1 except for Cd.

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