Bulletin of Environmental Contamination and Toxicology (2022) 109:1117–1125
https://doi.org/10.1007/s00128-022-03606-3

Potentially Toxic Metal Accumulation in Spinach (*Spinacia oleracea* L.) Irrigated with Industrial Wastewater and Health Risk Assessment from Consumption

Ilker Ugulu[1] · Shehnaz Bibi[2] · Zafar I. Khan[2] · Kafeel Ahmad[2] · Mudasra Munir[2] · Ifra S. Malik[2]

Received: 11 June 2022 / Accepted: 5 August 2022 / Published online: 19 August 2022

Abstract

This study aimed to determine the potentially toxic metal contents in soil and spinach samples in areas irrigated with industrial wastewater and to evaluate the potentially toxic metal accumulation in spinach samples according to pollution indices. Water, soil and spinach samples were analysed using atomic absorption spectrophotometer (Perkin-Elmer AAS-300). In this study, it was determined that the potentially toxic metal values in the spinach samples irrigated with groundwater and sugar industry wastewater varied between 1.59 and 1.84, 0.22–0.68, 0.56–1.14, 1.41–1.56, 1.62–3.23, 0.57–1.02, 0.86–1.33, 0.20–0.32 and 0.35–2.10 mg/kg for Cd, Co, Cr, Cu, Fe, Ni, Pb, Zn and Mn, respectively. It was concluded that the difference between the metal values in the spinach samples according to the irrigation sources was statistically significant, except for Cu and Pb ($p > 0.05$). According to the results of this study, there is no health risk for Pb, Co and Cr with HRI values below 1.0, while there is a risk for Cd, Cu, Fe, Ni, Mn and Zn. The much higher HRI values of Cd than 1 (196.8 and 169.6) suggested that this metal is likely to cause significant health problems in the region.

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

Industrialization has both direct and indirect negative effects on the environment (Ugulu et al. 2021a, b, c, d, e). One of the direct effects of this issue is the wastewater generated in the industrial production process. Wastewater contains potentially toxic metals and pollutants are released into water and soil, posing a serious risk to humans (Khan et al. 2021). These metals can cause metal toxicity, which can cause damage or decrease in the mental and central nervous system, lungs, kidneys, liver and other body organs. In addition, multiple sclerosis, physical, muscular and neurological degeneration processes that mimic Alzheimer's disease occur as a result of long-term exposure to potentially toxic metals. However, it has been reported that the main contributors to soil pollution are by-products of industrial processes, different industries such as sugar processing, energy and power, cement and petrochemicals (Ugulu 2015a, b).

✉ Ilker Ugulu
ilkerugulu@gmail.com

1 Faculty of Education, Usak University, Usak, Turkey

2 Department of Botany, University of Sargodha, Sargodha, Pakistan

The use of industrial wastewater and municipal wastewater for irrigation is widely used in farmland and suburban areas in many countries (Khan et al. 2019a, b, c). The discharge of wastewater into the environment and agricultural soil without treatment adds large amounts of organic and inorganic pollutants to these areas. In general, industrial wastewater carries many toxic substances, including trace metals. Long-term use of such wastewater in agricultural lands causes high levels of potentially toxic metal accumulation in soil and plants (Sharma et al. 2006). As a result of prolonged wastewater irrigation, vegetables and crops grown in metal-contaminated soil accumulate more potentially toxic metals than those grown in uncontaminated soil. Potentially toxic metals, which can be easily taken up by vegetable roots, can accumulate in the edible parts of vegetables at high levels (Karak et al. 2014).

Spinach (*Spinacia oleracea* L.) belongs to the Amaranthaceae family and is an eatable vegetable family which is mostly grown in Central and Western Asia spinach grows up to 30 cm tall. It is an annual plant. Spinach has great dietary value, especially when quickly boiled or frozen and steams. The plant contains manganese, magnesium, vitamin A and Vitamin C. It is a rich source of dietary fibre, vitamin B6, riboflavin, B vitamins, vitamin E, potassium and calcium like other green leafy vegetables. However, spinach contains a high level of oxalate which inhibits the absorption of iron (Nadeem et al. 2019).

In Pakistan, where agriculture is among the most important inputs of the country's economy, wastewater such as sewage water, urban wastewater and industrial wastewater are used in agricultural irrigation in various regions of the country (Khan et al. 2018a, b, c). It is seen that many studies have been carried out on the risks to human health of using wastewater in agricultural irrigation for many agricultural products produced in various regions of the country (Ahmad et al. 2018, 2019; Chen et al. 2021; Munir et al. 2019; Nadeem et al. 2019; Rasheed et al. 2020; Siddique et al. 2019; Ugulu et al. 2020; Yang et al. 2020; Tariq et al. 2021). This study aimed to determine the potentially toxic metal contents in soil and spinach samples in areas irrigated with industrial wastewater and to evaluate the potentially toxic metal accumulation in spinach samples according to pollution indices.

Materials and Methods

Sargodha District is surrounded by Jhelum District in the north and Chenab River in the east. Located on the south side is the Jhang District. It is separated from the Khushab District on its western side by the Jhelum River. Sargodha District has a total area of 5854 km^2 and consists of five tehsils; Bhalwal, Kot Momin, Sahiwal, Shahpur and Sillanwali. While the maximum temperature in Sargodha Region is 45°C in summer, it drops to 0°C in winter. Currently, 7 textile mills, 4 sugar mills, 12 flour mills and 24 peeling units operate in Sargodha (Khan et al. 2018a, b).

The areas of influence of Chishtia Sugar Mill Limited were selected for sampling in sample collection studies. Sugar Factory is located next to Farooka in tehsil Sillanwali. It was established in 1990 and is pure sugar industry. Chishtia Sugar Mill Limited, which has an annual installed capacity of 7000 Tcd, employs approximately 213 people in Farooka, Sillanwali (Khan and Khan 2019).

100 mL samples were taken from each source to determine the metal content of each of the sugar industry wastewater and groundwater sources used for irrigation of Site 1 and Site 2. The bottles were washed with acid polypropylene and 1 mL of HNO_3 was added to prevent microbial growth. Samples were chilled before further analysis.

The first of the soil samples taken from the designated locations was irrigated with groundwater and the second was irrigated with sugar industry wastewater. Soil samples were randomly selected and taken from the upper crust (0–2.5 cm). After all, samples were dried, they were passed through a 2 mm crush strainer. Soil samples were placed on croft paper and stored for examining vegetables. One gram soil sample taken for digestion was mixed with nitric acid (HNO_3) in a beaker. After the mixture was left to stand overnight, it was boiled in a digestion tube at 75°C for 1 h and hydrogen peroxide (H_2O_2) was added until the solution became clear. After digestion was completed, the digested material was removed from the digestion tube and filter paper was used for filtration. This extract was mixed with distilled water to make a volume of 50 mm.

Twenty-five samples were taken from the spinach grown in Site 1 and Site 2, which were irrigated with groundwater and industrial wastewater, respectively. Spinach samples were first washed with deionized water and then dried at 80°C. Samples of 1 g of each vegetable were placed in digestion tubes and nitric acid (HNO_3) was added to the tubes. The digestive tube was placed on a 75°C hot plate the next day. After 35 min, hydrogen peroxide (H_2O_2) was added to the tube and boiled until the resulting solution was clear. The digested material was removed from the digestion tube and filtered using Whatman No.42 filter paper and deionized water was added to increase its volume to 50 mm.

Water, soil and spinach samples were analysed using atomic absorption spectrophotometer (Perkin-Elmer AAS-300). Experimental procedures and analytical methods in the analyzes performed with AAS were performed according to the manufacturer's instrumentation and application guidelines following European Commission (2006) guidelines. The metals identified in the current research are Cd, Co, Cr, Cu, Fe, Ni, Pb, Zn and Mn. Chromium (Cr) and Nickel (Ni) were detected by the very sensitive hydride method. To avoid chemical pre-treatment, these elemental analyses usually use graphite furnace AAS (GFAAS), from which the molten sample can be directly calculated. However, GFAAS is not as sensitive as the hydride technique. Therefore, the use of particularly powerful hollow cathode lamps (super lamps) becomes important for precise measurements. In this study, analyzes were performed for Cr and Ni in aqueous standards (Table 1).

The values of the Limit of Detection (LOD) were evaluated according to standard methods reported by Armbruster et al. (1994). The standard deviation (SD) and

Table 1 The operating conditions of chromium (Cr) and nickel (Ni) analysis

Parameters	Chromium (Cr)	Nickel (Ni)
Wavelength (nm)	358	232
Slit width (nm)	0.7	0.2
Lamp intensity (Ma)	25	25
Airflow (L/min)	10	10
Acetylene flow (L/min)	3	3
Relative noise	1.0	1.0

signal-to-noise ratio values of the blank solution were determined as 10, and the value was defined as LOD. The detection limits for the analysed potentially toxic metals are given in Table 2.

Diagnostic marker standardization values from Merck (Germany) were used for instrument calibration. In the study, deionized water was applied and crystalline pupils were systematically cleaned. Specialized Position Quantifiable (SRM-2711 for soil and SRM NIST 1577b for plant) assessments were performed to confirm the constancy of the results. The average recoveries of SRM for soil were 107%, 103%, 98%, 97%, 97%, 98% and 91% for Pb, Cu, Co, Mn, Cd, Cr, Zn and Fe, respectively, and the mean recoveries of SRM for spinach were 105%, 99%, 94%, 102%, 102%, 93%, and 95%, respectively.

One-way analysis of variance was used to evaluate the differences in metal values in irrigation areas using IBM SPSS 24 (Statistical Package for Social Sciences) (Dogan et al. 2011). The differences between the values were statistically tested at 0.05, 0.01 and 0.001 levels (Yorek et al. 2010; Ugulu and Erkol 2013; Ugulu et al. 2021a, b). In addition, the similarity/difference relationships regarding the metal values in the samples were evaluated by Hierarchical Clustering Analysis.

The bioconcentration factor (BCF) is an index used to evaluate the level of metal accumulation in plants. In this study, BCF values were calculated with the following formula:

$$BCF = C_{veg}/C_{soil}$$

While C_{veg} stands for metal values in plant tissues (mg/kg, dry weight), C_{soil} refers to metal concentration in soil (mg/kg, dry weight) (Khan et al. 2019a, b).

The pollution load index (PLI) aims to provide an estimate of the pollution state based on the value of the metal in the soil. The following formula was used in this study (Liu et al. 2005):

Table 2 Detection limits of atomic absorption spectrophotometer (mg/L)

Element	Detection limit
Lead (Pb)	8 (Flame AA)
Cadmium (Cd)	12 (Flame AA)
Nickel (Ni)	11 (Flame AA)
Iron (Fe)	5 (Flame AA)
Copper (Cu)	15 (Flame AA)
Manganese (Mn)	18 (Flame AA)
Zinc (Zn)	1.5 (Flame AA)
Cobalt (Co)	9 (Flame AA)

$$PLI = \text{Metal value in examined soil}/\text{Reference value of soil metal}.$$

The reference values (mg/kg) of Cd (1.49), Cr (9.07), Cu (8.39), Fe (56.90), Ni (9.06), Zn (44.19) and Mn (46.75) for soil were taken according to Ugulu et al. (2021a, b, c, d, e).

Daily metal intake (DIM) is used to find health risks from food consumption. In this study, it was calculated with the following formula (Sajjad et al. 2009):

$$DIM = C_{metal} \times D_{food\ intake}/B_{average\ weight}$$

where C_{metal} represents the value of metals ingrains, $D_{food\ intake}$ represents the daily food intake and $B_{average\ weight}$ represents average body weight. In the calculation, the average daily vegetable intake rate for adults is 0.345 kg/person/day and the average body weight is 55.9 kg (Wajid et al. 2020).

HRI was used in this study to calculate potentially toxic metal exposure that could occur if radish samples were consumed by humans. The HRI is calculated by taking the ratio of the daily metal intake (DIM) in food products to the oral reference dose (RfD) (Khan et al. 2020a, b):

$$HRI = \text{Daily intake of metals (DIM)}/\text{Oral reference dose (RfD)}$$

The RfD values used in this study are 0.001, 0.04, 1.5, 0.04, 0.7, 0.02, 0.003, 0.3 and 0.04 mg/kg/day for Cd, Co, Cr, Cu, Fe, Ni, Pb, Zn and Mn, respectively (USEPA 1997).

Results and Discussion

In Pakistan, where agricultural production is an important economic sector, groundwater and wastewater from different sources are mixed in certain proportions and used in agricultural irrigation in the form of canal waters due to the scarcity of clean water (Wajid et al. 2020). The metal values in the groundwater and sugar industry wastewater samples used for irrigation purposes varied between 0.84 and 1.67, 0.08–0.22, 0.42–0.72, 0.45–0.85, 2.51–9.99, 1.21–1.92, 0.02–0.15, 1.82–9.98 and 0.64–0.91 mg/kg for Cd, Co, Cr, Cu, Fe, Ni, Pb, Zn and Mn, respectively (Fig. 1). The Fe and Zn concentrations for both samples were higher than other metals. According to statistical analysis, inter-sample values were not significant for Cd, Co, Cr, Cu, Mn, Ni and Pb ($p > 0.05$), but were statistically significant for Fe and Zn (Table 3). FAO, WHO, European Standard Guidelines (Chiroma et al. 2014) and USEPA (1997) set the maximum allowable limits for Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn in water as 0.01, 0.05, 0.5, 0.2, 5, 0.2, 0.2, 0.065 and 2 mg/L, respectively. When these values were compared with the findings, potentially toxic metal values in this study were above the maximum allowable limits in water, except for Mn. This evaluation shows that the metal values in poor-quality water used for irrigation are higher than they should be and pose a risk of pollution (Ugulu et al. 2009). While this risk is of industrial origin for sugar industry wastewater, it can be caused by many sources such as urban runoff, soil erosion, vehicular traffic, industry and aerosol particles for groundwater (Ugulu et al. 2008; Wajid et al. 2020).

Wastewaters contain potentially toxic metals that can easily accumulate in the soil, easily pass from soil to vegetables and cause contamination (Wajid et al. 2020). In this study, it was determined that the metal values in soil samples irrigated with groundwater and sugar industry wastewater varied between 1.84 and 2.09, 0.66–0.74, 1.14–1.75, 1.56–2.16, 32.31–40.15, 0.57–0.84, 1.33–1.51, 0.32–0.38 and 0.35–0.78 mg/kg for Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, and Zn respectively (Fig. 2). While the effect of irrigation was insignificant on Cd, Ni, Zn, Fe, Cu, Mn and Cr values in soil samples, a significant effect was found for Pb and Co values ($p < 0.05$) (Table 4). However, the potentially toxic metal values were lower than the maximum allowable limits of Co (9.1), Cr (9.07), Cu (8.39), Fe (56.9), Mn (46.74), Ni (9.06), Pb (3.50), Zn (44.19) mg//kg (CSEPA 1995; Dutch Target and Intervention Values 2000; Singh et al. 2010), while Cd values were higher than the maximum allowable limit (1.49 mg/kg) (Singh et al. 2010). Similar results were reported for Cd above the allowable limit for different plant samples and locations, seasons, wastewater types and local environments, so the current study is in line with previous findings (Khan et al. 2021).

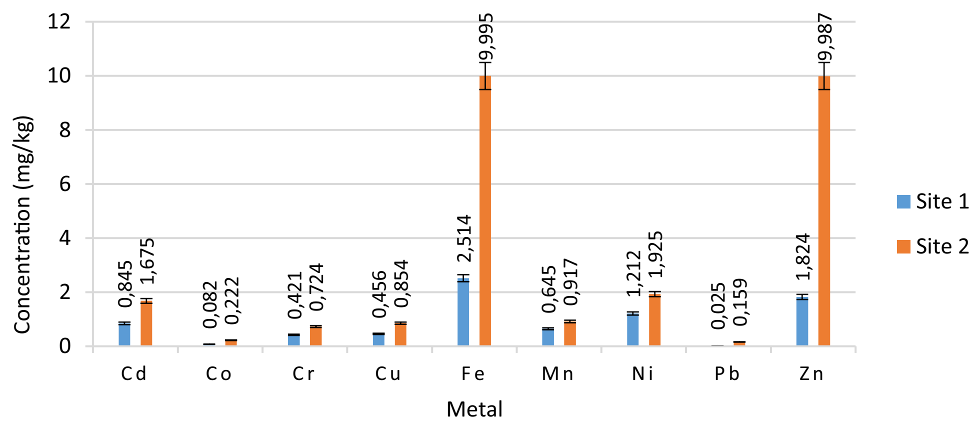

Fig. 1 The mean metal concentrations in water samples (n = 25)

Table 3 Analysis of variance for potentially toxic metal values in water samples

Source of variation	Degree of freedom	Mean squares								
		Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Treatments	1	0.215^{ns}	0.004^{ns}	0.002^{ns}	0.364 ^{ns}	6.189*	0.186^{ns}	0.750^{ns}	0.004 ^{ns}	0.006***
Error	6	0.052	0.033	0.001	0.074	0.813	0.039	0.307	0.020	0.001

ns non-significant

*, **, *** significant at 0.05, 0.01, and 0.001 levels

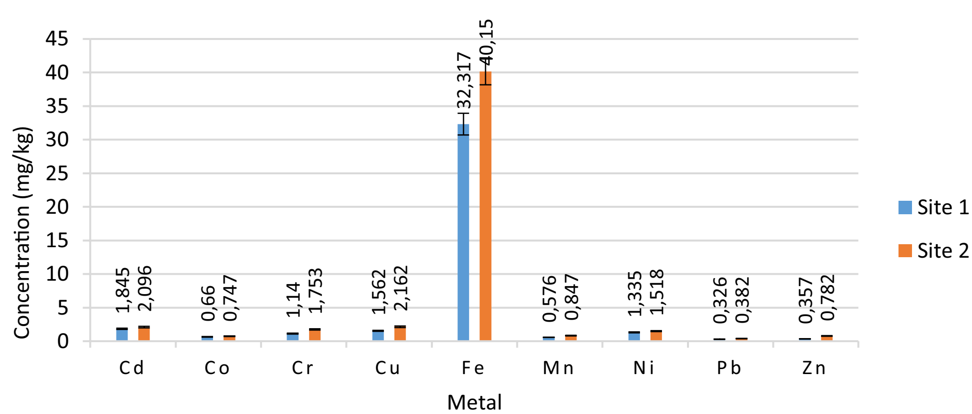

Fig. 2 The mean metal concentrations in soil samples (n = 25)

High potentially toxic metal concentrations in the habitat may result in higher translocation from soil to vegetables (Khan et al. 2021). In this study, the potentially toxic metal values in the spinach samples irrigated with groundwater and sugar industry wastewater varied between 1.59 and 1.84, 0.22–0.68, 0.56–1.14, 1.41–1.56, 1.62–3.23, 0.57–1.02, 0.86–1.33, 0.20–0.32 and 0.35–2.10 mg/kg for Cd, Co, Cr, Cu, Fe, Ni, Pb, Zn and Mn, respectively (Fig. 3). It was concluded that the difference between the metal values in the spinach samples according to the irrigation sources was statistically significant, except for Cu and Pb ($p > 0.05$) (Table 5). Current results for metals were below the maximum allowable limits reported by FAO/WHO (2001) for Co (50 mg/kg), Cr (2.3 mg/kg), Cu (73.3 mg/kg), Fe (425.5 mg/kg), Mn (500 mg/kg), Ni (67 mg/kg), Pb (0.3 mg/kg), Zn (99.4 mg/kg). However, Cd values in spinach samples were higher than the maximum allowable limit (0.2 mg/kg) reported by FAO/WHO (2001). Qadir et al. (2000) stated that the sources of Cd in environmental elements such as water, soil and plants are phosphate fertilizers, irrigation wastewater and wastes from industries. These sources support the high Cd values in the spinach samples in the study. However, according to a literature review conducted by Rai et al. (2019), the main route of transport of potentially toxic metals and other pollutants to crops in developing countries is irrigation with wastewater or sewage sludge.

Dendrogram for measured metals in the spinach samples according to Average Linkage cluster analysis revealed two main groups (Fig. 4). The first main group includes only Fe while the second main group includes the other 8 metals. Also, the second main group was divided into two subgroups with the first subgroup involving Cd, Cu and Zn, and the second subgroup involving the 5 metals. The second subgroup was further separated into two subgroups first of which contains only Pb and Co, and the second contains the remaining metals (Fig. 4). Ugulu et al (2021a, b, c, d, e), in a study on the effect of different organic fertilizers on potentially toxic metal accumulation in vegetables in Sargodha-Pakistan,

Table 4 Analysis of variance for potentially toxic metal values in soil samples

Source of variation	Degree of freedom	Metal								
		Pb	Cd	Ni	Fe	Cu	Mn	Cr	Zn	Co
Sites	1	0.06**	0.13ns	0.068ns	122.69ns	0.72ns	0.15ns	0.75ns	0.36ns	0.02***
Error	6	0.001	0.003	0.005	1.362	0.009	0.009	.0011	0.143	0.001

ns non-significant

*, **, *** significant at 0.05, 0.01, and 0.001 levels

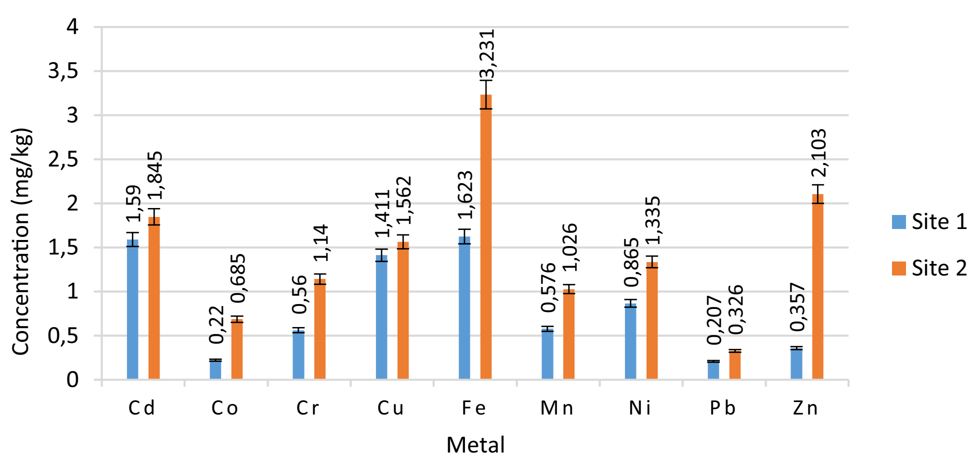

Fig. 3 The mean metal concentrations in spinach samples (n = 25)

Table 5 Analysis of variance for potentially toxic metal values in spinach samples

Source of variation	Degree of freedom	Metal								
		Pb	Cd	Ni	Fe	Cu	Mn	Cr	Zn	Co
Sites	1	0.02**	0.13ns	0.44ns	1884.2ns	0.04**	0.40ns	0.6ns	6.09ns	0.43ns
Error	6	0.001	0.009	0.009	.719	.048	.020	.010	.056	0.001

ns non-significant

*, **, *** significant at 0.05, 0.01, and 0.001 levels

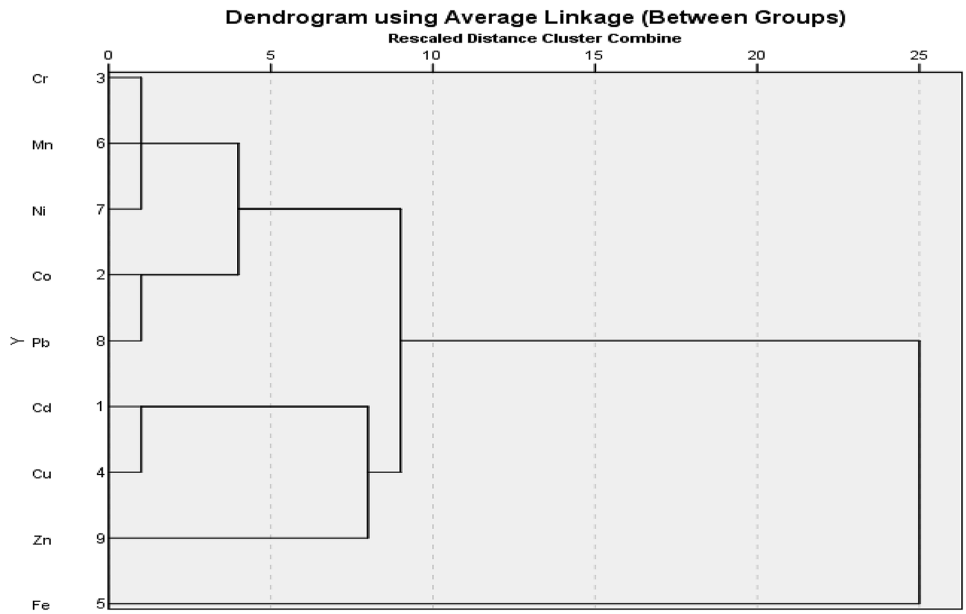

Fig. 4 Dendrogram constructed from totally 9 metals based on the spinach samples from two areas. It is generated with a hierarchical cluster analysis with Average Linkage (Between Groups)

determined that Fe and Zn accumulations were separated from other metals as a result of hierarchical cluster analysis. In this respect, the present study is similar to the findings and study area of Ugulu et al (2021a, b, c, d, e).

The bioconcentration factor can be used to express the bioavailability level of metals in the environment of a plant species (Ugulu et al. 2021c, d). In this study, the highest BCF value was found for Zn (2688) at Site 2 and the lowest for Fe (0.04) at Site 2 (Table 6). Zinc (Zn) is the second most common element in the structure of plants (Ugulu et al. 2021a, b, c, d, e). The high Zn concentration observed in plants can be attributed to the relative abundance of potentially toxic metals in the earth's crust in the upper layers of the soil (Zn > Cr > Pb > Ni > Cu > Cd) (Alloway 2013). However, long-term use of wastewater in agricultural irrigation may increase the content of Zn and other potentially toxic metals (Rattan et al. 2005). The high BCF values determined for Zn in this study support these findings. Jolly et al. (2013) also reported the higher bioconcentration factor of zinc observed in some vegetables. The BCF values determined for these vegetables are as follows: 0.263 (*Raphanus sativus*), 0.872 (*Amaranthus caudatus*), 0.189 (*Solanum melongena*) and 1.55 (*Beta vulgaris*). According to these findings, it can be said that leafy vegetables show higher BCF values among the vegetables examined.

In this study, the highest PLI value was observed for Fe (0.705) at Site 2 and the lowest PLI value for Mn (0.003) was observed at Site 1 (Table 6). An estimated PLI value of more than 1.0 indicates that the soil is contaminated with potentially toxic metals, while a value of less than 1.00 indicates that it is not contaminated (Khan et al. 2019a, b, c). PLI values of less than 1.0 determined for metals in this study showed that the land was not heavily contaminated with related potentially toxic metals (Khan et al. 2019a, b, c). Ugulu et al (2021a, b, c, d, e) investigated the effects of using organic wastes as fertilizer on potentially toxic metal accumulation in pepper in Sargodha city of Pakistan and reached the following PLI values for Cd, Co, Cr, Cu, Fe, Mn, Pb and Zn: 0.057–0.231, 0.049–0.638, 0.043–0.455, 0.081–0.700, 0.005–0.299, 0.105–0.511, 0.001–0.062 and 0.261–0.400, respectively. Except for Cd, the PLI values obtained in this study were lower than the values in the related study. In addition, the Zn, Fe, Cd, Co and Mn PLI values obtained in this study are lower than the values determined for contaminated vegetables from the wastewater irrigated areas of Varanasi, India (Singh et al. 2010).

Table 6 The mean bioconcentration factor values for vegetable/soil and Pollution load index for metals in soil of spinach

Study sites		Metal								
		Pb	Cd	Ni	Fe	Mn	Cu	Cr	Zn	Co
BCF	Site 1	0.341	0.468	0.485	0.072	1.008	0.551	0.112	1.758	1.026
	Site 2	0.5424	0.758	0.569	0.040	1.210	0.652	0.319	2.688	0.294
PLI	Site 1	0.040	0.615	0.1473	0.567	0.003	0.186	0.011	0.008	0.013
	Site 2	0.046	0.69	0.167	0.705	0.004	0.257	0.017	0.017	0.015

Table 7 Daily intake of metal and health risk index of spinach

Site	Metal	Pb	Cd	Ni	Fe	Mn	Cu	Cr	Zn	Co
Site 1	DIM (mg/kg/day)	0.001	0.196	0.031	138.7	0.105	0.078	0.019	0.357	3.33
	HRI	0.332	196.8	1.557	2.483	2.576	1.953	0.013	1.191	0.021
Site 2	DIM (mg/kg/day)	0.0007	0.166	0.020	1.217	0.188	0.705	0.009	2.1037	0.003
	HRI	0.211	169.6	1.009	1.739	4.588	1.764	0.006	7.012	0.006

The highest DIM value was 1.67 mg/kg/day for Zn at Site 2, and the lowest value was 0.0003 mg/kg/day for Pb at Site 1 (Table 7). WHO (1996), FAO/WHO (2001) and EFSA (2013) reported tolerable daily metal intake values of 0.0007, 0.0005, 0.3, 0.023, 0.43 and 0.8 mg/kg for Cd, Pb, Cr, Co, Zn and Fe. The DIM values of Cd, Pb, and Fe for both sites and the Co (Site 1) and Zn (Site 2) were higher than these tolerable values. Khan et al. (2008) obtained DIM values of 0.032, 0.008, 0.002, 0.0003, 0.005 and 0.005 for Zn, Cu, Pb, Cd, Cr, and Ni, respectively, in *S. oleracea* samples irrigated with wastewater in Beijing China. The current DIM values are higher than those obtained by Khan et al. (2008). The differences between the results may vary according to the wastewater content and characteristics, as well as the characteristics of the region and the facility.

The highest HRI value was found to be 196.8 for Cd at Site 1 and the lowest value to 0.006 for Co and Cr at Site 2 (Table 7). Increasing the use of wastewater for irrigation is a very important factor that increases metal accumulation in the soil and plants, causes an increase in the uptake of metals to the vegetative parts of plants and causes health risks (Nawaz et al. 2021). According to the results of this study, there is no health risk for Pb, Co and Cr with HRI values below 1.0, while there is a risk for Cd, Cu, Fe, Ni, Mn and Zn. The much higher HRI values of Cd than 1 (196.8 and 169.6) suggested that this metal is likely to cause significant health problems in the region. Cadmium (Cd) is defined as a very risky carcinogen for humans. It is also a potent and multi-tissue carcinogen for animals (Ugulu et al. 2019a, b). For this reason, it is thought that new studies on the health risks arising from metals and bioremediation in the region will be beneficial.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

References

Ahmad K, Kokab R, Khan ZI, Ashfaq A, Bashir H et al (2018) Assessment of heavy metals in wheat variety '"Chagi-2"' under short-term wastewater irrigation. Biologia (pakistan) 64(1):15–25

Ahmad K, Wajid K, Khan ZI, Ugulu I, Memoona H, Sana M, Nawaz K, Malik IS, Bashir H, Sher M (2019) Evaluation of potential toxic metals accumulation in wheat irrigated with wastewater. Bull Environ Contam Toxicol 102:822–828

Alloway BJ (2013) Heavy metals in soils. Springer, Dordrecht, Netherlands

Armbruster DA, Tillman MD, Hubbs LM (1994) Limit of detection (LQD)/limit of quantitation (LOQ): comparison of the empirical and the statistical methods exemplified with GC–MS assays of abused drugs. Clin Chem 40:1233–1238

Chen F, Khan ZI, Zafar A, Ma J, Nadeem M et al (2021) Evaluation of toxicity potential of cobalt in wheat irrigated with wastewater: health risk implications for public. Environ Sci Pollut Res 28(17):21119–21131. https://doi.org/10.1007/s11356-020-11815-8

Chiroma TM, Ebewele RO, Hymore FK (2014) Comparative assessment of heavy metal levels in soil, vegetables and urban grey wastewater used for irrigation in Yola and Kano. Int Refereed J Eng Sci 3:1–9

CSEPA (China State Environmental Protection Administration) (1995) GB 15618–1995 Environmental quality standard for soils. State Environmental Protection Administration of China.

Dogan Y, Ugulu I, Durkan N, Unver MC, Mert HH (2011) Determination of some ecological characteristics and economical importance of *Vitex agnus-castus*. Eurasia J Biosci 5:10–18. https://doi.org/10.5053/ejobios.2011.5.0.2

Dutch Target and Intervention Values (2000) Circular on target values and intervention values for soil remediation. pp: 1-51. Esdat Environmental Database Management Software. Available webpage (URL): https://www.esdat.net/environmental%20standards/dutch/annexs_i2000dutch%20environmental%20standards.pdf

EFSA, European Food Safety Authority (2013) Panel on additives and products or substances used in animal feed (FEEDAP). EFSA J 11(7):3287

European Commission (2006). Commission regulation (EC) No.1181/2006: setting maximum levels for certain contaminants in food stuffs. Official Journal of the European Union L 364, 20 December 2006, pp. 5-24. Available webpage (URL): http://eur-lex.europa.eu/legalcontent/EN/ALL/?uri=CELEX:02006R1881-20100701

FAO/WHO (2001) Codex Alimentarius Commission. Food additive and contaminants. Joint FAO/ WHO Food Standards Programme, ALINORM 01/ 12A, pp. 1–289.

Jolly YN, Islam A, Akbar S (2013) Transfer of metals from soil to vegetables and possible health risk assessment. Springer Plus 2:385–391

Karak T, Paul RK, Sonar I, Sanyal S, Ahmed KZ, Boruah RK, Das DK, Dutta AK (2014) Chromium in soil and tea (*Camellia sinensis* L.) infusion: does soil amendment with municipal solid waste compost make sense? Food Res Int 64:114–124

Khan MT, Khan IA (2019) Sugarcane biofuels: status, potential, and prospects of the sweet crop to fuel the world. Springer, Netherlands

Khan S, Cao Q, Zheng YM, Huang YZ, Zhu YG (2008) Health risks of heavy metals in contaminated soils and food crops irrigated with wastewater in Beijing. China Environ Pollut 152(3):686–692

Khan ZI, Ahmad K, Safdar H, Ugulu I, Wajid K, Bashir H, Dogan Y (2018a) Manganese bioaccumulation and translocation of in forages grown in soil irrigated with city effluent: An evaluation on health risk. Res J Pharmaceut Biol Chem Sci 9(5):759–770

Khan ZI, Ugulu I, Ahmad K, Yasmeen S, Noorka IR, Mehmood N, Sher M (2018b) Assessment of trace metal and metalloid accumulation and human health risk from vegetables consumption through spinach and coriander specimens irrigated with wastewater. Bull Environ Contam Toxicol 101:787–795. https://doi.org/10.1007/s00128-018-2448-8

Khan ZI, Ugulu I, Umar S, Ahmad K, Mehmood N, Ashfaq A, Bashir H, Sohail M (2018c) Potential toxic metal accumulation in soil, forage and blood plasma of buffaloes sampled from Jhang, Pakistan. Bull Environ Contam Toxicol 101:235–242. https://doi.org/10.1007/s00128-018-2353-1

Khan ZI, Safdar H, Ahmad K, Wajid K, Bashir H, Ugulu I, Dogan Y (2019a) Health risk assessment through determining bioaccumulation of iron in forages grown in soil irrigated with city effluent. Environ Sci Pollut Res 26:14277–14286

Khan ZI, Arshad N, Ahmad K, Nadeem M, Ashfaq A et al (2019b) Toxicological potential of cobalt in forage for ruminants grown in polluted soil: a health risk assessment from trace metal pollution for livestock. Environ Sci Pollut Res 26:15381–15389

Khan ZI, Nisar A, Ugulu I, Ahmad K, Wajid K, Bashir H, Dogan Y (2019c) Determination of cadmium concentrations of vegetables grown in soil irrigated with wastewater: evaluation of health risk to the public. Egypt J Bot 59(3):753–762. https://doi.org/10.21608/ejbo.2019.9969.1296

Khan ZI, Ugulu I, Zafar A, Mehmood N, Bashir H, Ahmad SM (2021) Biomonitoring of heavy metals accumulation in wild plants growing at soon valley, Khushab, Pakistan. Pak J Bot 53(1):247–252

Khan ZI, Safdar H, Ahmad K, Wajid K, Bashir H, Ugulu I, Dogan Y (2020a) Copper bioaccumulation and translocation in forages grown in soil irrigated with sewage water. Pak J Bot 52(1):111–119. https://doi.org/10.30848/PJB2020-1(12)

Khan ZI, Ugulu I, Sahira S, Mehmood N, Ahmad K, Bashir H, Dogan Y (2020b) Human health risk assessment through the comparative analysis of diverse irrigation regimes for Luffa (*Luffa cylindrica* (L.) Roem.). J Water, Sanit Hyg Dev 10(2):249–261. https://doi.org/10.2166/washdev.2020.132

Liu WH, Zhao JZ, Ouyang ZY, Söderlund L, Liu GH (2005) Impacts of sewage irrigation on heavy metal distribution and contamination in Beijing, China. Environ Inter 31(6):805–812. https://doi.org/10.1016/j.envint.2005.05.042

Munir M, Khan ZI, Ahmad K, Wajid K, Bashir H et al (2019) Transfer of heavy metals from different sources of fertilizers in wheat variety (Galaxy-13). Asian J Biol Sci 12(4):832–841. https://doi.org/10.3923/ajbs.2019.832.841

Nadeem M, Qureshi TM, Ugulu I, Riaz MN, An QU et al (2019) Mineral, vitamin and phenolic contents and sugar profiles of some prominent date palm (*Phoenix dactylifera*) varieties of Pakistan. Pak J Bot 51(1):171–178

Nawaz H, Anwar-ul-Haq M, Akhtar J, Arfan M (2021) Cadmium, chromium, nickel and nitrate accumulation in wheat (*Triticum aestivum* L.) using wastewater irrigation and health risks assessment. Ecotoxicol Environ Saf 208:111685

Qadir M, Ghafoor A, Murtaza G, Murtaza G (2000) Cadmium concentration in vegetables grown on urban soils irrigated with untreated municipal sewage. Environ Develop Sustain 2(1):13–21

Rai PK, Lee SS, Zhang M, Tsang YF, Kim K-H (2019) Heavy metals in food crops: health risks, fate, mechanisms, and management. Environ Int 125:365–385

Rasheed MJZ, Ahmad K, Khan ZI, Mahpara S, Ahmad T et al (2020) Assessment of trace metal contents of indigenous and improved pastures and their implications for livestock in terms of seasonal variations. Rev Chim 71(7):347–364. https://doi.org/10.37358/RC.20.7.8253

Rattan RK, Datta SP, Chonkar PK, Suribabu K, Singh AK (2005) Long-term impact of irrigation with sewage effluents on heavy metal content in soils, crops and groundwater—a case study. Agric Ecosys Environ 109(3–4):310–322

Sajjad K, Farooq R, Shahbaz S, Khan MA, Sadique M (2009) Health risk assessment of heavy metals for population via consumption of vegetables. World Appl Sci J 6:1602–1606

Sharma RK, Agrawal M, Marshall FM (2006) Heavy metals contamination in vegetables grown in wastewater irrigated areas of Varanasi, India. Bull Environ Contam Toxicol 77:312–318

Siddique S, Ahmad K, Khan ZI, Wajid K, Bashir H et al (2019) Sodium status of soil, forages, and small ruminants of Punjab. Pakistan. Pure Appl Biol 8(3):1950–1961. https://doi.org/10.19045/bspab.2019.80139

Singh A, Sharma RK, Agrawal M, Marshall FM (2010) Health risk assessment of heavy metals via dietary intake of foodstuffs from the wastewater irrigated site of a dry tropical area of India. Food Chem Toxicol 48:611–619

Tariq F, Xiukang W, Saleem MH, Khan ZI, Ahmad K et al (2021) Risk assessment of heavy metals in basmati rice: implications for public health. Sustainability 13:8513. https://doi.org/10.3390/su13158513

Ugulu I (2015a) Determination of heavy metal accumulation in plant samples by spectrometric techniques in Turkey. Appl Spectros Rev 50(2):113–151. https://doi.org/10.1080/05704928.2014.935981

Ugulu I (2015b) A quantitative investigation on recycling attitudes of gifted/talented students. Biotech Biotechnol Equip 29:20–26. https://doi.org/10.1080/13102818.2015.1047168

Ugulu I, Erkol S (2013) Environmental attitudes of biology teacher candidates and the assessments in terms of some variables. NWSA-Educ Sci 8(1):79–89

Ugulu I, Aydin H, Yorek N, Dogan Y (2008) The impact of endemism concept on environmental attitudes of secondary school students. Nat Monten 7(3):165–173

Ugulu I, Unver MC, Dogan Y (2019a) Potentially toxic metal accumulation and human health risk from consuming wild Urtica urens sold on the open markets of Izmir. Euro-Mediterr J Environ Integr 4:36. https://doi.org/10.1007/s41207-019-0128-7

Ugulu I, Khan ZI, Rehman S, Ahmad K, Munir M, Bashir H, Nawaz K (2019b) Trace metal accumulation in *Trigonella foenum-graecum* irrigated with wastewater and human health risk of metal access through the consumption. Bull Environ Contam Toxicol 103:468–475. https://doi.org/10.1007/s00128-019-02673-3

Ugulu I, Khan ZI, Rehman S, Ahmad K, Munir M, Bashir H (2020) Effect of wastewater ırrigation on trace metal accumulation in spinach (*Spinacia oleracea* L.) and human health risk. Pak J Anal Environ Chem 21(1):92–101. https://doi.org/10.2743/pjaec/2020.06.11

Ugulu I, Akhter P, Khan ZI, Akhtar M, Ahmad K (2021a) Trace metal accumulation in pepper (*Capsicum annuum* L.) grown using organic fertilizers and health risk assessment from consumption. Food Res Int 140:1992. https://doi.org/10.1016/j.foodres.2020.109992

Ugulu I, Khan ZI, Sheik Z, Ahmad K, Bashir H, Ashfaq A (2021b) Effect of wastewater irrigation as an alternative irrigation resource on heavy metal accumulation in ginger (*Zingiber officinale* Rosc.) and human health risk from consumption. Arab J Geosci 14:702. https://doi.org/10.1007/s12517-021-07073-8

Ugulu I, Khan ZI, Aslam Z, Ahmad K, Bashir H, Munir M (2021c) Potentially toxic metal accumulation in grains of wheat variety galaxy-2013 irrigated with sugar industry wastewater and human health risk assessment. Euro-Mediterr J Environ Integr 6:38. https://doi.org/10.1007/s41207-020-00203-w

Ugulu I, Khan ZI, Safdar H, Ahmad K, Bashir H (2021d) Chromium bioaccumulation by plants and grazing livestock as affected by the application of sewage irrigation water: implications to the food chain and health risk. Int J Environ Res 15:261–274. https://doi.org/10.1007/s41742-021-00311-7

Ugulu I, Ahmad K, Khan ZI, Munir M, Wajid K, Bashir H (2021e) Effects of organic and chemical fertilizers on the growth, heavy metal/metalloid accumulation, and human health risk of wheat (*Triticum aestivum* L.). Environ Sci Pollut Res 28:12533–12545. https://doi.org/10.1007/s11356-020-11271-4

Ugulu I, Baslar S, Dogan Y, Aydin H (2009) The determination of colour intensity of *Rubia tinctorum* and *Chrozophora tinctoria* distributed in Western Anatolia. Biotech Biotechnol Equip 23(SE):410–413. https://doi.org/10.1080/13102818.2009.10818451

USEPA (1997) Exposure Factors Handbook. Volume II-Food Ingestion Factors. EPA/ 600//P-95/002Fa. Office of Research and Development, US Environmental Protection Agency, Washington, DC.

Wajid K, Ahmad K, Khan ZI, Nadeem M, Bashir H, Chen F, Ugulu I (2020) Effect of organic manure and mineral fertilizers on bioaccumulation and translocation of trace metals in maize. Bull Environ Contam Toxicol 104:649–657

WHO (1996) Trace elements in human nutrition and health. World Health Organization, Geneva, Switzerland

Yang Y, Khan ZI, Ahmad K, Arshad N, Rehman SU et al (2020) Does the chromium element in forages and fodders grown in contaminated pasture lands cause toxicity in livestock: assessing the potential risk. Rev Chim 71(7):397–405. https://doi.org/10.37358/RC.20.7.8257

Yorek N, Ugulu I, Sahin M, Dogan Y (2010) A qualitative investigation of students' understanding about ecosystem and its components. Nat Monten 9(3):973–981