



Potentially toxic metal accumulation in grains of wheat variety Galaxy-2013 irrigated with sugar industry wastewater and human health risk assessment

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Abstract

Wheat plays a major role in feeding the global population. Therefore, given its potential impact on human health, it is very important to determine toxic metal accumulation in the various varieties of wheat. This study was carried out to investigate potentially toxic metal accumulation in the wheat variety Galaxy-2013 following various industrial wastewater irrigation treatments, and to examine various indices such as the bioconcentration factor, enrichment factor, and health risk index in order to better understand how metals are transported and accumulate throughout the food chain. Atomic absorption spectrophotometry was used to evaluate the concentrations of iron (Fe), copper (Cu), chromium (Cr), cadmium (Cd), and cobalt (Co) in the wheat. A multipurpose pot experiment was conducted to determine metal accumulation in wheat samples irrigated with industrial wastewater in the Botanical Garden of the Botany Department at the University of Sargodha. The concentrations of the potentially toxic metals Co, Cd, Cr, Cu, and Fe in the harvested wheat samples ranged from 0.720 to 1.075, from 0.316 to 0.526, from 0.111 to 0.950, from 0.603 to 0.665, and from 1.617 to 1.884 mg/kg, respectively. These concentrations were higher than the safe levels specified by WHO, FAO, and US EPA, except for Cd. However, the health risk index for Cd was found to be above the permissible limit. Pearson correlation coefficients indicated that there were correlations between the potentially toxic metal contents in the soil and those in the plants: a significant positive correlation was observed for Cd, while the correlations for Cr, Cu, and Fe were positive and nonsignificant.

Keywords Biomonitoring · Health risk · Potentially toxic metal · Wastewater

Introduction

Uncontrolled industrialization and urbanization by nations around the globe have dangerous effects on the environment (including the overexploitation of natural resources) as well as human health (Ugulu 2015a, b; Yorek et al. 2016; Khan et al. 2019a, b). The increasing rate of urbanization has driven industrial development, and both urban areas and industry are major producers of waste materials that disturb the environment (Ahmad et al. 2019; Munir et al. 2019). The

volume of urban and industrial wastewaters has increased in parallel with urbanization and industrialization, and these wastewaters pose a major threat to the environment. In many regions of the world, they are discharged untreated into water bodies in neighboring regions, causing serious environmental problems. On the other hand, industrial and domestic wastewaters are used for agricultural irrigation in many countries in which there are shortages of freshwater (Ahmad et al. 2018).

Agriculture is one of the most important drivers of Pakistan's economy (Nadeem et al. 2019). Although agriculture is important in Pakistan, water shortages mean that farmers frequently use industrial wastewater for irrigation purposes (Khan et al. 2019a, b; Munir et al. 2019). While industrial wastewater contains nutrients, it also includes various types of contaminants, such as inorganic and organic molecules, microorganisms, and radioactive elements (Khan et al. 2018a, b). When industrial and urban effluents are discharged directly into the environment, they have serious negative effects on ecosystem

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elements and agricultural products (Ugulu et al. 2009; Sahin et al. 2016). Industrial wastewater and potentially toxic metals damage fertile land by adding chemical compounds to the soil, which leads to greater yield losses (Khan et al. 2018c, d).

Various studies on the subject have shown that the use of industrial and sewage wastewaters in agricultural irrigation can result in potentially toxic metal accumulation in soil and plants (Siddique et al. 2019). High levels of organic matter are present in the upper layer of the soil. However, in agricultural areas close to cities or industrial facilities, which tend to be the agricultural areas in which wastewater is used for irrigation, there are high trace metal concentrations in the upper layer of the soil that originate from wastewater (Khan et al. 2019c, d). Since the roots of many crops grow in this zone, the upper soil layer is an important influence on the substances that are taken up by plants from the soil (Wajid et al. 2020).

Plants are one of the most important components of both human and animal nutrition. Wheat (*Triticum aestivum* L.) has more nutritional value than other cereals and helps to fulfill the dietary requirements of one-third of the global population. It is a major source of the protein, vitamins, and minerals that are necessary for human health. However, wheat crops that are irrigated with wastewater show significant accumulation of toxic metals, so it is potentially hazardous for humans to consume these crops (Perveen et al. 2011; Ugulu et al. 2016). Toxic metals can degrade human mental and central nervous system function, negatively affect blood composition, damage vital internal organs such as the liver, kidneys, and lungs, and disrupt metabolic reactions (Khan et al. 2021).

Healthy human nutrition is one of the most popular topics of recent years (Ugulu et al. 2020). Thus, given their potential impact on human health, it is very important to determine the levels of toxic metals in the various varieties of wheat that play a major role in feeding the global population. Literature reviews on this subject indicate that studies on the accumulation of potentially toxic metals in wheat variety Galaxy-2013 and the effect of this accumulation on human health are scarce. Therefore, the present study was performed to determine the levels of the potentially toxic metals Cd, Cr, Fe, Co, and Cu in Galaxy-2013 wheat irrigated with industrial wastewater, and to examine various indices such as the bioconcentration factor, enrichment factor, and health risk index in order to improve our understanding of how metals are transported and accumulate throughout the food chain.

Materials and methods

Study area

The present research was carried out during the years 2015–2016 in the Botanical Garden of the

Botany Department at the University of Sargodha (32°8'0"N, 73°7'0"E), northeastern Punjab, Pakistan (Fig. 1). Sargodha is the fifth most contaminated urban area of Punjab according to Punjab Environment Protection Department officials. While the summer season is very hot in the city of Sargodha, the winter season is quite cold. The Jhelum River flows to the north and west of the city, and the Chenab River passes to the east of the city.

Plant cultivation and sample preparation

Healthy seed samples of the wheat variety Galaxy-2013 were collected in the year 2015. About 6 kg of soil were added to each of 12 plastic pots 30 cm in height and 40 cm in diameter, and 20 seeds were placed in each pot. Pot trials were then conducted in the natural environment (November–April) for 6 months. The seeds were initially watered with tap water until the first leaves appeared, after which they were irrigated with different wastewater treatments. The pots were split into four groups: T-I: control (irrigated with 100% groundwater), T-II: irrigated with 30% industrial wastewater and 70% groundwater, T-III: irrigated with 60% industrial wastewater and 40% groundwater, and T-IV: irrigated with 90% industrial wastewater and 10% groundwater. The industrial wastewater originated from Ramzan Sugar Mill in Jhang. Wheat grains were harvested in April 2016, about 120 days after planting the seeds.

Next, the grain samples were washed with a mixture of distilled water and 5% HCl, placed in an oven at a temperature of 105 °C to dry, and crushed to a fine powder using an electrical grinder before being dried and weighed. Soil samples were collected from the top 15–18 cm of the soil profile. After being dried outdoors, the soil samples were oven dried at 72 °C for 4 days.

The soil and seed samples were digested using a wet digestion method outlined by Allen et al. (1986). One gram of each soil and wheat sample was digested with a mixture of HNO₃, H₂SO₄, and HClO₄ (5:1:1) at 80 °C until a transparent solution was obtained. After cooling the solution, it was filtered with Whatman filter paper #42 and the final volume of the solution was made up to 50 mL. It was stored in plastic bottles until further analysis. The concentrations of potentially toxic metals in the samples were measured after digestion with HNO₃ and H₂O₂ followed by atomic absorption spectrophotometry (Allen et al. 1986).

Metal determination

Atomic absorption spectrophotometry (AA-6300, Shimadzu, Japan) was used to assess the concentrations of the potentially toxic metals iron (Fe), copper (Cu), chromium (Cr), and cadmium (Cd). The atomic absorption spectrophotometer was equipped with a graphite furnace and D2



Fig. 1 Map of Sargodha

corrector (Perkin–Elmer Model 503) in order to determine the Co content. The iron in the organic layer was analyzed under the standard conditions defined in the manufacturer’s

instrumentation and application manual. The stock solutions specified in the standard conditions for metals were created and used. The experimental procedures and analytical

methods used for AAS were taken from the manufacturer's guide to instrumentation and applications, with reference to the guidelines of the European Commission (2006), the report of El-Ansary and El-Leboudy (2015), and Liu et al. (2020).

Physicochemical parameters

The physicochemical properties of the soil and water samples, such as the amount of organic matter, the electrical conductivity (EC), the pH, and the Ca, Mg, Cl, and available P and K levels were determined. The pH of a 1:2.5 soil solution (created using deionized water) was tested using a HI 3221 pH meter (Mclean 1982). Titration was used to determine the levels of Ca^{2+} , Mg^{2+} , and Cl^- . Soil organic matter was measured by a loss-on-ignition method, ashing at 550 °C for 4 h (Page 1982). Available K and P were determined via the methods of Schollenberger and Simon (1945) and Olsen and Sommers (1982), respectively.

Statistical analysis

Statistical analyses were performed using the Statistical Package of Social Sciences (SPSS), and one-way analysis of variance (ANOVA) was applied to evaluate the concentrations of potentially toxic metals in water, soil, and food grains. Pearson's correlation coefficient was evaluated to determine the relationships between the concentration of each metal in the soil and its concentration in wheat grains. The correlation analysis applied was a bivariate method that examined the relationships between different parameters using the least significant difference test at probability levels of 0.05, 0.01, and 0.001 (Ugulu 2020).

Bioconcentration factor

The bioconcentration factor (BCF) is a measure of the accumulation of a potentially toxic metal in a plant as a result of the transfer of the metal from the soil to the plant. The following formula is used to calculate the bioconcentration factor:

$$\text{BCF} = C_{\text{veg}}/C_{\text{soil}}, \quad (1)$$

where C_{veg} refers to the metal concentration in plant tissues (mg/kg, fresh weight) and C_{soil} refers to the metal concentration in the soil (mg/kg, dry weight) (Liu et al. 2005).

Daily intake of metals

One of the methods that is used to find consumption-related health risks is the daily intake of metals (DIM). This was calculated using the following equation described by Wang et al. (2005):

$$\text{DIM} = C_{\text{metal}} \times D_{\text{food intake}}/B_{\text{average weight}}, \quad (2)$$

where C_{metal} represents the metal concentration in grains, $C_{\text{food intake}}$ represents the daily food intake, and $B_{\text{average weight}}$ represents the average body weight. An average daily vegetable intake rate of 0.345 kg/person/day and an average body mass of 55.9 kg for adults were used, based on data in the literature (Wang et al. 2005).

Health risk index

The health risk index (HRI) indicates the threat to the health of humans who consume contaminated food. In this study, it was used to calculate human exposure to potentially toxic metals due to the consumption of metal-contaminated wheat. The HRI is calculated as the ratio of the DIM from food crops to the oral reference dose (Stephens et al. 2001):

$$\text{HRI} = \text{daily intake of metals}/\text{oral reference dose}. \quad (3)$$

Results and discussion

Physicochemical parameters of the irrigation water

The electrical conductivity (EC) is an important indicator as it can be used to check the ionic content of water. In this study, EC values of irrigation water samples across all wastewater treatments varied from 19.3 to 50 dS/m, with mean values per treatment of: T-I, 0.79; T-II, 19.3; T-III,

Table 1 Mean values of physicochemical parameters of the water used for irrigation in each treatment performed in this study

Treatment	EC (dS/m)	$\text{Ca}^{2+} + \text{Mg}^{2+}$ (mg/L)	Na^+ (mg/L)	HCO_3^- (mg/L)	Cl^- (mg/L)
T-I	0.79	7.8	0.1	6.2	1.1
T-II	19.3	45	27	38.2	45
T-III	35	540	1120	540	95.5
T-IV	50	620	1400	894	194
Standard limit	> 3 ^a	400 + 60 ^a	900 ^a	600 ^a	11 ^a

^a Source: FAO (1985)

35; T-IV, 50 dS/m. The values for the wastewater treatments were higher than 7.8 dS/m, the value reported by Pandey and Singh (2015), and much higher than the EC value (1.032 dS/m) determined by Alghobar and Suresha (2015) for wastewater used for irrigation purposes. US regulations state that high-quality irrigation water should have EC < 0.7 dS/m, while acceptable irrigation water should have EC < 3 dS/m. Thus, the average EC values for the irrigation water used in treatments T-II, T-III, and T-IV were above the permissible limit.

Combined Ca^{2+} and Mg^{2+} concentrations in the irrigation water samples ranged from 7.8 to 620 mg/L, with mean concentrations per treatment of: T-I, 7.8; T-II, 45; T-III, 540; T-IV, 620 mg/L. Na^+ concentrations ranged from 0.1 to 1400 mg/L, with mean concentrations per treatment of: T-I, 0.1; T-II, 27; T-III, 1120; T-IV, 1400 mg/L. Bicarbonate concentrations were 6.2–894 mg/L, with mean concentrations per treatment of: T-I, 6.2; T-II, 38.2; T-III, 540; T-IV, 894 mg/L. Chloride concentrations ranged from 1.1 to 194 mg/L, with mean concentrations of: T-I, 1.1; T-II, 45; T-III, 95.5; T-IV, 194 mg/L (Table 1). These concentrations of the major cations Ca^{2+} , Mg^{2+} , and Na^+ in the wastewater treatments are higher than the corresponding concentrations in sugarcane (48, 43, and 27 mg/L, respectively) reported by Alghobar and Suresha (2015). On the other hand, the concentrations of major anions such as HCO_3^- and Cl^- in the wastewater treatments in the present study were found to be higher than those determined by Alghobar and Suresha (2015): 296 and 93 mg/L, respectively.

Physicochemical parameters of soil samples taken after wheat cultivation

The soils collected from the four treatments after wheat cultivation had a clay loam texture. The soil pH ranged between 8.1 and 8.3, with average values per treatment of: T-I, 8.1; T-II, 8.2; T-III, 8.3; T-IV, 8.2. The soil EC ranged between 1.58 and 1.80 dS/m, with average EC values per treatment of: T-I, 1.80; T-II, 1.58; T-III, 1.58; T-IV, 1.80 dS/m. The organic matter (OM) contents of the soil samples ranged between 1.11 and 1.25%, with mean percentages per treatment of: T-I, 1.25; T-II, 1.11; T-III, 1.18; T-IV, 1.24%. Soil phosphorus (P) values ranged between 1.71 and 2.91 mg/

kg, with mean values per treatment of: T-I, 2.91; T-II, 1.72; T-III, 1.71; T-IV, 1.90 mg/kg. Soil potassium (K) ranged between 3.40 and 5.90 mg/kg, with mean values per treatment of: T-I, 5.22; T-II, 4.90; T-III, 3.40; T-IV, 5.90 mg/kg (Table 2). Numerous studies have reported that the soil pH decreases significantly after irrigation with wastewater (Li et al. 1987), possibly due to organic acid production and organic matter decomposition (Khan et al. 2020c).

Potentially toxic metal concentrations in irrigation water

In the present study, the concentrations of the potentially toxic metals Co, Cd, Cu, Cr and Fe in irrigation water samples ranged from 1.823 to 2.463, from 0.333 to 1.036, from 0.354 to 1.440, from 0.523 to 1.183, and from 0.533 to 0.926 mg/L, respectively (Table 3). When the metals were ranked based on their abundances in the water samples, we found that $\text{Co} < \text{Cr} < \text{Cu} < \text{Fe} < \text{Cd}$ for T-I, $\text{Co} < \text{Cr} < \text{Fe} < \text{Cu} < \text{Cd}$ for T-II, $\text{Co} < \text{Cr} < \text{Fe} < \text{Cu} < \text{Cd}$ for T-III, and $\text{Fe} < \text{Co} < \text{Cu} < \text{Cr} < \text{Cd}$ for T-IV (Fig. 2). While the Cd concentration was high in water samples from all the treatments, the Cu, Cr, and Fe concentrations increased as the percentage of wastewater in the irrigation water increased. Metal accumulation was highest in the irrigation water used in the T-IV treatment, which included the most industrial wastewater. However, the results of one-way ANOVA indicated that treatment had a nonsignificant effect ($p > 0.05$) on the concentrations of the metals (Cd, Cr, Cu, Co, and Fe) in the irrigation water (Table 4).

The maximum allowable limits for Co, Cd, Cu, Cr, and Fe in water are 0.05, 0.01, 0.01, 0.55, and 0.50 mg/L, respectively, as defined by WHO, FAO, Standard Guidelines in Europe (Chiroma et al. 2014), and US EPA (2002). The accumulated levels of potential toxic metals in the irrigation water samples in the present study were higher than those maximum limits except in the case of Fe. Studies investigating this issue have revealed that increased acidity of the irrigation water increases potentially toxic metal accumulation (Wajid et al. 2020). Therefore, the high potentially toxic metal concentrations measured in the irrigation water samples in the present study may be due to the low pH of the wastewater. However, the Fe and Cr concentrations were

Table 2 Mean values of various physicochemical parameters of the soils collected from the four irrigation treatments after wheat cultivation

Treatment	Physicochemical parameter					
	pH	EC (dS/m)	OM (%)	Available P (mg/kg)	Available K (mg/kg)	Textural class
T-I	8.10	1.80	1.25	2.91	5.22	Loam
T-II	8.20	1.58	1.11	1.72	4.90	Loam
T-III	8.30	1.58	1.18	1.71	3.40	Loam
T-IV	8.20	7.89	1.50	1.90	5.90	Loam

Table 3 Mean concentrations of metals in the irrigation water and in the soil and wheat samples taken after cultivation

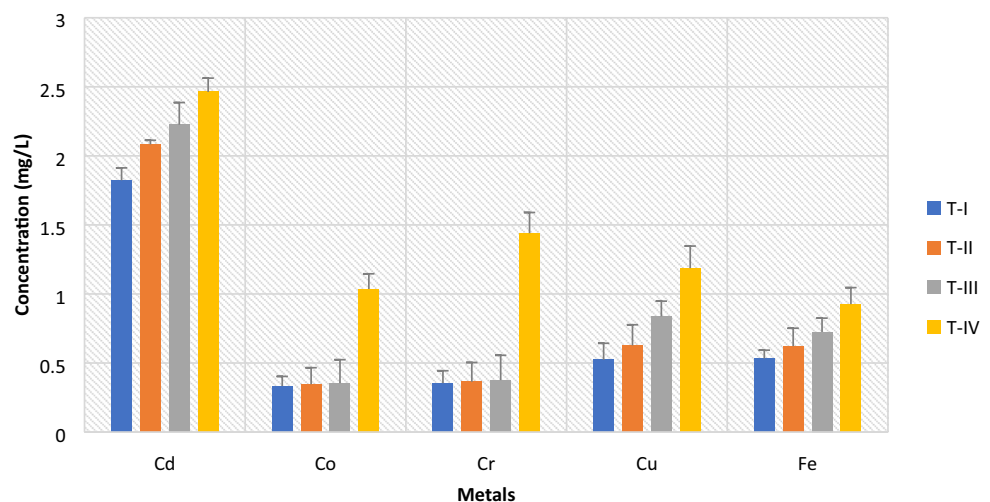
Metal	T-I	T-II	T-III	T-IV
Concentration in irrigation water (mg/L)				
Cd	1.823 ± 0.09	2.083 ± 0.03	2.226 ± 0.16	2.463 ± 0.10
Co	0.333 ± 0.07	0.346 ± 0.12	0.354 ± 0.17	1.036 ± 0.11
Cr	0.354 ± 0.12	0.364 ± 0.14	0.376 ± 0.18	1.440 ± 0.15
Cu	0.523 ± 0.06	0.627 ± 0.15	0.838 ± 0.11	1.187 ± 0.16
Fe	0.533 ± 0.11	0.622 ± 0.13	0.725 ± 0.10	0.926 ± 0.12
Concentration in soil post-cultivation (mg/kg)				
Cd	1.824 ± 0.19	1.908 ± 0.10	1.815 ± 0.02	1.900 ± 0.05
Co	0.688 ± 0.90	0.730 ± 0.08	0.591 ± 0.06	0.561 ± 0.09
Cr	0.931 ± 0.07	0.948 ± 0.06	0.740 ± 0.05	1.260 ± 0.11
Cu	1.643 ± 0.06	1.508 ± 0.09	1.193 ± 0.90	1.523 ± 0.02
Fe	2.204 ± 0.11	2.101 ± 0.10	2.831 ± 0.10	2.565 ± 0.09
Concentration in wheat grains (mg/kg)				
Cd	0.868 ± 0.09	1.075 ± 0.07	0.720 ± 0.09	0.922 ± 0.05
Co	0.420 ± 0.08	0.322 ± 0.09	0.316 ± 0.08	0.526 ± 0.08
Cr	0.950 ± 0.07	0.150 ± 0.10	0.111 ± 0.07	0.135 ± 0.10
Cu	0.630 ± 0.06	0.665 ± 0.06	0.611 ± 0.06	0.603 ± 0.06
Fe	1.617 ± 0.09	1.778 ± 0.09	1.698 ± 0.09	1.884 ± 0.09

Table 4 ANOVA results for the effects of treatment on metal concentrations in irrigation water and in soil and wheat grain samples taken after cultivation

Metal	Cd	Co	Cr	Cu	Fe
Water	0.215 ^{ns}	0.738 ^{ns}	0.867 ^{ns}	0.257 ^{ns}	0.086 ^{ns}
Soil	0.007 ^{ns}	0.019 ^{ns}	0.139 ^{ns}	0.111 ^{**}	33.81 ^{ns}
Wheat grains	0.64 ^{ns}	0.30 ^{ns}	0.002 ^{***}	0.002 ^{ns}	0.430 ^{***}

ns nonsignificant

******, ******* Significant at the 0.01 and 0.001 levels, respectively

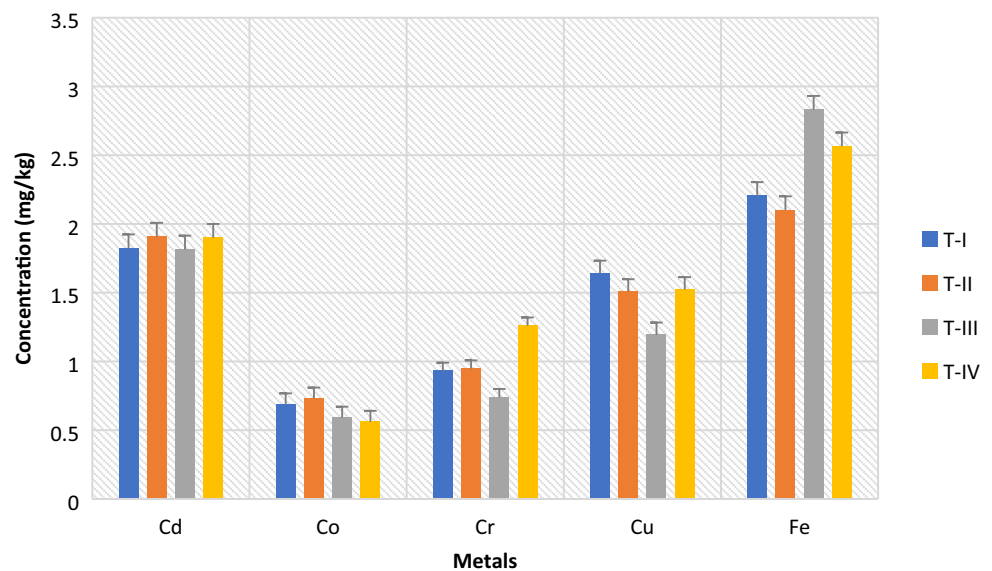
Fig. 2 Concentrations of metals in the irrigation water used in the four irrigation treatments

lower whereas the Cd and Cu levels were higher than the corresponding values recorded by Antil (2012): 5.6, 3.5, 0.97, and 0.46 mg/L, respectively. The concentrations of Cu, Cd, Co, and Cr (but not Fe) were also higher than the corresponding values measured by Alghobar and Suresha (2015) when sugarcane crop samples were irrigated with wastewater. In an experiment to investigate the effect of wastewater on soil and crops, Alghobar and Suresha (2015) observed concentrations of 2.93, 0.05, 0.047, 0.055, and 0.32 mg/L for Cu, Cd, Co, Cr, and Fe, respectively, in the wastewater.

Mean concentrations of potentially toxic metals in soil samples taken after wheat cultivation

In the present study, the Cd, Co, Cr, Cu, and Fe concentrations in the soil samples ranged from 1.815 to 1.908, from 0.561 to 0.730, from 0.740 to 1.260, from 1.193 to 1.931, and from 2.101 to 2.836 mg/kg, respectively (Table 3). When the metals were ranked based on their abundances in the soil samples, we found that $Co < Cr < Cu < Cd < Fe$ for T-I, $Co < Cr < Cu < Cd < Fe$ for T-II, $Co < Cr < Cu < Cd < Fe$ for T-III, and $Co < Cr < Cu < Cd < Fe$ for T-IV (Fig. 3). When the treatments were compared in terms of the overall potentially toxic metal concentration in samples, none of the treatments were found to result in a much higher potentially toxic metal concentration than the other treatments. However, different treatments led to the accumulation of different metals. The mean Co concentrations in the soil samples were low whereas the Fe, Cd, Cu, and Cr concentrations were high regardless of the treatment applied. The results from ANOVA showed that treatment had a nonsignificant effect ($p > 0.05$) on the concentrations of Cd, Cr, Co, and Fe in the soil samples, whereas treatment exerted a significant effect on the Cu concentration ($p < 0.05$) (Table 4).

Fig. 3 Concentrations of metals in soil samples taken following the four irrigation treatments



The maximum allowable concentrations of Co, Cd, Cu, Cr, and Fe in soil are defined as 50, 3, 100, 100, and 50,000 mg/kg, respectively, by US EPA (1997). The Co, Cd, Cu, Cr, and Fe concentrations in the soil samples examined in this work were below the allowable limits defined by US EPA (1997), regardless of the treatment applied. Also, the mean Cd, Cr, Cu, and Fe concentrations in the soil samples were lower than the corresponding values measured by Asdeo (2014) and Rattan et al. (2005). These findings indicate that there is low absorption of potentially toxic metals from the soil, so the metal concentrations in the soil samples were in the safe range (Ugulu et al. 2019a, b).

Mean concentrations of potentially toxic metals in wheat grains

In the present study, the concentrations of the potentially toxic metals Co, Cd, Cr, Cu, and Fe in wheat samples ranged from 0.720 to 1.075, from 0.316 to 0.526, from 0.111 to 0.950, from 0.603 to 0.665, and from 1.617 to 1.884 mg/kg, respectively (Table 3). When the metals were ranked based on their abundances in the wheat grains, we found that $Co < Cr < Cu < Cd < Fe$ for T-I, $Cr < Co < Cu < Cd < Fe$ for T-II, $Cr < Co < Cu < Cd < Fe$ for T-III, and $Cr < Co < Cu < Cd < Fe$ for T-IV (Fig. 4). The Cr concentrations in grains obtained with the T-II, T-III, and T-IV treatments were lower than those in grains obtained with the T-I treatment, while the Fe, Cd, and Cu concentrations in the grains were high with all treatments. Treatment had a nonsignificant effect ($p > 0.05$) on the concentrations of Cd, Cu, and Co in the grains, whereas treatment had a significant effect on Cr and Fe concentrations (Table 4).

The maximum allowable concentrations of Co, Cd, Cu, Cr, and Fe in vegetables are defined as 50, 0.1, 73, 50, and 425 mg/L, respectively, by WHO, FAO, Standard Guidelines in Europe (Chiroma et al. 2014), and US EPA (2002). The concentrations of potentially toxic metals in the grains studied here were higher than the maximum permissible levels in vegetables, except for Cd. The main factors that affect the levels of potentially toxic metals taken up by plants from the soil are the pH, temperature, and the cation exchange capacity of the soil, the levels of other metals in the soil, chemical selectivity, and the plant species (Khan et al. 2020a, b, c).

Ahmad et al. (2019) studied potentially toxic metal accumulation in wheat variety Chagi-4 caused by irrigation with sewage water in Sargodha, Pakistan. They observed that the mean concentrations of metals in Chagi-4 wheat ranged from 0.03 to 0.2 for Co, from 1.2 to 1.6 for Cd, from 0.3 to 0.5 for Cr, from 0.7 to 1.4 for Cu, and from 0.8 to 1.6 for Fe (mg/kg), respectively. All metal concentrations in the wheat (Galaxy-2013) grown in the present study were higher than those observed in the Chagi-4 variety, except for Cu. The metal concentrations measured in wheat grains in the present study were also higher than those in the wheat grains analyzed by Asdeo (2014). The differences in metal concentrations between these studies may be due to the different wastewaters used to irrigate the wheat varieties, or differences in the metal intake characteristics of these varieties.

Bioconcentration factor (BCF)

BCF values were calculated for all the metals of interest in each treatment. The highest value was observed for Cr, and the smallest for Cu. After ranking the BCF values, we found that $Cr > Co > Fe > Cd > Cu$ for T-I, $Cr > Fe > Co > Cu > Cd$ for T-II, $Cr > Co > Fe > Cd > Cu$

Fig. 4 Concentrations of metals in the *Triticum aestivum* grains obtained using the four irrigation treatments

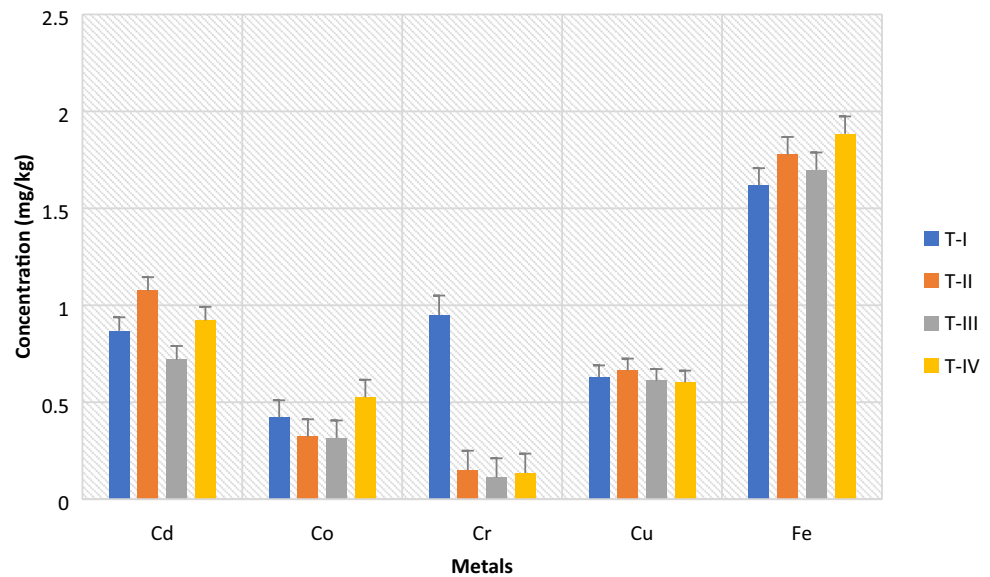


Table 5 Bioconcentration factor values for heavy metals in Galaxy-2013 wheat grains that were obtained under various wastewater irrigation treatments

Treatment	Heavy metal				
	Cd	Co	Cr	Cu	Fe
T-I	0.475	0.628	1.020	0.383	0.539
T-II	0.563	0.441	1.138	0.440	0.592
T-III	0.396	0.534	1.150	0.512	0.566
T-IV	0.485	0.926	1.107	0.395	0.628

for T-III, and $Cr > Co > Fe > Cd > Cu$ for T-IV (Table 5). The BCF is the most accurate indicator of the tendency of a metal to be transferred from the soil to a vegetable. All of the BCF values for Cr were > 1 , meaning that this metal is readily available to and relatively likely accumulate in vegetables (Khan et al. 2020c). The results therefore suggest that the wheat variety Galaxy-2013 accumulates Cr. In the present study, the BCF values for Cr and Cu were higher than the corresponding values reported by Verma et al. (2015). The BCF values of Cr, Co, Cu, and Fe were also higher than those reported by Alghobar and Suresha (2015). Thus, it appears that Cr, Co, Cu, and Fe have a high tendency to move from the soil to the wheat variety Galaxy-2013. Higher BCF values were also observed for Cd, Cr, and Cu in the present study than in the studies performed by Asdeo (2014) and Jia et al. (2010).

Daily intake of metals (DIM)

The possible exposure of humans to each potentially toxic metal due to the consumption of wheat variety Galaxy-2013 was estimated in order to evaluate the health risk from that metal. In the present study, the sole pathway for the studied metals into humans was assumed to be the consumption of wheat grains. The DIM values for the metals of interest were estimated based on the average wheat grain consumption of an adult. High DIM values were observed in all the treatments for Fe, Cr, Cd, and Cu but not for Co. When the metals were ranked based on their DIM values, we found that $Fe > Cr > Cd > Cu > Co$ for T-I, $Fe > Cu > Cd > Co > Cr$ for T-II, $Fe > Cd > Cu > Co > Cr$ for T-III, and $Fe > Cd > Cu > Co > Cr$ for T-IV (Table 6). The DIM values for these metals were lower than the corresponding values reported by Antil (2012), indicating that all of the wheat grain samples were safe for consumption.

Health risk index (HRI)

Health risk index values for the potentially toxic metals in Galaxy-2013 wheat grains were estimated by performing health risk assessments (US EPA 2002; Cui et al. 2004; Wang et al. 2005). If the HRI of a metal in a specific food crop is < 1 , the concentration of the metal in the food is low enough for the food to be safe for human consumption, whereas a HRI of > 1 is harmful and dangerous (US EPA 2002). For the wheat grains grown in treatment T-I, the health risk index values of the metals were ranked as follows: $Cd > Cr > Fe > Cu > Co$. The corresponding rankings for the wastewater treatments were as follows: $Cd > Cr > Fe > Cu > Co$ (T-II); $Cd > Cr > Fe > Cu > Co$ (T-III); and $Cd > Cr > Fe > Cu > Co$ (T-IV) (Table 6). The

Table 6 Daily intake of metals and health risk index values for those metals due to the consumption of *Triticum aestivum*

	Cd	Co	Cr	Cu	Fe
Daily intake of metals					
Treatment					
T-I	0.004	0.002	0.0054	0.0036	0.009
T-II	0.006	0.001	0.0008	0.0038	0.010
T-III	0.004	0.001	0.0006	0.0035	0.009
T-IV	0.005	0.003	0.0007	0.0034	0.010
Health risk index					
Treatment					
T-I	4.991	0.056	1.092	0.090	0.092
T-II	6.181	0.043	0.172	0.095	0.102
T-III	4.140	0.042	0.127	0.087	0.097
T-IV	5.302	0.070	0.155	0.086	0.108

HRI values for Cr, Fe, Cu, and Co were < 1 in all treatments, indicating that the accumulated concentrations of these metals in Galaxy-2013 wheat grains were below their maximum permissible limits (i.e., not hazardous to human health). However, it appears that the consumption of crops that have been irrigated with wastewater and or grown near wastewater sources could be dangerous to human health as it could result in exposure to high levels of Cd.

Correlation

Pearson's bivariate correlation coefficient was used to determine the association between the concentration of a particular potentially toxic metal in soil post-cultivation and its concentration in Galaxy-2013 wheat grains. In the present study, the Cd concentrations in soil and grains were found to be significantly positively correlated, whereas the corresponding correlations for Cr, Cu, and Fe concentrations in soil and grains were weak (Table 7). The significant correlations of potentially toxic metals indicate that they may have originated from irrigation with wastewater. The correlation between Fe and Cr concentrations showed that they both originated from the tannery industry. However, the strong association of Cd, Zn, and Cu concentrations also indicates that these metals originated from the same anthropogenic sources, such as the paint industry and the municipal sewage system.

Table 7 Correlation between the concentration of a particular metal in the soil post-cultivation and its concentration in wheat grains

Metal	Correlation between concentration in soil and concentration in grains
Cd	0.595*
Co	- 0.361
Cr	0.309
Cu	0.395
Fe	0.378

Values shown are Pearson's correlation coefficients. * Significant at the 0.05 level

Conclusion

Wastewater irrigation is common practice when insufficient freshwater resources are available. Wastewaters may contain nutrients that are essential for the growth of plants, but they can also contain toxic metals.

In the present study, the concentrations of potentially toxic metals in irrigation water samples were higher than their permissible limits reported by WHO, FAO, US EPA, except for Fe. While the Co, Cd, Cu, Cr, and Fe concentrations in soil samples taken after wheat cultivation remained below their permissible limits under all treatment conditions, the concentrations of all the metals in the wheat grains were higher than the permissible limits, except for Cd. However, the health risk index value of Cd was found to be > 1. This value indicates that irrigating Galaxy-2013 wheat with wastewater from the Jhang region of Pakistan may lead to the accumulation of dangerous levels of Cd in the resulting wheat grain. Therefore, precautionary measurements should be taken to check that the wastewater in this region is treated properly before it is applied to agricultural lands.

Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest regarding the publication of this paper.

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