Health risk assessment of heavy metals in wheat using different water qualities: implication for human health

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Abstract In the recent years, the use of sewage water for irrigation has attracted the attention of arid and semi-arid countries where the availability of fresh water is poor. Despite the potential use of sewage water in crop irrigation as effective and sustainable strategy, the environmental and human risks behind this use need to be deeply investigated. In this regard, an experiment was carried out under field conditions in Nursery, University College of Agriculture Sargodha, to evaluate the possible health risks of undesirable metals in wheat grains. Wheat variety Sarang was cultivated and irrigated with different combinations of ground (GW) and sewage water (SW). The concentrations of heavy metals (Cr, Cd, Ni, and Pb) and trace elements (Cu, Zn, and Fe) in wheat grains as well as in soil were determined. Moreover, the pollution load index (PLI), accumulation factor (AF), daily intake of metals (DIM), and health risk index (HRI) were calculated. Results showed that the concentration trend of heavy metals was $Pb < Cr < Cu < Ni < Cd < Zn < Fe$ and $Cr < Cu < Pb < Cd < Ni < Fe < Zn$ in soil and wheat, respectively. Among metals, Cd concentration in wheat exceeded the permissible limits regardless water quality, whereas Pb concentration in grain was within the acceptable levels as suggested by World Health Organization, when 100 % of SW was used for irrigation. Similar observation was reported for Cd concentration in the soil when wheat was irrigated with 100 %

SW. In comparison to soil, the edible part of wheat presented lower concentration of all studied metals, except for Zn which was much higher compared to the tested soil samples. The higher concentration of Zn was responsible for increasing the DIM of Zn where, in average, the highest value was reported, particularly in 75 % SW treatment. This was reflected also in HRI where the maximum value was reported for Zinc under the same treatment. Higher value of HRI for wheat cultivated on polluted soils suggested that appropriate management of cultivated area is necessary for food safety and thus for public health. The results are expected to create awareness among the public on the safety of consuming food products grown in particular areas.

Introduction

Wheat (Triticum aestivum L.) is the most widely crop grown in the world. Pakistan is well known as an agricultural country and its agriculture sector continues to play a vital role in the national economy (Adnan et al. 2009). Wheat production represents the leading food grain in Pakistan where the country is classified among the 10th wheat producer countries worldwide. Wheat, known as "Gandum," is an essential ingredient for population as it constitutes by 60 % of the daily diet in Pakistan. Area under wheat has shown an increase of 0.5 % over last year and the production stood at 24.3 million tonnes, against the target of 25.5 million tonnes. To date, there is about 60 % yield gap in wheat, which needs to be narrowed. The major reasons for low productivity and instability include shortage of irrigation water. In fact, large fraction of agriculture areas in Pakistan falls within arid and semi-arid zones

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(Shah et al. 2011; Baig et al. 2013). About 75 % of the total cultivated area is irrigated, while the rest is rainfed (PCST, Pakistan Council for Science and Technology 2005). Being the key element of agricultural production, the availability of sufficient water for crop irrigation can reduce the impact of water scarcity (Falkenmark and Dolden 2008). Today, due to constraint in availability of fresh water for irrigation, wastewater especially sewage water is being used by farmers to satisfy crop water needs. Many studies confirm that treated sewage wastewater can be useful as an additional water resource for irrigation (Mehrdadi et al. 2007; Palese et al. 2009; Khan et al. 2016a) and as a big source of important minerals and nutrients which can reduce the cultivation costs due to synthetic N fertilizer use (Lone et al. 2003; Qadir et al. 2008). Although the reuse of sewage wastewater can be beneficial to agriculture and considering that safeguard the environment and the production of clean and safe food are the basic requirements of human existence, there are some environmental and health burdens that have been considered (Khan et al. 2016b). For instance, untreated sewage water irrigation is believed to be the major source of increasing soil and crop metals. It contains different types of poisonous compounds, and therefore, the extensive use of wastewater for irrigation purposes of food crops causes accumulation of these toxic compounds in plants (Singh et al. 2004). As metals cannot be broken down, when concentrations within the plant exceed optimal levels, they adversely affect the plant both directly and indirectly. Some of the direct toxic effects caused by high metal concentration include inhibition of cytoplasmic enzymes and damage to cell structures due to oxidative stress (Assche and Clijsters 1990; Jadia and Fulekar 2009), while the important feature of indirect toxic effect is the replacement of essential nutrients at cation exchange sites of plants (Taiz and Zeiger 2002).

In agriculture fields, some heavy metals, such as Cu, Fe, Ni, and Zn, are required in few amount by organisms. However, excessive amounts of these elements can become harmful and can negatively affect crop productivity. Since the complete removal of these toxic metals from the soil surface is not possible, they will find their way to enter in the food chain (Wilson and Pyatt 2007). Because antagonistic and synergistic behaviors exist among heavy metals, the presence of one heavy metal may affect the availability of another in soil and hence in plant. Salgare and Acharekar (1992) reported that the inhibitory effect of Mn on the total amount of mineralized C was antagonized by the presence of Cd. Similarly, Cu and Zn as well as Ni and Cd have been reported to compete for the same membrane carriers in plants (Clarkson and Luttge 1989). In contrast, Cu was reported to increase the toxicity of Zn in spring barley (Luo and Rimmer 1995). This implies that the interrelationship between heavy metals is quite complex.

In Pakistan, the province of Punjab is the focus of major agricultural planning and development efforts being the main

source of agricultural production in the country. However, in the last few years, Punjab's dominance in wheat production has greatly reduced due to stagnation in wheat production of the province. This stagnation was due to different issues including lack of sufficient watering system, low yields and quality, costly and otherwise intensive cultivation techniques, and less utilization of current innovation in wheat production. To reach the production target, National Research System has been developing wheat varieties for different ecological zones through PARC National Uniform Testing System (NUWYT). One of the new rust-resistant varieties (NIA Sarang-2013) was recommended to be cultivated in rainfed area of Punjab. In fact, in order to sustain wheat production in Pakistan, water source management and investment in water for irrigation through viable alternatives can help to achieve this goal. However, the increase of public concern on the viability and the effects of wastewater use on soil and human health, through food chain, are still under criticism. In this regard, in this study, we aimed to explore the potential for soil and wheat pollution irrigated with sewage wastewater and to evaluate the health hazards of heavy metals.

Materials and methods

Study area

This study was conducted under pot conditions in Nursery, University College of Agriculture Sargodha, province of Punjab (Pakistan). The study location is about 9.7 km north from University of Sargodha. Climate is characterized primarily as desert or near desert. The country stands among the most arid countries with an annual rainfall of below 240 mm (Farooq et al. 2007). Climate conditions in Sargodha are usually characterized by very hot summer temperature and temperate weather in winter. The extreme temperature in summer reaches 50 °C while 12 °C represents the lowest temperature recorded in winter.

Experiment set up

Sarang variety of wheat was sown at the end of December 2014 in plastic pots using three replicates with a total of 15 pots in each replicate. Each pot was filled up with 2.5 kg of fertile soil and then ten seeds were sown per pot. Different water qualities were applied to wheat pots using ground/fresh water (GW) and untreated municipal sewage water (SW) alone or in combination with different concentrations. The experimental pots were irrigated with five treatments: T-I (100 % GW), T-II (75 % GW+25 % SW), T-III (50 % GW+ 50 % SW), T-IV (25 % GW+75 % SW), and T-V (100 % SW). Sewage water was taken from sewage water ponds, which was located in the front of Agricultural College, University of

Sargodha, Sargodha. After maturation, four plants per pot were left while others were eradicated. Crop was harvested at the end of April 2015. Grain samples were taken from each pot where they were detached from husks and then ground in grinder to get wheat grain flour. The resulted wheat flour was kept in the oven for 3 days at temperature of 104 °C. After 3 days, samples were digested using wet digestion method (Bowman 1989) for the determination of heavy metals in wheat samples.

Sample preparation and metals determination

Samples of fine grain flour dried in oven and samples of soil (about 2 g) were prepared by digesting in H_2O_2 and HNO_3 in 2:1 on a hot plate for about 1 h. Later, 2 ml of H_2O_2 were added to each digested sample. The digestion method sustained until the mixture of digestion for each sample comes to be clear. Then, sample mixture was filtered and diluted using distilled water up to 50 ml (Khan et al. 2016a, b). In order to determine metal and metalloid concentrations in the digested grains and soil samples, an atomic absorption spectrophotometer (model AA-6300 Shimadzu) was used. To proceed with the analysis, the standards of investigated heavy

metals (Cd, Cu, Cr, Ni, Fe, Zn, and Pb) were prepared (Khan et al. 2016a, b). Results of heavy metal concentrations were expressed as mean value (mg kg^{-1}) on dry weight (± SE) of three sub-samples collected from each treatment.

Indexes calculation

To reach the objectives of our study, the pollution load index (PLI), translocation factor (AF), daily intake (DIM), and health risk index (HRI) were calculated as follows:

Translocation factor The ability of heavy metals to translocate from the soil to the fruits or grains of agriculture crops is referred to using an accumulation factor (AF). Previous studies have calculated this factor using different terms, i.e., bioconcentration factor (BCF) (Raskin et al. 1994; Cui et al. 2004) and transfer factor (TF) (Chojnacka et al. 2005; Bi et al. 2010; Wang et al. 2006). For the calculation of AF in this study, heavy metal concentrations in wheat grains from each treatment were used. The AFs of Cd, Zn, Pb, Cu, Cr, Ni, and Fe were then calculated following the methodology described by Li et al. (2012). The following equation was used for the calculation:

$$AF = \text{Metal concentration in edible parts of crop/Metal concentration in the soil}$$

Pollution load index Contamination levels in soil are analyzed using pollution load index (PLI) proposed by Tomlinson et al. (1980) and Angulo (1996). Based on the concentration factor of each metal in the soil, PLI gave us an estimation to the metal contamination status and the necessary action that should be taken. In this study, we estimate PLI for each treatment following the methodology described by Liu et al. (2005). The equation used for the estimation is as follows:

$$PLI = \text{concentration of metal in soil/reference value of metal in soil}$$

Daily intake of metals For finding out consumer-based health risks, certain methods are taken into consideration. In this study, the daily intake of metal method was used to estimate health risks (see also Balkhair and Ashraf 2016). The daily intake of metals (DIM, mg kg^{-1} person^{-1} day^{-1}) was calculated as in the following equation:

$$DIM = C_{metal} \times C_{factor} \times C_{intake}/B_{weight}$$

where C_{metal} represents concentration of metal ingrains, C_{factor} represents conversion factor, C_{food} intake represents

the daily food intake, and $B_{average\ weight}$ represents average body weight.

Health risk index It was measured to find overall risks of exposure to all heavy metals via ingestion of particular food crops. This shows the risk to people, which consume contaminated foodstuff. It is defined as ratio of daily intake of metals in food crops to the oral reference dose (USEPA, US Environmental Protection Agency 2002). Therefore, health risk index was calculated as in the following equation:

$$\text{Health Risk Index (HRI)} = \text{daily intake of metals (DIM)}/$$
$$\text{oral reference dose (RfD)}$$

HRI higher than 1 for any metal in plant's edible part(s) is considered to be not safe for human health which can cause a serious health risk. However, HRI <1 means that the exposure to heavy metals by population is assumed to be safe.

Statistical analysis

The average concentrations of metal and metalloid in the wheat grains and soil were obtained. For both soil and grains

data, the SPSS software was used and one-way ANOVA was applied. Correlation between wheat grains and soil with respect to each metal concentration was determined.

Results and discussion

Concentration of metals

The concentrations of heavy metals (Cr, Cd, Ni, and Pb) and trace elements (Cu, Zn, and Fe) in wheat grains and soil are reported in Fig. 1. Results indicated that concentration of heavy metals in both soil and grains did not follow a regular trend. However, most of the metals showed higher concentrations when SW was used for irrigation, with only some exceptions.

The concentrations of heavy metals (as mg/kg) obtained in soil samples showed high concentrations, except for Zn which was much higher in wheat. In fact, Zn in grain was not satisfactorily predicted by total soil contents of Zn. Among the investigated metals, higher levels of Fe and Zn were observed in both soil and grain compared to the other metals. Most of metal levels in the SW irrigation water (used as a reference) resulted beyond the suggested reference values for maximum permissible level in irrigation water (Table 1). The same Table indicates also that Cd and Cu levels in GW exceed the maximum permissible level for irrigation water. In the soil, all studied elements were found to be below the maximum allowable limits compared to reference values (Table 2).

Concentration of metals in the water

The heavy metal concentrations in water used for wheat irrigation are reported in Table 1. To establish a baseline data for heavy metals found in our irrigation water, the average data from different studies on heavy metal concentrations in GW (Tariq et al. 2006) as well as in SW (Murtaza et al. 2010) from different nearby cities of Pakistan were used (Table 1). Since safe limits for heavy metals in irrigation water, soil, and food crops have not been established yet in Pakistan (Hassan et al.

Table 1 Average heavy metal concentration of different water combinations used for irrigation and average heavy metal concentration in ground (GW) and sewage (SW) water compared to the permissible maximum level (PML)

Treatment	Cd	Cr	Cu	Fe	Ni	Zn	Pb
T-I	0.530	0.332	2.422	6.181	0.669	10.836	0.871
T-II	0.598	0.410	1.629	6.519	0.527	10.845	0.635
T-II	0.467	0.252	1.589	4.488	0.784	12.826	0.635
T-IV	0.731	0.511	0.945	3.534	0.882	5.755	0.608
T-V	0.541	0.695	0.638	4.160	0.803	2.395	0.664
Irrigation water							
Metal	SW[a]	GW[a]	SW[b]		GW[c]	PML[d]	
Cd	0.584	0.530	0.041		0.03	0.010	
Cr	0.467	0.332	0.586		0.09	0.55	
Cu	1.200	2.422	0.223		0.36	0.20[e]	
Fe	4.675	6.181	1.167		0.08	5.00[e]	
Ni	0.749	0.669	0.471		0.88	1.40	
Zn	7.955	10.836	0.210		0.1	2.00[e]	
Pb	0.635	0.871	0.259		0.66	5.00[e]	

Data are expressed as mg l⁻¹

[a] Present study

[b] Murtaza et al. (2010)

[c] Tariq et al. (2006)

[d] Chiroma et al. (2014)

[e] Pescod (1992)

2013), heavy metals in this study were compared to permissible maximum limits from WHO/FAO and Ewers (1991), Standard Guidelines in Europe, taken from Chiroma et al. (2014) and Pescod (1992).

Concentration of metals in the soil

After the analysis of soils within treatments, heavy metal traces were recorded in all samples. A wide range of soil heavy metal concentrations were observed (Table 2). The results ranged from 1.19–2.5, 0.11–1.9, 0.25–1.93, 4.79–9.70, 0.53–2.59, 2.76–7.50, and 0.65–1.38 mg kg⁻¹ for Cd, Cr, Cu, Fe, Ni,

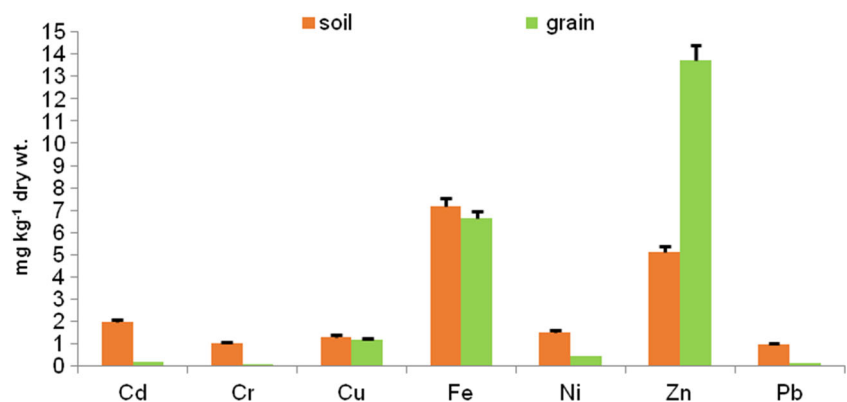**Fig. 1** Average heavy metal and trace element concentration in soil and grain

Table 2 Metal concentrations (mg kg^{-1} dry weight ± SE) in soil under the different treatments

Metals	Treatments					PML (µg/g)[a]
	T-I	T-II	T-III	T-IV	T-V	
Cd	1.69 ± 0.54	2.26 ± 0.38	2.25 ± 0.27	1.197 ± 0.12	2.5 ± 0.48	3
Cr	0.99 ± 0.096	1.34 ± 0.08	1.90 ± 0.26	0.92 ± 0.17	0.11 ± 0.27	100
Cu	0.97 ± 8.73	1.59 ± 0.29	1.93 ± 0.150	1.80 ± 0.23	0.25 ± 0.17	100
Fe	8.73 ± 0.88	9.70 ± 0.73	7.49 ± 0.71	5.08 ± 0.16	4.79 ± 0.79	50,000
Ni	0.96 ± 0.26	1.56 ± 0.11	1.92 ± 0.38	2.59 ± 0.24	0.53 ± 0.36	50
Zn	7.50 ± 0.23	6.51 ± 0.37	5.45 ± 0.36	2.76 ± 0.107	3.4 ± 0.21	300
Pb	0.650 ± 0.06	0.98 ± 0.15	1.38 ± 0.24	1.17 ± 0.163	0.767 ± 0.09	100

PML permissible maximum limit, *SE* standard error

[a] Sources: Ewers 1991 and Chiroma et al. (2014)

Zn, and Pb, respectively. The heavy metal concentrations were below the PML for soil (Ewers 1991 and Chiroma et al. 2014). The most prevalent heavy metals were Fe and Zn (7.16 and 5.12 mg kg^{-1}, respectively). Their concentrations were higher in soils irrigated with GW (T-I) and lower in T-V for Fe and in T-IV for Zn, respectively. In a previous study, Kansal et al. (1996) found that utilization of SW for irrigation of food crops extensively raises the accumulation of Zn, Cu, Pb, and Cd in soil from different localities of Punjab (Pakistan). In particular, the authors found that the concentration of Zn and Fe was greater in soils receiving SW of factories. This was in agreement with the average data reported in our study (Fig. 1), indicating that the concentrations of Zn and Fe are the highest in soil compared to the other metals.

In overall, the amount of Pb in soil was found to be the lowest among studied metals with the lowest value being reported in T-I. The other metals (Cd, Cr, Cu, and Ni) were reported in close concentrations (ranging between 1.05 and 1.98 mg kg^{-1}). Results obtained show that the mean concentrations of metals and metalloids in the soil of T-I treatment were Pb<Ni<Cu<Cr<Cd<Zn<Fe, whereas in T-II Pb<Cr<Ni<Cu<Cd<Zn<Fe. However, in T-III treatment, the mean concentrations were as follows: Pb<Ni<Cr<Cu<Cd<Zn<Fe. Finally, in T-IV and T-V, the metals levels were Cr<Pb<Cu<Cd<Ni<Zn<Fe and Cr<Cu<Pb<Ni<Cd<Zn<Fe, respectively (Table 2).

Concentration of metals in wheat

The comparison of mean concentration of metals in wheat grains under the five treatments indicated that the mean concentration (mg kg^{-1}) of Cr was the lowest, whereas Zn concentration was the highest (Table 3). As in the soil, the analysis data revealed that the most prevalent heavy metals in wheat were Fe and Zn (Fig. 1). However, the Zn concentration in the grain was higher than Fe (an opposite behavior was observed in the soil). This higher amount of Zn in wheat grain was far below the PML; therefore, the relatively small amount of Zn did not cause health problems. In fact, Pakistan agricultural research council (PARC) stated that small amount of Zinc in food products is necessary for human health. In particular, wheat variety having 50 % Zn has health benefits. Karatas et al. (2006) found that the average amount of Zn (22.31 mg kg^{-1}) was highest in wheat grains irrigated with wastewater. This was

Table 3 Metal concentrations (mg kg^{-1} dry wt. ± SE) in wheat grain irrigated with five different treatments

Metals	Treatments					PML (µg/g)[a]
	T-I	T-II	T-III	T-IV	T-V	
Cd	0.19 ± 0.037	0.18 ± 0.025	0.19 ± 0.03	0.16 ± 0.01	0.16 ± 0.024	0.10
Cr	0.063 ± 0.023	0.06 ± 0.036	0.08 ± 0.018	0.059 ± 0.02	0.078 ± 0.02	50[b]
Cu	1.04 ± 0.23	1.21 ± 0.154	1.97 ± 1.09	0.83 ± 0.058	0.97 ± 0.018	73
Fe	7.84 ± 1.7	8.43 ± 2.20	7.52 ± 3.33	4.16 ± 0.30	5.29 ± 1.25	425
Ni	0.468 ± 0.035	0.47 ± 0.03	0.36 ± 0.036	0.47 ± 0.11	0.46 ± 0.08	67
Zn	12.20 ± 2.68	15.14 ± 2.06	12.80 ± 1.48	16.85 ± 6.65	11.57 ± 0.36	100
Pb	0.11 ± 0.04	0.11 ± 0.007	0.12 ± 0.047	0.05 ± 0.02	0.29 ± 0.15	0.3

PML permissible maximum limit, *SE* standard error

[a] Source: Chiroma et al. (2014)

[b] Source: US-EPA, Environmental Protection Agency, US 1997

Table 4 Analysis of variance of data for metal contents in soil and wheat grain

Metals	Soil	Wheat grain
Cd	0.135***	0.001[ns]
Cr	0.036***	0.0001[ns]
Cu	0.064**	0.605[ns]
Fe	21.02***	10.055[ns]
Ni	0.011*	0.007[ns]
Zn	35.48***	14.688[ns]
Pb	0.040***	0.024[ns]

ns non-significant

*Significant at 0.05

**Significant at 0.01

***Significant at 0.001

Table 5 Heavy metal translocation factors (on dry weight basis) for wheat grown under different irrigated soils

Treatment	Cd	Cr	Cu	Fe	Ni	Zn	Pb
T-I	0.273	0.513	1.067	0.247	0.692	0.975	0.325
T-II	0.710	0.181	1.108	0.266	0.849	1.210	0.532
T-III	0.769	1.212	1.909	0.386	0.692	0.951	0.318
T-IV	0.851	0.476	1.036	1.197	0.798	2.174	0.316
T-V	0.308	0.374	1.298	0.240	0.858	2.947	0.124

not in line with our findings; however, the concentration value of Zn in wheat grain was lower. The lower value reported for Zn might be due to the fact that, in present study, there was less accumulation of toxic metals from smoke of industries. The lead level observed in the present study was similar to the findings of Karatas et al. (2006).

Among the treatments, the distribution of Zn indicated that the maximum levels in wheat grain samples were found in T-IV, whereas the highest concentration for Fe was reported in T-II (8.43 mg kg^{-1}). The other metals (Cd, Cu, Ni and Pb) reported a close average which ranged between the lowest (0.136 mg kg^{-1}) for Pb and the highest (1.204 mg kg^{-1}) for Cu. Trace of Cr metal was reported with the lowest concentration (0.068 mg kg^{-1}) among the studied metals in wheat grain samples (Fig. 1). Despite its lower amount compared to other metals, treatments with different combination of GW and SW show no significant effect on Cr concentration. Its presence in wheat grain across the treatments shows the lowest concentrations being reported in T-IV.

The general trend of the results shown in Table 3 indicated that the mean concentrations of Cd as well as Cu decreased, while Pb reached the maximum level when the amount of sewage water irrigation increased. In T-I, the concentration of metals and metalloids in grains was Cr<Pb<Cd<Ni<Cu<Fe<Zn. The

same metal concentration order was also reported in T-II and T-III. In T-IV, the mean concentration of metals and metalloids in grains was Pb<Cr<Cd<Ni<Cu<Fe<Zn. In wheat irrigated with 100 % SW, the mean concentration of metals and metalloids was as follows: Cr<Cd<Pb<Ni<Cu<Fe<Zn (Table 3).

For all studied metals (Cr, Cd, Cu, Ni, Fe, Zn, and Pb), the results showed no significant effect ($P > 0.05$) on metal and metalloid concentrations in wheat, while a significant effect on the metal concentration in the soil was observed (Table 4).

Calculation of parameters

Based on heavy metal concentration in soil and in wheat grain samples, the pollution load index (PLI), translocation/accumulation factor (AF), daily intake of metals (DIM), and health risk index (HRI) were calculated (Fig. 2).

Translocation factor

The study of heavy metal accumulation from SW by the crops is important, highlighting a direct possible effect on human health through the food chain. The data analysis of AF indicates that there was variability within treatments and across metals. It is believed that soil-metal levels, soil cation exchange capacity, soil types, and pH as well as crop varieties affect the uptake of metals by plants (Barancikova et al. 2004).

The present results suggest a general increase of metal uptake by wheat grains irrigated with SW. The maximum

Fig. 2 Comparison of mean average studied metals for the calculated parameters
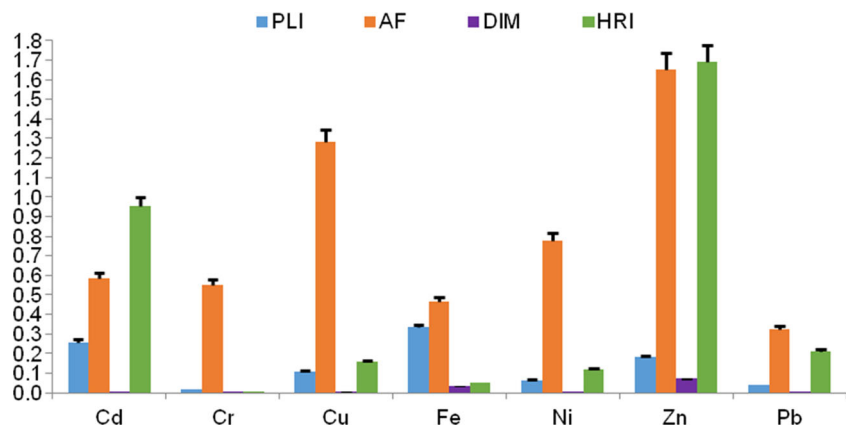
Table 6 Pollution load index values for wheat

Treatment	Cd	Cr	Cu	Fe	Ni	Zn	Pb
T-I	0.467	0.0135	0.117	0.557	0.074	0.283	0.043
T-II	0.176	0.038	0.130	0.557	0.061	0.283	0.059
T-III	0.172	0.007	0.123	0.342	0.057	0.305	0.047
T-IV	0.131	0.014	0.095	0.036	0.066	0.017	0.022
T-V	0.343	0.023	0.089	0.172	0.059	0.009	0.032

Table 8 Health risk index values

Treatment	Cd	Cr	Cu	Fe	Ni	Zn	Pb
T-I	1.011	0.0002	0.138	0.059	0.123	1.503	0.172
T-II	0.981	0.00023	0.160	0.063	0.126	1.867	0.169
T-III	1.046	0.0003	0.261	0.056	0.095	1.578	0.186
T-IV	0.887	0.0002	0.110	0.031	0.125	2.077	0.085
T-V	0.839	0.0004	0.129	0.040	0.122	1.427	0.445

accumulation value from soil to plants of metals was for Zn, indicating a strong accumulation of the respective metal by wheat (Table 5), while the lowest AF was reported for Pb. The higher value of Zn accumulation resulted in higher concentration in wheat grain compared to the other metals. Similar result was obtained by Jolly et al. (2013) who found that Zn had the highest AF in vegetables.

Data in Table 5 show that the AF values were lower for Pb, Fe, Cr, and Cd and higher for Zn, Cu, and Ni, respectively (with some exceptions). The variation in AF values could be due to differences in water quality used to irrigate wheat and the associated metal concentration. The trends of AF for heavy metals in wheat grain of T-I were Cu>Zn>Ni>Cr>Pb>Cd>Fe and in the order of Zn>Cu>Ni>Cd>Pb>Cr>Fe for T-II treatment. However, in T-III, the AF order was Cu>Cr>Zn>Cd>Ni>Pb>Fe, while for T-IV and T-V were Zn>Cu>Cd>Ni>Cr>Pb>Fe and Zn>Cu>Pb>Ni>Cr>Cd>Fe, respectively.

Pollution load index

Contamination level in soil can be explored using pollution load index (Angulo 1996). This index provides a simple and comparative means for assessing the quality of different combinations of water irrigation. As described by Tomlinson et al. (1980), a value of zero indicates no risk, whereas a value of one and values above one would indicate progressive deterioration of the site irrigated with this water quality. In the present study, PLI values for water-irrigated soil under the different treatments are reported in Table 6. All values were below one and ranged between the minimum of 0.007 for Cr in T-III and the maximum of 0.557 for Fe in T-I and T-II, respectively.

Among the studied metals, the PLI of Cr and Fe reported the lowest and the highest, respectively. In the case of average value across treatments, Fe and Zn were the highest distributed metals in both soil and wheat grain. In average, PLI values for the different metals have followed the order of: Cr<Pb<Ni<Cu<Zn<Cd<Fe. According to Ahmad et al. (2010), the order of PLI for heavy metals in soil was Pb>Cr>Ni>Cd>Fe>Cu>Zn. This was against the PLI order in the present study, where PLI value for Pb, Cr, and Ni was less and Zn, Fe, and Cu was higher. The order of contamination in T-I was Cr<Pb<Ni<Cu<Zn<Cd<Fe. In T-II, the order was Cr<Pb<Ni<Cu<Cd<Zn<Fe, whereas in T-III, T-IV, and T-V the order was Cr<Pb<Ni<Cd<Zn<Cu<Fe, Cr<Pb<Cd<Ni<Cu<Fe<Zn and Cr<Pb<Ni<Cd<Cu<Fe<Zn, respectively (Table 6).

Daily metal intake

To assess the health risk of a pollutant element, we estimated the possible exposure level of humans to each element through food chain pathways. In the present study, the intake pathway of the studied metals was assumed to be the consumption of wheat grain. In this regard, the DIM values were estimated according to the average wheat grain consumption by adults. The obtained data are reported in Table 7. Across treatments, the analysis between values of DIM revealed that Fe and Zn had the higher intake, whereas Cr and Pb showed the lower daily intake values. The DIM values obtained in this

Table 7 Values of daily metal intake (DIM, mg kg^{-1} person^{-1} day^{-1}) in wheat

Treatment	Cd	Cr	Cu	Fe	Ni	Zn	Pb
T-I	0.001	0.0003	0.005	0.041	0.0024	0.064	0.0006
T-II	0.000	0.0003	0.006	0.044	0.0025	0.080	0.0005
T-III	0.001	0.0004	0.010	0.039	0.002	0.067	0.0007
T-IV	0.000	0.0003	0.004	0.022	0.003	0.089	0.0003
T-V	0.008	0.0004	0.005	0.028	0.002	0.061	0.001

Table 9 Metal and metalloid correlation between soil and wheat

Correlation	
Metals	**Soil-grains**
Cd	-0.007
Cr	-0.267
Cu	0.605
Fe	0.962**
Ni	0.515
Zn	-0.198
Pb	-0.089

**Correlation was significant at $P < 0.01$

study (Table 7) were close to those reported by Mahmood and Malik (2014), where DIM was found to be higher for Zn, Ni, and Cu and lower for Cr, Pb, and Cd. The order of DIM and metalloids considering the average of the five treatments was Zn>Fe>Cu>Ni>Cd>Pb>Cr (Fig. 2).

Health risk index

Human risk assessment caused by eating polluted food through food chain pathway was carefully measured by health risk index (USEPA, US Environmental Protection Agency 2002; Cui et al. 2004 and Wang et al. 2005). The quantification of this index is of great importance especially in countries where the wastewater irrigation practice remains unregulated. If the value of HRI of a specific food material is <1, it was assumed to be safe for human consumption. However, if it is >1, serious threat to human health is possible (USEPA, US Environmental Protection Agency 2002).

In our study, the HRI was calculated under each treatment and metal. Results from the HRI calculation are presented in Table 8. These results revealed that HRI for Zn was the highest in all treatments, indicating a possible human health risk via the intake of food crop. This was in agreement with the findings of Bansal and Singh (2015) who found that the HRI value for Zn was higher than 1 in food materials. The HRI value for Cd in T-I and T-III also exceeded the permissible values, which may pose a potential threat to human health. The HRI of other metals (i.e., Cr, Cu, Fe, Ni, and Pb) reported lower-than-permissible values under all treatments, indicating no risk for human health. Among the studied elements, Cr reported the lowest values for HRI; therefore, metals (i.e., Cr, Cu, Fe, Ni, and Pb) indicate safe consumption for human. The order for health risk index of the metals in T-I and T-II was Cr<Fe<Ni<Cu<Pb<Cd<Zn. The order for health risk index of the metals in T-III was Cr<Fe<Ni<Pb<Cu<Cd<Zn. Metal concentration was in order of Cr<Fe<Pb<Cu<Ni<Cd<Zn and Cr<Fe<Ni<Cu<Pb<Cd<Zn in T-IV and T-V, respectively (Table 8).

Correlation soil-wheat and metal concentration

Results on correlation between soil-metal concentration and wheat-metal concentration showed positive non-significant correlation of Cu and Ni, and negative and non-significant correlation of Cd, Cr, and Zn. The only positive and significant correlation between wheat grains and soil was found in Fe (Table 9). In present study, results of the correlation for Fe and Cu were comparable to previous studies conducted by Ahmad et al. (2010), revealing that the correlation between soil and food crop was significant and positive for Fe, while it was non-significant for Cu.

Conclusions

Irrigation with polluted water may contaminate readily the soil and cultivated land. Wheat grain and soil samples showed significant deviation in mean concentrations of significant metals. The accumulation of heavy metals from soil to plant varied according to metals and treatment; however, they did not follow any particular pattern. The results of risk assessment suggest that Zn and Cd contamination in wheat grain had a potential health risk for consumers. Thus, with the increase of population and industrialization, the heavy metal concentrations in wastewater would linearly increase influencing also water quality. Despite the high concentration of some metals found also in ground water, this study should support the future political plans to increase the public awareness about the use of irrigation water with hazardous substances. This study can also help to establish a baseline data regarding environmental safety and suitability of sewage irrigation in future crop production. However, more wide-ranging sampling is needed to study such areas, and further investigation on contamination of other cereals is required.

References

Adnan S, Rashed M, Azmat HAK (2009) Water balance conditions of Potohar and Baluchistan Plateau from 1931 to 2008. World Appl Sci J 7(2):162–169

Ahmad MK, Islam S, Rahman S, Haque MR, Islam MM (2010) Heavy metals in water, sediment and some fishes of Buriganga River, Bangladesh. Int J Environ Res 4:321–332

Angulo E (1996) The Tomlinson pollution load index applied to heavy metal "mussel-watch" data: a useful index to assess coastal pollution. Sci Total Environ 187:19–56

Assche F, Clijsters H (1990) Effects of metals on enzyme activity in plants. Plant Cell Environ 24:1–15

Baig MB, Shahid SA, Straquadine GS (2013) Making rainfed agriculture sustainable through environmental friendly technologies in Pakistan: a review. Int Soil Water Conservation Res 1(2):36–52

Balkhair KS, Ashraf MA (2016) Field accumulation risks of heavy metals in soil and vegetable crop irrigated with sewage water in western region of Saudi Arabia. Saudi J Biol Sci 23:S32–S44

Bansal OP, Singh G (2015) Investigation of heavy metal status in soil and vegetables grown in sewage effluent water irrigated soils of Aligarh: a five year study. Res J Agri Environ Sci 2(2):10–15

Barancikova G, Madaras M, Rybar O (2004) Crop contamination by selected trace elements. J Soils Sediments 4:37–42

Bi X, Ren L, Gong M, He Y, Wang L, Ma Z (2010) Transfer of cadmium and lead from soil to mangoes in an uncontaminated area, Hainan Island, China. Geoderma 155(1–2):115–120

Bowman RA (1989) A rapid plant digestion method for analysis of P and certain cations by inductively coupled plasma emission spectrometry. Commun Soil Sci Plant Anal 20:539–553

Chiroma TM, Ebewele RO, Hymore FK (2014) Comparative assessment of heavy metal levels in soil, vegetables and urban grey waste water used for irrigation in Yola and Kano. Int Refereed J Eng Sci (IRJES) 3:01–09

Chojnacka K, Chojnacki A, Górecka H, Górecki H (2005) Bioavailability of heavy metals from polluted soils to plants. Sci Total Environ 337(1–3):175–182

Clarkson DT, Luttge U (1989) Mineral nutrition: divalent cations, transport and compartmentation. Progress Botany 51:93–112

Cui YJ, Zhu YG, Zhai RH, Chen DY, Huang YZ, Qui Y, Liang JZ (2004) Transfer of metals from near a smelter in Nanning, China. Environ Int 30:785–791

Ewers U (1991) Standars, guidelines and legislative regulations concerning metals and their compounds. In: Merian E (ed) Metals and their compounds in the environment: ocurrence, analysis and biological relevance. VCH, Weinheim, pp. 687–711

Falkenmark M, Dolden D (2008) Wake up to realities of River Basin closure. Water Resour Dev 24(2):201–215

Farooq U, Munir A, Jasra AW (2007) Natural resource conservation, poverty alleviation, and farmer partnership. The Pakistan Development Review, Part I 46(4):1023–1049

Hassan NU, Mahmood Q, Waseem A, Irshad M, Faridullah, Pervez A (2013) Assessment of heavy metals in wheat plants irrigated with contaminated wastewater. Pol J Environ Stud 22(1):115–123

Jadia CD, Fulekar MH (2009) Phytoremediation of heavy metals: recent techniques. Afr J Biotechnol 8(6):921–928

Jolly, Y.N., Islam, A., and Akbar, S. 2013. Transfer of metals from soil to vegetables and possible health risk assessment. Springer Plus, 2: 385.

Kansal BD, Kumar R, Sokka R (1996) The influence of municipal wastes and soil properties on the accumulation of heavy metals in plants. In: Heavy metals in the environment. CEC Consultants Ltd., Edinburg, pp. 413–416

Karatas M, Dursun S, Guler E, Ozdemir C, Argun ME (2006) Heavy metal accumulation in wheat plants irrigated by wastewater. Cellul Chem Technol 40(7):575–579

Khan ZI, Ahmad K, Ashraf M, Parveen R, Bibi Z, Mustafa I, Yaqoob R et al (2016a) Risk assessment of heavy metal and metalloid toxicity through a contaminated vegetable (Cucurbita maxima) from wastewater irrigated area: a case study for a site-specific risk assessment in Jhang, Pakistan. Human Ecol Risk Assess Int J 22(1):86–98

Khan ZI, Ahmad K, Ashraf M, Shoaib N, Parveen R, Bibi Z, Ullah MF et al (2016b) Assessment of toxicological health risk of trace metals in vegetables mostly consumed in Punjab, Pakistan. Environ Earth Sci 75(5):1–5

Li Q, Chen Y, Fu H, Cui Z, Shi L, Wang L, Liu Z (2012) Health risk of heavy metals in food crops grown on reclaimed tidal flat soil in the Pearl River Estuary. China J Hazard Mater 227–228:148–154

Liu WH, Zhao JZ, Ouyang ZY, Soderlund L, Liu GH (2005) Impacts of sewage irrigation on heavy metals distribution and contamination in Beijing, China. Environ Int 31:805–812

Lone MI, Saleem S, Mahmood ST, Saifullah K, Hussain G (2003) Heavy metal contents of vegetables irrigated by sewage/tube well water. Int J Agric Biol 5:533–535

Luo Y, Rimmer DL (1995) Zinc-copper interaction affecting plant growth on a metal-contaminated soil. Environ Pollut 88(1):79–83

Mahmood A, Malik RN (2014) Human health risk assessment of heavy metals via consumption of contaminated vegetables collected from different irrigation sources in Lahore, Pakistan. Arab J Chem 7(1): 91–99

Mehrdadi N, Joshi HG, Nasrabadi T, Hoveidi H (2007) Application of solar energy for drying of sludge from pharmaceutical industrial waste water and probable reuse. Int J Environ Res 1(1):42–48

Murtaza G, Ghafoor A, Qadir M, Owens G, Aziz MA, Zia MH, Saifullah (2010) Disposal and use of sewage on agricultural lands in Pakistan: a review. Pedosphere 20(1):23–34

Palese AM, Pasquale V, Celano G, Figliuolo G, Masi S, Xiloyannis C (2009) Irrigation of olive groves in southern Italy with treated municipal waste water: effects of microbiological quality of soil and fruits. Agric Ecosyst Environ 129:43–51

PCST, Pakistan Council for Science and Technology. 2005 Development and management of water resources. Report of the Expert Committee, Islamabad. pp 1–256

Pescod MD (1992) Wastewater treatment and use in agriculture. In: Food and agricultural organization (FAO) irrigation and drainage paper 47. FAO, Rome

Qadir M, Wichelns D, Raschid-Sally L, McCornick PG, Derchsel P, Bhari A, Minhas PS (2008) The challenges of wastewater irrigation in developing countries. Agric Water Manag 97:561–568

Raskin I, Kumar PBAN, Dushenkov S, Salt DE (1994) Bioconcentration of heavy metals by plants. Curr Opin Biotechnol 5(3):285–290

Salgare SA, Acharekar C (1992) Effect of industrial pollution on growth and content of certain weeds. J Nat Conserv 4:1–6

Shah AH, Gill KH, Syed NI (2011) Sustainable salinity management for combating desertification in Pakistan. Int J Water Resour Arid Environ 1(5):312–317

Singh KP, Mohon D, Sinha S, Dalwani R (2004) Impact assessment of treated/untreated wastewater toxicants discharge by sewage treatment plants on health, agricultural, and environmental quality in wastewater disposal area. Chemosphere 55:227–255

Taiz L, Zeiger E (2002) Plant physiology, Fifth edn. Sinauer Associates, Sunderland

Tariq M, Ali M, Shah Z (2006) Characteristic of industrial effluents and their possible impacts on quality of underground water. Soil Environ 25(1):64–69

Tomlinson L, Wilson G, Harris R, Jeffrey DW (1980) Problems in the assessments of heavy-metal levels in estuaries and formation of a pollution index. Helgoländer Meeresun 33:566–575

USEPA (US Environmental Protection Agency) (2002) Exposure factors handbook. Volume II-food ingestion factors. EPA/600//P-95/002Fa. Office of Research and Development, Washington

US-EPA, Environmental Protection Agency (US) (1997) Exposure factors handbook. Volume II-food ingestion factors. EPA/600//P-95/002Fa. Office of Research and Development, Washington

Wang X, Sato T, Xing B, Tao S (2005) Health risks of heavy metals to the general public in Tianjin, China via consumption of vegetables and fish. Sci Total Environ 350(1):28–37

Wang G, Su M, Chen Y, Lin F, Luo D et al (2006) Transfer characteristics of cadmium and lead from soil to the edible parts of six vegetable species in southeastern China. Environ Pollut 144:127–135

Wilson B, Pyatt FB (2007) Heavy metal dispersion, persistence, and bioaccumulation around an ancient copper mine situated in Anglesey. UK. Ecotoxicol Environ Saf 66:224–231