



Evaluation of Potential Toxic Metals Accumulation in Wheat Irrigated with Wastewater

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Abstract

The present study was carried out to ascertain the level of various metals in wheat variety (Chagi-4) irrigated with diverse doses of wastewater. The concentration of metals in soil, water and wheat grain samples was examined through an atomic absorption spectrophotometer. In wheat grains, the mean values of metals (mg/kg) varied from 0.06 to 0.2 for Pb, 1.2 to 1.6 for Cd, 0.6 to 0.9 for Ni, 0.8 to 1.6 for Fe, 0.4 to 1.0 for Mn, 0.7 to 1.4 for Cu, 0.3 to 0.5 for Cr, 0.1 to 0.9 for Zn and 0.03 to 0.2 for Co, correspondingly. Measured concentrations were found within the permissible limit given by FAO/WHO except for cadmium whose concentration exceeded an acceptable limit 0.2 mg/kg suggested by FAO/WHO. It might be due to high soil pH, which hinders the efficient transfer of metals between different mediums. Wastewater irrigated soil, wheat and water had high metal values, but the low rate of transfer was noticed from soil to grains. Higher bioconcentration factor was obtained for manganese and cadmium; cadmium had even higher pollution load index, which could indicate the contamination status of soil. Therefore, regular monitoring of wastewater is necessary to prevent the excessive build-up of metals.

Keywords Wastewater · Permissible limit · Bioconcentration factor · Potential toxic metal · *Triticum aestivum*

Water is an indispensable part of our life, but it is a vulnerable and limited resource that has qualitative susceptibility and quantitative limits (Ugulu 2015; Khan et al. 2018a). It is predicted that, by the year of 2050 more than 60% of the world's population face the problem of water scarcity (Rijsberman 2006). The places where scarcity of freshwater is very high, wastewater is an alternative source for irrigating crops (Khan et al. 2018b). In many countries, wastewater is not legal for irrigation of croplands because wastewater is contaminated with trace metals such as Cr, Pb, Mn, Zn, Co,

and Cu and causes various toxic effects on plants, animals, and humans (Ugulu et al. 2016; Khan et al. 2018c). Continuous use of treated and untreated wastewater in agricultural land considerably increases the potential toxic metal content of soil, cereals and vegetables and these metals are further transferred to food chain causing various health hazards to human (Durkan et al. 2011; Ugulu et al. 2012; Dogan et al. 2014a).

Wheat (*Triticum aestivum* L.) is a staple food in Pakistan and a major component of the diet. Wheat is grown all over the Pakistan ranging from 23° North to 37° North, from 61° East to 76° East. Wheat is cultivated over an area of 9,042,000 ha and annual production amounts to 2,300,000 tons with an average production of 2714 kg/ha in Pakistan (GOP 2012). Wheat is fulfilling the dietary needs of one third of the world's population and has more nutritional value as compared to other cereals. Wheat provides a good quantity of protein, vitamins and minerals (Shewry 2009).

Large amounts of metals are taken up by a wheat crop irrigated with wastewater and cause various health hazards to human (Ahmad et al. 2018; Nadeem et al. 2019). Toxic effects of metals result in lower energy levels, damaged or reduced mental and central nervous system functions and

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damage to blood composition, liver, lungs, kidneys and other important organs (Dogan et al. 2014b; Unver et al. 2015).

In this study, we hypothesized that wastewater irrigation may inhibit or enhance the uptake of metals by wheat plant. Therefore, the present study was planned with the objectives: (1) to analyse the physicochemical parameters of the soil, (2) to explore potential toxic metal content in wheat irrigated with wastewater, (3) to evaluate the transfer of metals from soil to the wheat plant, (4) to determine the pollution load index (PLI).

Materials and Methods

The experiment was carried out at Botanical Garden in Sargodha City, Pakistan during 2015–2016. The district has extreme climatic conditions in summer with a maximum temperature fluctuating between 45 and 50°C. Winters are cooler with temperature ranging from 4 to 25°C.

Wheat variety (Chagi-4) grains were sown in November, 2015 in plastic pots (15 cm in height and 25 cm in diameter) and harvested in April, 2016. Each pot was filled with about 6 kg of soil. Twelve pots with three replicates of various treatments were prepared. 10 seeds were sown in each pot. These pots were irrigated with domestic wastewater with different combinations: T-I (ground water), T-II (50% ground water + 50% wastewater), T-III (25% ground water + 75% wastewater), and T-IV (wastewater). The pots were irrigated twice a week. The untreated domestic wastewater was taken from residential wastewater treatment plant in Sargodha. The experiment was laid down in completely randomized design (CRD). Threshing was done in April, 2016. Soil samples were taken from the upper profile of soil. Samples of soil and grains were dried in an oven for 48 h at 105°C. After drying grain samples were ground into powder.

The physicochemical characters of water and soil samples such as electrical conductivity (EC), pH, calcium, magnesium, and organic matter was determined. The electrical conductivity and pH of samples were calculated by EC meter and pH meter. Concentrations of Ca^{+2} and Mg^{+2} were determined by using the titration method.

Soil organic matter (OM) was determined by Walkley and Black acid digestion method (Page 1982). Available K and P were calculated by following Olsen and Sommers (1982).

Acid/wet digestion method was used for chemical analysis of potential toxic metals. Water samples (50 mL) were digested with 10 mL of concentrated HNO_3 at 80°C (APHA 2005). The sample was made up to 50 mL by adding deionized water. One gram of ground powder of each soil and grains was put into a 50 mL beaker. 10 mL analytical grade H_2SO_4 and 4 mL HClO_4 were added in the beaker and digested. Final volume was made up to 50 mL by adding deionized water.

In this study, lead (Pb), cadmium (Cd), nickel (Ni), iron (Fe), manganese (Mn), copper (Cu), chromium (Cr), zinc (Zn), and cobalt (Co) were analysed. The concentration of metals in all samples of soil, water, grains was analysed by flame atomic absorption spectrophotometer (AAS; GBC 932). An atomic absorption spectrophotometer equipped with a graphite furnace and D2 corrector (Perkin–Elmer Model 503) was used to determine Co contents. Operating conditions for determination of metals are given in Table 1. Precision and accuracy of analyses was guaranteed through repetitive samples against National Institute of Standard Technology, Standard reference material (SRM 1643e for water, SRM 2709 for soil, CRM-NIST 1567a for wheat) for all metals. The results were found within $\pm 2\%$ of the certified value. The glass wares were placed in 10% nitric acid overnight and rinsed several times with deionized water before used to prevent it from contamination.

Bioconcentration factor (BCF) calculated in the current study was based on the total metal content of the plant and metal content of soil (Cui et al. 2004). Pollution load index (PLI) was calculated as metal content of soil against standard metal contents (Liu et al. 2005).

Analysis of variance was implemented for treatments in support of water, soil and wheat grains with the help of the software package SPSS 21. The correlation between soil and wheat grains regarding each potential toxic metal was calculated.

Table 1 Operating conditions for the analysis of metals using flame atomic absorption spectrometry

Element	Pb	Cd	Ni	Fe	Mn	Cu	Cr	Zn	Co
Wavelength (nm)	283.3	228.8	232.0	248.3	279.5	324.8	422.7	213.9	240.7
Slit width (nm)	0.7	0.7	0.2	0.2	1.0	0.7	0.7	0.7	0.2
Lamp current (mA)	10	8	12	12	9	6	10	8	7
Air flow rate (L/min)	15	15	15	15	15	15	15	15	15
Acetylene flow rate (L/min)	2.0	1.8	1.6	0.9	1.0	1.8	2.8	2.0	1.5
Burner height (mm)	7	7	7	7	7	7	9	7	7

Table 2 Physicochemical parameters of water samples (n = 12) used to irrigate wheat. Sources: ^aMWE (2005), ^bFAO (1985)

Treatment	Physicochemical parameters			
	EC (dS/m)	Ca ²⁺ , Mg ²⁺	Na ⁺	Cl ⁻
	meq/L			
T-I	2.51 ± 1.3	6.46 ± 1.4	10.99 ± 1.4	6.83 ± 1.1
T-II	4.50 ± 1.8	11.36 ± 1.6	30.46 ± 1.6	20.23 ± 1.5
T-III	6.91 ± 1.4	18.33 ± 1.4	40.26 ± 1.8	31.33 ± 1.12
T-IV	8.18 ± 2.1	25.79 ± 1.7	51.13 ± 1.7	45.54 ± 1.6
Standard limits	5.1 ^a	200 ^a , 150 ^a	900 ^b	–

Results and Discussion

Physicochemical parameters of water observed in the present study were shown on the Table 2. The values of EC ranged from 2.51 to 8.18 dS/m among all treatments. The values of Ca²⁺ and Mg²⁺ among four treatments were 6.46, 11.36, 18.33 and 25.79 meq/L respectively. Level of Na⁺ was highest (51.13 meq/L) at T-IV while the minimum level (10.99 meq/L) was present at T-I. The range of chloride (Cl⁻) in four treatments was 6.83–45.54 meq/L (Table 2). Electrical conductivity determines the total ionic concentration of water. The values of EC in present investigation were lower while calcium, magnesium and sodium were higher as compared to the values reported by Kumar et al. (2015). Yang et al. (2015) reported higher values of electrical conductivity while lower concentrations of chloride ion as compared to the present investigation.

Physicochemical parameters of soil observed in the present study are shown on the Table 3. Loamy soil was

observed in four treatments with pH ranging from 8 to 7.6. Highest pH value was found at T-I while the lowest values were observed at T-IV. Mean concentrations of EC among the four treatments ranged from 9.28 to 16.51 dS/m. The range of OM was between 0.76%–1.18%. The values of P were 7.56, 11.5, 14.06, 14.06 and 16.1 mg/kg respectively. The mean values of available K were 313, 329, 347, and 358 mg/kg respectively (Table 3). Organic content and pH are main physicochemical properties of soil. Soil pH directly influences the accessibility of potential toxic metals in the soil and capacity of plants to uptake metals (Zeng et al. 2011). Wastewater irrigation considerably decreases the pH of soil. Low pH values increase the availability of potential toxic metals in soil (Ugulu and Baslar 2010; Zhao et al. 2015). In the current study, values of available P and available K were lower while pH and EC were higher as compared to the findings of Salakinkop and Hunshal (2014).

Treatments significantly affected concentrations of Co, Ni, Cr, Fe, Cu, Mn, Pb, Cd in grains, Cd, Zn, Co, Mn, Fe, Cu, Pb, Cr, Ni, Fe in water and Fe, Zn, Mn, Cr, Pb, Cd, Cu, Co in soil (Table 4).

In water, the mean values of potential toxic metals (mg/kg) were higher for Ni and lower for Pb (Table 5). Wastewater irrigation is common practice in developing countries and causes a metal surge in crops grown there to become the part of the food chain (Singh and Garg 2006). In the present investigation, Co, Pb, Ni, Mn, Cd, Cu, Cr, Zn, and Pb values in water were above the permissible limit suggested by WWF (2007) and (FAO 1985). The values of Pb, Cd, Ni, Fe, Mn, Cu, Cr, and Zn in the present investigation were higher than the values reported by

Table 3 Post-harvest physicochemical parameters of soil samples (n = 12)

Treatment	Physicochemical parameters						Texture
	pH	EC (dS/m)	Organic matter (%)	Available phosphorus (mg/kg)	Available potassium (mg/kg)		
T-I	8.0 ± 0.03	9.28 ± 0.01	0.76 ± 0.01	7.56 ± 0.12	313 ± 0.15	Loam	
T-II	7.5 ± 0.12	10.25 ± 10.01	0.84 ± 0.01	11.35 ± 0.16	329 ± 0.57	Loam	
T-III	7.7 ± 0.14	12.71 ± 0.08	1.12 ± 0.01	14.06 ± 0.08	347.6 ± 0.88	Loam	
T-IV	7.6 ± 0.08	16.51 ± 0.18	1.18 ± 0.08	16.1 ± 0.11	358.6 ± 1.20	Loam	

Table 4 Analysis of variance of potential toxic metals in water, soil and grains

Metal	Mean square								
	Pb	Cd	Ni	Fe	Mn	Cu	Cr	Zn	Co
Water	0.003***	0.024***	0.049**	37.425**	0.052**	0.027***	0.005 ^{ns}	38.211**	0.016***
Soil	0.022***	0.009***	0.070 ^{ns}	95.065**	0.004***	0.328***	0.052**	0.322**	0.020***
Wheat grains	0.023***	0.113**	0.045*	0.360**	0.249***	0.333***	0.020**	0.339 ^{ns}	0.020***

^{ns} non-significant

*, **, *** Significant at 0.05, 0.01, 0.001 levels

Table 5 Mean concentration of metals in irrigation water, soil and in grains of *T. aestivum*
Sources: ^aWWF (2007), ^bFAO (1985), ^cUSEPA (2010), ^dEuropean Union (2006), ^eUSEPA (1997), ^fFAO/WHO (2001)

Metal	Treatment				Permissible limit
	T-I	T-II	T-III	T-IV	
Irrigation water (mg/L)					
Pb	0.141 ± 0.12	0.282 ± 0.17	0.367 ± 0.23	0.51 ± 0.3	0.10 ^a
Cd	1.521 ± 0.2	1.691 ± 0.27	1.856 ± 0.38	1.879 ± 0.25	0.01 ^a
Ni	1.020 ± 0.11	1.046 ± 0.24	1.080 ± 0.16	1.300 ± 0.23	0.20 ^a
Fe	2.212 ± 0.42	4.989 ± 0.49	7.598 ± 0.4	9.451 ± 0.7	5 ^a
Mn	0.384 ± 0.2	0.463 ± 0.3	0.572 ± 0.5	0.683 ± 0.3	0.20 ^a
Cu	0.542 ± 0.17	0.610 ± 0.38	0.678 ± 0.21	0.767 ± 0.32	0.20 ^a
Cr	0.551 ± 0.16	0.579 ± 0.2	0.588 ± 0.27	0.653 ± 0.3	0.10 ^b
Zn	1.612 ± 0.28	5.324 ± 0.6	7.789 ± 0.5	9.425 ± 0.21	2 ^b
Co	0.321 ± 0.09	0.369 ± 0.13	0.450 ± 0.21	0.471 ± 0.17	0.05 ^a
Soil (mg/kg)					
Pb	0.193 ± 0.07	0.301 ± 0.09	0.350 ± 0.1	0.391 ± 0.12	300 ^c
Cd	1.903 ± 0.1	1.935 ± 0.14	1.968 ± 0.15	2.030 ± 0.17	3 ^c
Ni	1.260 ± 0.08	1.315 ± 0.12	1.358 ± 0.12	1.605 ± 0.19	50 ^d
Fe	2.795 ± 0.1	3.356 ± 0.13	3.873 ± 0.14	4.044 ± 0.2	21000 ^e
Mn	0.538 ± 0.06	0.576 ± 0.09	0.600 ± 0.12	0.630 ± 0.12	2000 ^d
Cu	1.198 ± 0.08	1.461 ± 0.13	1.711 ± 0.17	1.968 ± 0.21	50 ^c
Cr	1.053 ± 0.09	1.146 ± 0.11	1.188 ± 0.14	1.368 ± 0.15	50 ^f
Zn	1.406 ± 0.13	1.640 ± 0.15	1.696 ± 0.2	2.186 ± 0.1	200 ^c
Co	0.610 ± 0.09	0.661 ± 0.11	0.708 ± 0.12	0.800 ± 0.15	100 ^f
Grain (mg/kg)					
Pb	0.068 ± 0.02	0.123 ± 0.02	0.170 ± 0.04	0.273 ± 0.03	0.3 ^f
Cd	1.248 ± 0.04	1.333 ± 0.05	1.396 ± 0.07	1.695 ± 0.06	0.2 ^f
Ni	0.631 ± 0.04	0.721 ± 0.05	0.801 ± 0.04	0.921 ± 0.07	67 ^f
Fe	0.825 ± 0.07	1.231 ± 0.07	1.326 ± 0.08	1.666 ± 0.06	425.5 ^f
Mn	0.400 ± 0.06	0.696 ± 0.07	0.883 ± 0.08	1.076 ± 0.08	500 ^f
Cu	0.711 ± 0.05	0.846 ± 0.08	1.108 ± 0.1	1.468 ± 0.06	73.3 ^f
Cr	0.386 ± 0.03	0.423 ± 0.07	0.466 ± 0.06	0.573 ± 0.09	2.3 ^f
Zn	0.101 ± 0.04	0.343 ± 0.07	0.398 ± 0.07	0.901 ± 0.09	99.4 ^f
Co	0.031 ± 0.02	0.081 ± 0.04	0.148 ± 0.04	0.218 ± 0.06	50 ^f

Balkhair and Ashraf (2016). Iqbal et al. (2016) reported higher values of Cr, Pb, and the lower value of Ni, Cd than the present research.

In soil samples, the mean values (mg/kg) of potential toxic metals fluctuated from 0.19 to 0.39 for Pb, 1.90 to 2.03 for Cd, 1.26 to 1.60 for Ni, 2.79 to 4.04 for Fe, 0.53 to 0.63 for Mn, 1.19 to 1.96 for Cu, 1.05 to 1.36 for Cr, 1.40 to 2.18 for Zn and 0.61 to 0.80 for Co respectively (Table 5). The mean values of all metals fell within the maximum permissible limit (MPL) of Pb, Cd, Ni, Fe, Mn, Cu, Cr, Zn, and Co recommended by USEPA (1997), FAO/WHO (2001), European Union (2006) and USEPA (2010). This could be due to the high pH of soil because transfer of potential toxic metals from soil to plant mainly depends on soil pH. Availability of metals in plant decreases at pH value greater than 6.5 (Harrison and Waites 1998). Ni, Cd, and Cr contents were above and that of Zn was below the reported values by Iqbal et al. (2016). Mahmoud and Ghoneim (2016) reported

high contents (mg/kg) of Fe, Zn, Mn, Cd, Pd, Ni, Cu, in soil than the present study. Liu et al. (2016) reported high contents of Pb, Cr and low contents of Cd than the present findings. Li et al. (1994) suggested that plants absorb Cd more readily as compared to other metals and the level of Cd in plants exceeds the maximum value that is dangerous to human health.

In wheat grains, the mean values of metals (mg/kg) varied from 0.06 to 0.2 for Pb, 1.2 to 1.6 for Cd, 0.6 to 0.9 for Ni, 0.8 to 1.6 for Fe, 0.4 to 1.0 for Mn, 0.7 to 1.4 for Cu, 0.3 to 0.5 for Cr, 0.1 to 0.9 for Zn and 0.03 to 0.2 for Co, correspondingly (Table 5). Concentration of Cd in the present investigation was greater than MPL 0.2 mg/kg given by FAO/WHO (2001). Due to its high mobility Cd is readily taken up by plants. To the human body, accumulation of cadmium leads to certain disorders, including liver and nervous system and cardiovascular diseases (Tataruch and Kierdorf 2003). Total concentrations of potential toxic metals

detected in wheat grain were lower than the MPL except for Cd (FAO/WHO 2001). This might be due to inconsistency in physical and chemical properties of soil and variation in ability of the plant to uptake metals (Khan et al. 2018d). In current results, Cd concentration was higher and Cu, Zn were lower than the values reported by Yu et al. (2016). The results from the present study and earlier report given by Liu et al. (2005) confirmed that plants grown on wastewater irrigated soil were contaminated with potential toxic metals and cause various health hazards in human. The absorption and accumulation of potential toxic metals in plant tissue depend on many factors such as pH, OM, nutrient availability, moisture and temperature, however, the presence of organic matter content has been reported to increase the uptake of metals in wheat plants (Rupa et al. 2003; Dogan et al. 2010).

A positive and significant correlation of Pb, Cd, Ni, Fe, Mn, Cu, and Co showed a strong association (Table 6). This might be due to their common source in wastewater. The positive and non-significant relationship of Cr and Zn showed a weak relationship of metals. This showed the imbalance of Cr and Zn between soil and wheat grains. The correlation between soil and wheat grain depends on the accessibility of metals in soil. Current results were similar to the findings of Ahmad et al. (2018).

The translocation competency of potential toxic metals from soil to edible parts of plants was determined by

BCF. The results illustrated that Mn showed the highest BCF value, while Co showed the lowest value among all treatments (Table 7). Bioconcentration factor is the key component to determine the human exposure to potential toxic metals via the food chain. The BCF values for Mn was higher than 1 in T-II, T-III and T-IV while those of all other metals were less than 1 in all treatments. BCF factor ≤ 1 demonstrated that the plant could just absorb metals however, did not accumulate metals; BCF > 1 demonstrated that plants might potentially and readily accumulate metals (Singh et al. 2011).

To estimate the soil contamination status with metals, PLI was determined. In the present investigation, the value of PLI for Cd was higher among all treatments (Table 8). The PLI was calculated as the concentration of metals in wastewater irrigated soil with respect to reference values. The value of PLI of all potential toxic metals was below than the reference values (mg/kg) of Pb (8.15), Cd (1.49), Ni (9.06), Fe (56.90), Mn (46.75), Cu (8.39), Cr (9.07), Zn (44.19) and Co (9.1) (Dutch Standards 2000; Dosumu et al. 2005; Singh et al. 2010). Pollution load index due to Cr was high, but due to Mn, Fe, Pb, and Cd was less at present work than given by Ashfaq et al. (2015). The value of PLI for Cd was higher than one indicating that environmental risk can be caused by Cd.

In the backdrop of reducing freshwater resources and shortage of rains due to global climate change, the focus

Table 6 Correlation between soil and grain potential toxic metal concentrations

Correlation									
Metals	Pb	Cd	Ni	Fe	Mn	Cu	Cr	Zn	Co
Soil-Grains	0.782**	0.905**	0.615*	0.891**	0.741**	0.772**	0.940 ^{ns}	0.512 ^{ns}	0.863**

^{ns} non-significant

*, **Correlation is significant at 0.05 and 0.01 levels

Table 7 Bioconcentration factor for wheat grain/soil system

Treatment	Metal								
	Pb	Cd	Ni	Fe	Mn	Cu	Cr	Zn	Co
T-I	0.3523	0.6558	0.5453	0.2951	0.7434	0.5934	0.3665	0.0718	0.0508
T-II	0.4086	0.6888	0.5710	0.3668	1.2083	0.5790	0.3691	0.2091	0.1225
T-III	0.4857	0.7093	0.6187	0.3423	1.4716	0.6475	0.3922	0.2346	0.2090
T-IV	0.6982	0.8349	0.6189	0.4119	1.7079	0.7459	0.4188	0.4121	0.2725

Table 8 Pollution load index for potential toxic metals in soil

Treatment	Metal								
	Pb	Cd	Ni	Fe	Mn	Cu	Cr	Zn	Co
T-I	0.0236	1.2771	0.1390	0.0491	0.0115	0.1427	0.1160	0.1550	0.0670
T-II	0.0369	1.2986	0.1451	0.0589	0.0123	0.1741	0.1263	0.1808	0.0726
T-III	0.0429	1.3208	0.1498	0.0680	0.0128	0.2039	0.1309	0.1869	0.0778
T-IV	0.0479	1.3624	0.1771	0.0710	0.0134	0.2345	0.1508	0.2410	0.0879

is shifting on alternate sources of irrigation like municipal wastewater. This study has highlighted potential implications and threats of using wastewater for crop production. Bio-availability of metals to plants results in buildup of metals in edible plant tissues up to toxic levels. In wheat grains, level all metals were found within the permissible limit given by FAO/WHO, except for Cd whose concentration exceeded the permissible levels and high pollution load index was reported for Cd indicating toxicity and contamination of grains. Continuous soil monitoring is required to keep a check on metal levels in wheat crop.

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