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Assessment of Heavy Metals in Wheat Variety ‘‘Chagi-2’’ under Short-Term Wastewater Irrigation

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ABSTRACT

The increased utilization of wastewater has raised the level of heavy metals in the crops. Concentrations of heavy metals (Cu, Pb, Cd, Ni, Mn, Zn, Cd, Co, and Fe) in soil, water, and wheat grains using different treatments of wastewater and ground water were determined in present pot experiment conducted in Sargodha, Pakistan. The current study also focused on the evaluation of bioconcentration factor and pollution load index of the contaminated soil. Most of the metals observed in wastewater were present above the permissible limit set by WWF. Cd concentration increased considerably under wastewater irrigation in soil and wheat grains. Zn showed highest bioconcentration. Wastewater irrigated soil exhibited the maximum Pollution Load Index (PLI) values as compared to control treatment. It is recommended that wastewater must be treated properly before agricultural use to reduce accumulation of heavy metals in the cereal crops and implement safe practices for wastewater usage.
Key Words: Bioconcentration factor: Wastewater: Permissible maximum limit: Wheat **(***Triticum aestivum* L.).

INTRODUCTION

The wastewater irrigation not only affects the soil properties but also promotes increased heavy metal uptake by the crops thereby posing threats to the standard and safety of food owing to the high levels of heavy metals in the agricultural soil (Muchuweti*et al.,* 2006; Khan *et al.*, 2018). The major source of heavy metal contamination of groundwater and soil is wastewater irrigation (Bassanino *et al*., 2007; Ahmad *et al.,* 2018). Anthropogenic activities are also major route of heavy metal contamination (Dogan *et al.,* 2014; Ugulu 2015; Ugulu *et al.*, 2016). Heavy metals are present in large amount in the environment as a result of natural and human activities and affecting human life in various ways (Wilson &Pyatt, 2007; Ugulu*et al.*, 2012; Unver *et al*., 2015).

Major source of food are the cereals in many countries. Wheat (*Triticum sp.*), corn (*Zea mays*), barley (*Hordeum vulgare*), oat (*Avena sativa*), rice (*Oryza sativa*) and rye (*Secale cereale*) are the principal cereals, making the human diet complex. But wheat is widely spread and consumed (Araujo *et al*., 2007). Cereals are enriched with vitamins, fibers and trace minerals, and are supposed to be involved controlling many diseases. Today, their daily consumption is thus suggested. Among the important varieties of wheat, most cultivated ones are soft wheat (*Triticum aestivum*) and hard wheat (*Triticum durum*). *Triticum aestivum* can be grown in temperate or hot areas and in comparison, to hard wheat, it has more phosphorus, starch, iron, vitamin B and fats .

Heavy metals accumulate in the environment due to their long biological half-life and non-biodegradable nature (Cui *et al*., 2005; Durkan et al., 2011). High pH of the soil increases heavy metal mobility (Kukier *et al*., 2004 and Basta *et al*., 2005). Some of the most problematic heavy metals are

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arsenic, chromium, lead, nickel, cadmium, mercury, beryllium, selenium and manganese (Topbas *et al.,* 1998). 15 to 20 times reduced enzymatic activity was reported in the soil due to increased concentration of heavy metals (Chander *et al*., 1995).

The high concentration of heavy metals in the wheat due to its high consumption can create potential health risk (Doe *et al*., 2013). Usually top soil exhibit high concentrations of heavy metals like Zn, Pb, Cd, Ni, and Cr using irrigated wastewater (Mishra *et al*., 2009). The sewage irrigation may increase electrical conductivity (EC) but it doesn't have any bad impact on the soil salinity (under optimum range). The heavy metals differ in their bioavailability and mobility from soil to the edible parts of the crops (Ahmad & Goni, 2010). This study aims at the assessment of heavy metals concentration in the grains of wheat variety Chagi-2 under different treatments of wastewater irrigation and to analyze mobility of heavy metals from soil to edible portion of crop and to observe metal pollution index.

MATERIALS AND METHODS

To analyze concentration of different heavy metals in water, soil and wheat, a pot experiment was conducted in the Botanical Garden of Department of Botany, University of Sargodha, Sargodha, Pakistan. In the current study, different physico-chemical properties of soil and water samples were also determined. The Sargodha has a burning climate in summer (upto 50^{o}C) and cold in winter (upto 12^{o}C). The conducted experiment had four treatments: T-I (GWI), T-II (50% GWI: 50% WWI), T-III (25% GWI: 75% WWI), and T-IV (WWI). One pot with two replicates was filled with 2.5 kg soil. Each treatment had two replicates Seeds of wheat variety Chagi-2 were sown at the end of Nov, 2015 and harvested at the end of April, 2016. Wastewater was collected from the sewage pond near University of Sargodha, Sargodha, Pakistan.

Sample Preparation

The seed samples were collected from each pot after harvesting. The soil samples were taken from the upper 3-5 cm of the soil layer from each pot. Both the seed and soil samples were kept in oven and dried at 72^{o}C for few days after drying in the air. After removing from the oven, grains were separated from the spikes and ground in an electrical grinder. The samples were then subjected to wet digestion method (Allen *et al*., 1986).

Digestion

The respective samples (about 1 g) were placed in a small conical flask and digested with 15 ml mixture of HNO_3, H_2SO_4 and $HClO_4$ (5:1:1) at 80^{o}C on a hot plate to obtain transparent solution. Digestion continued until a colorless solution appear and allowed it to cool. After cooling, all the samples were filtered, and diluted in measuring flask upto 50 ml using distilled water and stored in plastic bottles.

Digestion of Water Samples

The ground water and wastewater samples were digested by following the method of APHA (2005).

Determination of Metals

The concentrations of different heavy metals (Co, Cu, Zn, Mn, Fe, Pb, Ni, Cr, and Cd) in the digested samples were assayed by Atomic Absorption Spectrophotometer. The standard solutions of different metals were also prepared to obtain a calibration curve for each metal.

Statistical Analysis

Using Statistical Package for Social Sciences (SPSS 16), mean concentrations of metals were calculated in water, soil and wheat grain samples. All the results were subjected to one way ANOVA. To assess soil-plant interaction, Pearson's correlation coefficient was applied. It is a bivariant method. The significant differences between mean values were seen at 0.05, 0.01, and 0.001 probability levels (Steel & Torrie, 1980).

Bioconcentration Factor (BCF)

To evaluate the transport of metals (mg/kg) from soil to the plant parts, a BCF was used following Cui *et al*., (2004).

BCF= Concentration of metal in edible part/
Concentration of metal in soil

Pollution Load Index (PLI)

The pollution load index was used to investigate concentration of metals (mg/kg) in soil (Liu *et al*., 2005).

PLI = Metal concentration in investigated soil/
Reference value of metal in soil

Table I: Reference values of metals in soil

Metals	Reference Values (mg/kg)
Co	5.23[a]
Cu	8.39[b]
Ni	9.06[b]
Zn	44.9[b]
Pb	8.15[b]
Mn	25.5[c]
Fe	56.9[d]
Cr	64.27[e]
Cd	2.8[c]

Source [a] Shad *et al*. (2014); [b] Singh *et al*. (2010); [c] Hassan *et al*. (2013); [d] Dosumu*et al*. (2005); [e] Bao *et al*. 2014)

RESULTS

Analysis of variance exhibited non-significant effect (P>0.05) of different treatments on the concentrations of Co, Cu, Ni, Pb, Mn, and Cd, while significant effect (P<0.05) was observed on Zn, Fe, and Cr in water samples. There was a significant effect (P<0.05) of treatments on soil Cu, Co, Ni, Pb, Mn, and Cr, whereas non-significant effect (P>0.05) on soil Zn, Fe and Cd. A significant effect (P<0.05) of treatments was observed on Mn concentration in grains, while reverse was true for Co, Cu, Ni, Zn, Pb, Fe, Cr, and Cd (Table II).

Table II: Analysis of variance for metal concentrations in water, soil, and grains.

	Mean Squares								
Metals	Co	Cu	Ni	Zn	Pb	Mn	Fe	Cr	Cd
Grains	0.004[ns]	0.176[ns]	0.015[ns]	62.06[ns]	0.002[ns]	1.744[***]	0.212[ns]	0.046[ns]	0.030[ns]
Water	0.02[ns]	0.01[ns]	0.009[ns]	0.076[***]	0.066[ns]	0.007[ns]	0.023[***]	0.019[**]	0.032[ns]
Wheat grown in soil	0.01[**]	0.2[***]	0.7[***]	0.03[ns]	0.06[**]	0.02[***]	55.3[ns]	0.09[**]	0.1[ns]

******, *******significant at 0.01 and 0.001 levels, respectively; ns= non-significant

Pysico-chemical parameters of water

In water, electrical conductivity (dS/m) varied from 1.47 to 9.20 with mean concentration of 1.47 (T-I), 5.16 (T-II), 7.23 (T-III), and 9.2 (T-IV) respectively. The range of Ca^{+}+Mg^{+}(mg/L) was 162.1 to 261.3 with mean concentration of 162.1 (T-I), 187.7 (T-II), 213.3 (T-III), and 261.3 (T-IV) respectively. Other parameters like sodium, bicarbonate, chloride, and sodium adsorption ratio (SAR) were ranged from 76.6-208.5, 76-518.5, 128.2-206.5 mg/L, and 1.66-4.60, respectively (Table III).

Metals in water

The sequence of concentration of metals in T-I was: Cd>Ni>Zn>Fe>Cr>Cu>Mn>Co>Pb. In T-II, the order of concentration of metals was noticed as: Cd>Ni>Cr>Fe>Zn>Cu>Mn>Co>Pb, in T-III was: Cd>Ni>Fe>Cr>Zn>Cu>Mn>Co>Pb, and in T-IV was: Cd>Zn>Ni>Fe>Cr>Cu>Mn>Co>Pb (Figure 1). The range of metals concentration was recorded as: Co, 0.31 to 0.37; Cu,0.52 to 0.57; Ni, 0.89 to 1.02; Zn, 0.56 to 0.9; Pb, 0.02 to 0.32; Mn, 0.39 to 0.5; Fe, 0.54 to 0.74; Cr, 0.53 to 0.72; and Cd, 1.48 to 1.72 mg/L, respectively (Table IV).

Metals in soil

The sequence of concentration of metals in soil in T-I and T-II was: Fe>Cd>Ni>Cu>Cr>Co>Zn>Mn>Pb and Fe> Cd> Cu> Ni> Cr> Zn> Co> Mn> Pb, respectively. In T-III, decreasing order of concentration of metals in soil was: Fe>Cd>Cu>Ni>Cr>Co>Mn>Zn>Pb, and in T-IV was: Fe>Ni>Cd>Cu>Cr>Co>Mn>Pb>Zn (Fig., 2). The range of metals concentration in soil in all treatments was: Co, 0.68 to 0.86; Cu, 1.22 to 1.92; Ni, 1.31 to 2.36; Zn, 0.13 to 0.61; Pb, 0.3 to 0.41;

Mn, 0.6 to 0.81; Fe, 3.04 to 3.96; Cr, 1.15 to 1.59; and Cd, 1.5 to 2.05 mg/kg, respectively (Table V).

Metals in grains

The sequence of concentration of metals in wheat grains in T-I and T-II was: Zn>Cd>Fe>Cu>Ni>Cr>Mn>Co>Pb and Zn>Cd>Fe>Ni>Cu>Cr>Mn>Co>Pb, respectively. InT-III, decreasing order of concentration of metals in wheat grains was: Cd>Fe>Zn>Ni>Cu>Mn>Cr>Co>Pb, and in T-IV was: Mn>Fe>Zn>Cd>Cu>Ni>Cr>Co>Pb (Fig., 3). The range of metals concentration in soil at all treatments was: Co, 0.15 to 0.23; Cu, 0.93 to 1.46; Ni, 0.96 to 1.09; Zn, 1.16 to 8.58; Pb, 0.03 to 0.09; Mn, 0.37 to 2.12; Fe, 1.37 to 1.96; Cr, 0.43 to 0.7; and Cd, 1.49 to 1.72 mg/kg, respectively (Table VI).

Table III: Mean concentrations for physico-chemical parameters of water

Physico-chemical parameters	EC (dS/m)	Ca^{+}+Mg^{+} (mg/L)	Na^{+} (mg/L)	HCO_3^{-} (mg/L)	Cl^{-} (mg/L)	SAR
T-I	1.47±0.09	162.1±0.72	208.5±0.03	380.2±0.06	149.3±0.09	1.66±0.14
T-II	5.16±0.36	187.7±0.05	76.6±0.17	518.5±0.14	128.2±0.08	2.00±0.31
T-III	7.23±0.25	213.3±0.02	132.1±0.02	76±0.09	167.3±0.27	3.48±0.17
T-IV	9.20±0.11	261.3±0.01	187.5±0.06	88±0.06	206.5±0.19	4.60±0.82
Mean squares	3.274[ns]	5.247[**]	2917[ns]	41.51[ns]	1672[*]	1.104[***]

[*], [**], and [***] significant at 0.05,0.01 and 0.00 levels, respectively; ns= non-significant

Table IV: Metals concentration (mg/L) in irrigation water.

Metals	T-I	T-II	T-III	T-IV	PML (mg/kg)
Co	0.31±0.09	0.33±0.05	0.34±0.06	0.37±0.09	0.05[a]
Cu	0.52±0.1	0.53±0.09	0.56±0.07	0.57±0.05	0.20[a]
Ni	0.95±0.09	0.99±0.08	1.02±0.07	0.89±0.06	0.20[a]
Zn	0.57±0.06	0.56±0.08	0.61±0.09	0.90±0.11	2[b]
Pb	0.023±0.03	0.02±0.04	0.05±0.02	0.32±0.08	0.1[a]
Mn	0.39±0.08	0.41±0.09	0.50±0.11	0.42±0.12	0.2[b]
Fe	0.54±0.09	0.57±0.13	0.64±0.15	0.74±0.1	5.0[a]
Cr	0.53±0.06	0.59±0.09	0.64±0.13	0.72±0.12	0.1[b]
Cd	1.48±0.09	1.58±0.12	1.63±0.14	1.72±0.2	0.01[a]

Metals	T-I	T-II	T-III	T-IV	PML (mg/kg)
Co	0.68±0.21	0.82±0.22	0.80±0.17	0.86±0.19	9.1[a]
Cu	1.22±0.16	1.50±0.21	1.68±0.2	1.92±0.19	8.39[b]
Ni	1.32±0.24	1.31±0.18	1.43±0.14	2.36±0.17	9.06[b]
Zn	0.61±0.09	0.91±0.18	0.41±0.15	0.13±0.13	44.19[b]
Pb	0.30±0.11	0.36±0.09	0.37±0.13	0.41±0.16	350[c]
Mn	0.60±0.14	0.62±0.19	0.653±0.2	0.81±0.21	46.75[b]
Fe	3.04±0.19	3.31±0.23	3.82±0.25	3.96±0.2	56.9[d]
Cr	1.15±0.2	1.30±0.19	1.30±0.15	1.59±0.19	9.07[b]
Cd	1.50±0.19	1.96±0.2	1.95±0.17	2.05±0.14	1.49[b]

Source: [a] WWF (2007); [b] FAO (1985); PML= Permissible Maximum Limit

Table V: Metals concentration (mg/kg) in soil grown with *T. aestivum.*

Metals	T-I	T-II	T-III	T-IV	PML[a] (mg/kg)
Co	0.15±0.11	0.22±0.13	0.23±0.12	0.21±0.13	50
Cu	0.97±0.23	0.93±0.43	1.07±0.4	1.46±0.31	73.3
Ni	0.96±0.3	0.97±0.31	1.09±0.3	1.08±0.3	67
Zn	8.58±0.4	7.16±0.32	1.16±0.29	1.74±0.25	99.4

Source:[a] Dutch Standard (2000); [b] Singh *et al*., (2010); [c] MEPC(1995); [d]Dosumu *et al*., (2005); PML= Permissible Maximum Limit

Table VI: Metals concentration (mg/kg) in grains of *T. aestivum*"Chagi-2"

Pb	0.09±0.2	0.09±0.29	0.03±0.19	0.08±0.24	0.2[b]
Mn	0.37±0.2	0.67±0.2	1.03±0.2	2.12±0.2	500
Fe	1.37±0.2	1.41±0.2	1.58±0.2	1.96±0.2	425.5
Cr	0.43±0.17	0.70±0.23	0.54±0.19	0.68±0.24	2.3
Cd	1.49±0.21	1.55±0.29	1.64±0.32	1.72±0.26	0.2

Source: [a] FAO/WHO (2001); [b] FAO/WHO (1984); PML= Permissible Maximum Limit

Correlation between soil and wheat grains

The results showed that positive significant correlation was exhibited by Pb, Mn and Cr, while Co, Cu, Ni, Fe and Cd have positive non-significant correlation but Zn has negative significant correlation between soil and wheat grains (Table VII).

Bioconcentration factor (BCF)

Bioconcentration factors are shown in Table VIII. It was analyzed for transfer of metals from soil to wheat grains. The order of bioconcentration factor for metals in T-I was in the decreasing order of Zn>Cd>Cu>Ni>Mn>Fe>Cr>Pb>Co while order observed in T-II and T-III was: Zn>Mn>Cd>Ni>Cu>Cr>Fe>Co>Pb, and in T-IV, the sequence was seen as: Zn>Mn>Cd>Cu>Fe>Ni>Cr>Co>Pb (Table VIII).

Pollution Load Index (PLI)

The order of the PLI in T-I was: Cd>Cu>Ni>Co>Fe>Pb>Mn>Cr=Zn. in T-II, the PLI was found in the order of: Cd>Cu>Co>Ni>Fe>Pb>Zn>Cr=Mn. PLI in T-III was in the sequence of: Cd>Cu>Ni>Co>Fe>Pb>Mn>Cr>Zn, whereas in T-IV it was in the order of: Cd>Ni>Cu>Co>Fe>Pb>Mn=Cr>Zn (Table IX).

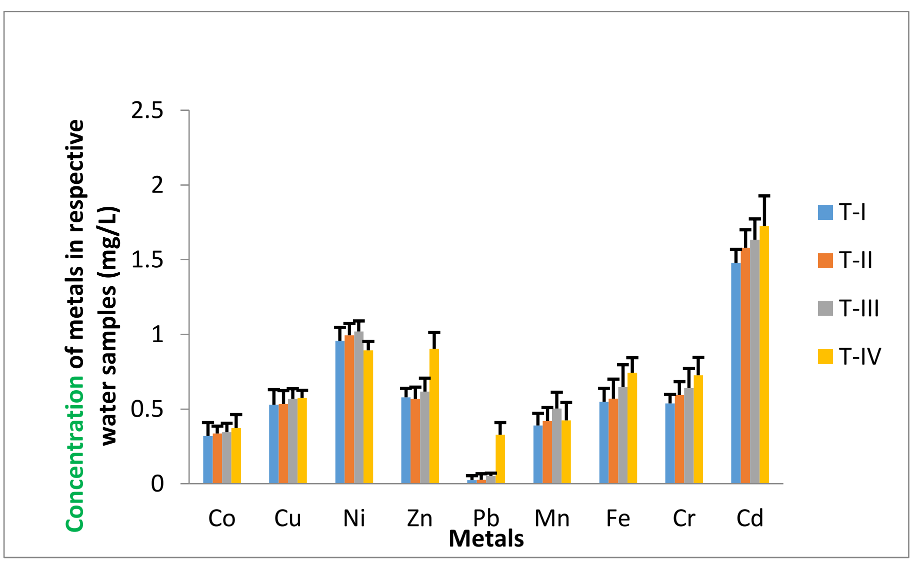

Fig., 1: The variation of metals concentration in irrigation water.

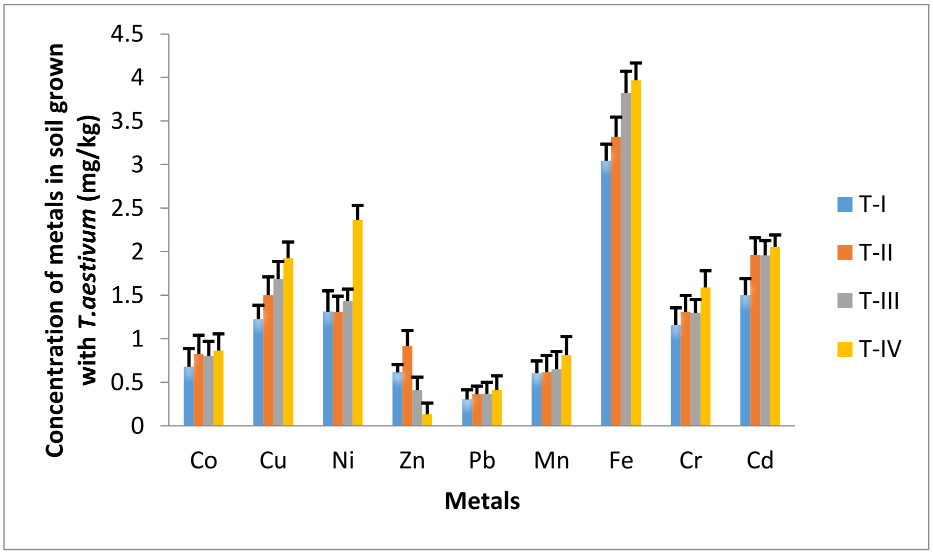

Fig., 2: The variation of metals concentration in soil.

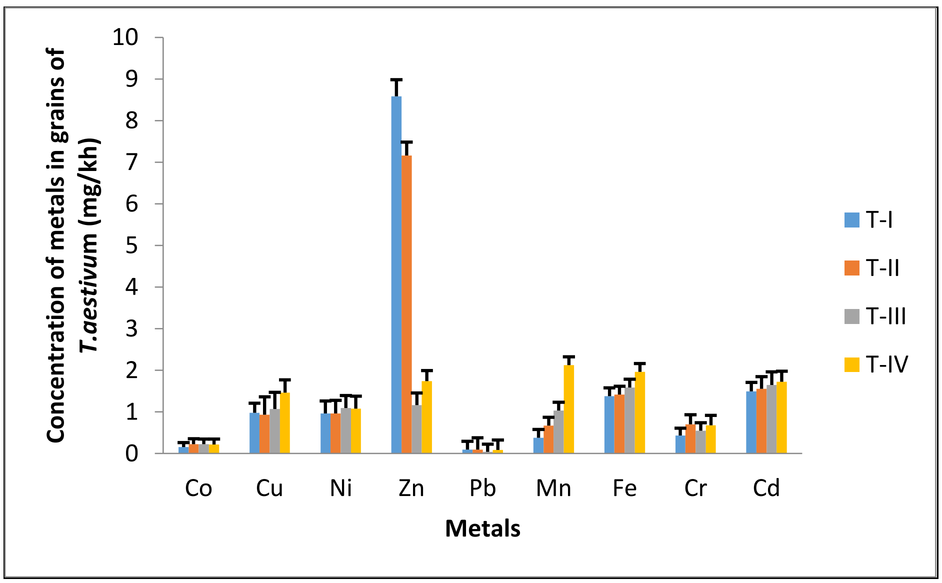

Fig., 3: The variation of metals concentration in grains of *T. aestivum*.

Table VII: Correlation between soil and grains metal concentrations.

Correlation	
Metals	Soil-Grain
Co	0.465
Cu	0.356
Ni	0.456
Zn	-0.631*
Pb	0.581*
Mn	0.869**
Fe	0.008
Cr	0.656*
Cd	0.321

*****, ******Correlation is significant at the 0.05 and 0.01 levels, respectively.

Table VIII: Bioconcentration factors for soil-plant systems.

Treatments	Co	Cu	Ni	Zn	Pb	Mn	Fe	Cr	Cd
I	0.22	0.79	0.73	13.92	0.29	0.62	0.45	0.37	0.99
II	0.27	0.62	0.73	7.82	0.24	1.08	0.42	0.54	0.79
III	0.28	0.63	0.76	2.83	0.10	1.57	0.41	0.42	0.84
IV	0.25	0.76	0.45	13.09	0.20	2.60	0.49	0.43	0.83

Table IX: Pollution load index for metals in soil.

Treatments	Co	Cu	Ni	Zn	Pb	Mn	Fe	Cr	Cd
I	0.13	0.15	0.14	0.01	0.03	0.02	0.05	0.01	0.54
II	0.16	0.18	0.14	0.03	0.04	0.02	0.06	0.02	0.7
III	0.15	0.20	0.16	0.01	0.04	0.03	0.07	0.02	0.69
IV	0.17	0.23	0.26	0.002	0.05	0.03	0.07	0.03	0.73

DISCUSSION

The permissible range of EC for water is 0.4-0.6 dS/m according to WHO. All the water samples had EC above the normal range of EC. The higher value of EC is an easy indication of sulphate, chloride, potassium or sodium in water (Nazir *et al*., 2015). It was suggested by Singh *et al*. (2012) that wastewater often had high concentration of dissolved salts. The amount of dissolved calcium and magnesium ions in water is regarded as hardness of water (Nazir *et al*., 2015). Almost all water samples were within the normal range (50-250 mg/L) of hardness except T-IV. Very high values of other parameters have been reported by Nahhal *et al*., (2013) as compared to the present study.

According to the findings of Singh *et al*., (2004), high levels of toxic heavy metals, for example, Cu, Ni, Zn, Pb, As and Mo are present in wastewater along with various nutrients and natural matter. Hassan *et al*., (2013) reported low values of Ni, Pb, Cr, and Cd while high values of Zn and Fe but Cu and Mn in the wastewater were in similarto the present study. The current finding shows high value of Co than the values given by Alghobar *et al*., (2014). It was found that the concentrations of more heavy metals like zinc and iron were below the maximum permissible limit as set by WHO because may be it is also present in low amount from where it added in wastewater. Cadmium had the highest concentration in wastewater.

Soil Cd, Cr, Cu, Ni, and Pb values observed in the present study were lower from the findings of Hassan *et al*., (2013). Concentration of Fe, Cd, and Zn were not affected under short-term wastewater irrigation in the soil. In the wastewater irrigated soil, Fe, Co, Cu, Cd, Cr, Pb, Mn, and Ni were found in high concentration, while Zn had low value in comparison with soil irrigated with ground water. Ni, Zn, Mn, Cr, Cu, and Cd were present within permissible range in the soil according to Singh *et al*., (2010). Soil physico-chemical properties were also affected by the wastewater irrigation. Ghosh *et al*., (2012) found that as the heavy metals are within acceptable range, but long term wastewater irrigation can elevate their concentration in the soil. Shad *et al*., (2014) recorded high value of cobalt as compared to present study.

Only Cd was present above the permissible limit while other metals (Cr, Cu, Pb, Mn, Ni, Zn, Co, Fe) had safe limit in the wheat grains as set by FAO/WHO (2001). Hassan *et al*., (2013) observed low value of Cd and high values of Mn, and Fe as compared to the present investigation in wheat grains. Wastewater frequently increases Co concentration in the cereals (Khaskhoussy *et al*., 2013). Copper bioavailability is highly reduced when pH of the soil is above 7. In current analysis, Cu and Zn concentrations were quite lower than the values given by Jamali *et al*., (2009). According to Kabata-Pendias& Mukherjee (2007), plants that grow in uncontaminated places usually have low levels of heavy metals, Pb is one of them. The toxic level of Mn may cause cognitive disorders and impaired motor skills. It may be analyzed that elevated level of manganese in the plants is due to its geological origin (Al-Othman *et al*., 2013). Bermudez *et al*., (2011) found that cadmium concentration in wheat grains was dependent on the soil pH, and it also determined its uptake by plants.

The high correlation coefficient indicates a good association between soil and plants (Sharma & Raju, 2013). Present result was similar to that of Szabo &Czeller (2009) in case of Cu. The positive value of correlation coefficient indicates strong association, while negative value represents weak relationship between soil and plants. Negative significant correlation was found in case of Zn.

The highest bioconcentration factor was observed in Zn, interpreting that it is abundantly available to the plants. Chao *et al*., (2007) also investigated higher transfer factor from soil to the plant for Zn. The bioconcentration factor was used to analyze the bioavailability of heavy metals in the plant parts in comparison with the soil. Ratio greater than 1 indicates high accumulation of metals in plant parts than soil (Kisku *et al*., 2000).

To examine the extent of heavy metal contamination in soil, pollution load index was evaluated. Among the studied metals, the maximum PLI values were reported in wastewater irrigated soils. If the PLI value was above 0.50, it can be said that the metals are being contaminated by anthropogenic pollution and thus need proper environmental monitoring of that area (Sponza & Karaoglu, 2002).

CONCLUSION

Current results showed the Cd concentration in soil, and crop while Co, Cu, Ni, Mn, Cr, and Cd in water were above the permissible limits. Water's EC, sodium adsorption ratio (SAR), Ca^{+2}, Mg^{+2}, and Cl^{-} increased with increased concentration of wastewater irrigation. As heavy metals concentration were lower in the crop but long term wastewater irrigation can accumulate heavy metals in the edible parts of crops leading to serious health risks causing(Cancer, Hepatitis etc) via food chain. The highest transfer factor was seen in Zn for soil-plant system. Cd showed the highest PLI value of present analysis resulting in contamination of the soil. Current research suggest that regular monitoring of municipal wastewater discharge and treatment must be carried out in order to avoid heavy metals toxicity in the soil and plants growing there.

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