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Sugar industry effluents as a source of soil fertility and potential toxicological risk of heavy metals in food crop

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ABSTRACT

Concentrations of heavy metals such as manganese, zinc, lead and nickel in the environment are currently increasing, mainly due to human activities. Zinc is essential element for several biochemical processes in plants. Any of these metals, at high concentrations in soil, can cause severe damage to physiological and biochemical activities of plants. Scarcity of fresh water in agricultural area enforced farmers to use industrial effluent and domestic wastewater for irrigation purpose. Ramzan sugar mill industry located at Chiniot discharges high amount of effluent which is used by farmers for irrigation purpose. Current experiment was conducted in Sargodha, Punjab, Pakistan to assess the level of different heavy metals such as Mn, Ni, Pb and Zn in wheat variety (Chagi-4) irrigated with varying quantity of sugar industry effluent. The water, soil and wheat grain samples were analyzed for heavy metals by Atomic Absorption Spectrophotometer. Concentrations of Mn (0.29-0.61), Ni (0.79-1.02), and Pb (0.01-0.42) mg/L in water samples were higher than the permissible limit of Mn (0.2), Ni (0.20), and Pb (0.1) mg/L given by FAO, while value (mg/L) of Zn (0.61-0.80 mg/L) was within the acceptable limit recommended for irrigation. In grain samples, values of all heavy metals such as Mn (0.18-0.75), Ni (0.32-0.77), and Zn (0.52-0.98) mg/kg were within acceptable range of Mn (500), Ni (67), Zn (99.4 mg/kg) suggested by FAO/WHO except for Pb whose concentration (0.19-0.83) mg/kg exceeded the permissible limit (0.3 mg/kg) given by FAO/WHO. The values of all heavy metals in water were beyond the acceptable limit but in wheat grains concentrations of heavy metals were within acceptable limit except for Pb, which indicate the lesser transfer of these metals from soil to the wheat plant. The analysis reveals that regular monitoring of sugar industry effluent is necessary to prevent the excessive buildup of metals in food chain which has broader implications in sustainable agricultural water management.

Keywords: Industrial wastewater, Bioconcentration factor, *Triticum aestivum*

INTRODUCTION

Study of heavy metal pollution is gaining much more importance all over the world. Some heavy metals essential for growth of plants and animals are called micronutrients. Other heavy metals are non-essential for growth of plants and animals and cause various problems in plants and animals when present in excess amounts (Mapanda *et al.,* 2010; Mahmood, 2010).

When industrial wastewater is used for irrigation purpose, these non-essential heavy metals are taken up by plants and become the part of food chain (Ahmad *et al.,* 2018a). In Pakistan,

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the industrial effluent and municipal wastewater are drained directly into irrigation canals, streams and rivers without any treatment and accumulate in canals, rivers and reach agricultural land (Nafees *et al.,* 2011). Contamination of water by untreated wastewater is the main reason of increased level of non-essential heavy metals (Manzoor *et al.,* 2006).

Wheat (*Triticum aestivum* L.) is widespread cereal crop of the world. Wheat is a staple food in Pakistan. Wheat is a chief source of food for most of the human population in the world (Khan *et al.,* 2016). Globally, wheat is the leading source of vegetable protein in human food, having a higher protein content than other major cereals such as maize (corn), and rice (Otokunefor & Obiukwu, 2005).

The concentration of heavy metals in wheat crop increase after long-term use of industrial wastewater irrigation, which pose serious threats to human health by entering in the food chain (Abdu *et al.,* 2011; Ahmad *et al.,* 2018b).

Prolong intake of heavy metals exert an adverse effect on animals and human health (Dogan *et al.,* 2014; Ugulu *et al.*, 2016). Heavy metals enter in the food chain by consumption of contaminated food crop and cause carcinogenic and non-carcinogenic (a headache, liver disease and neurological disorders) health hazards in human when their concentration exceeds the acceptable limit (USEPA, 2000). In human, chronic intake of metals causes genotoxic, developmental, gastrointestinal, dermal, cardiovascular, hematological, neurological, respiratory, reproductive and immunological disorders (Lin *et al.,* 2013).

This study was conducted with objectives to determine the concentration of heavy metals (Zn, Mn, Ni, and Pb) in wheat irrigated with industrial wastewater and to also calculate the bioconcentration factor for each metal.

MATERIALS AND METHODS

Study area

The current research was carried out at Department of Botany, University of Sargodha, Punjab, Pakistan. Sargodha district has extreme climatic conditions. In summer the maximum temperature goes up to 50 ℃ and minimum up to 12 ℃ in winter.

Plant cultivation and harvesting

A pot trial was conducted in a natural environment from November 2015 to April 2016. Firstly twelve pots were taken and filled with 4 kg of soil each. In each pot, 10 seeds of wheat variety (Chagi-4) were sown. The plants were irrigated with sugar industry effluent in different concentration: T-!; 100% groundwater, T-II; 30% industrial wastewater and 70% groundwater, T-III; 60% industrial wastewater and 40% groundwater and T-IV; 90% industrial wastewater and 10% groundwater. The industrial effluent was collected from Ramzan Sugar Mill Industry situated in District Chiniot. Drip irrigation was done twice a week.

At maturity, morphological parameters of plants were determined. Harvesting was done in April 2016. After harvesting, seeds were separated from the husk. Soil samples were taken from the upper profile of soil. Both soil and grains samples were oven dried at 72 ^{o}C and were crushed into a fine powder with the help of an electrical grinder.

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Analysis of physicochemical properties of water and soil samples

The physicochemical properties of soil and water samples such as organic matter, electrical conductivity (EC), pH, calcium, magnesium, chloride and available P were determined. Electrical conductivity was determined by a method described by Richard (1954). pH of samples was determined by pH meter (Mclean, 1982). Titration method was used for determination of Ca^{+2} and Mg^{+2} and Cl^{-}. Organic matter of soil was determined by Walkley and Black acid digestion method (Page, 1982). Available P and K were determined by following Olsen & Sommers (1982).

Digestion of soil and grain samples

The soil and grain samples (each 1 g) were processed with 15 mL mixture of HNO_3, $HClO_4$, and H_2SO_4 in 5:1:1 at 80 ^{o}C for 2h until the digestion solution became colourless. Filtered the digest and diluted it with distilled water to make 50 mL volume (Allen *et al.,* 1986).

Digestion of water samples

Digestion of Sugar industry wastewater and ground water was done by method described by APHA (2005). 10 mL con. HNO_3 and 50 mL water sample was taken in a beaker and maintained it on a hot plate at 80 ^{o}C. When mixture was reduced to 20 mL added more 5 mL HNO_3 and kept on heating until transparent solution was obtained. Filtration was done by Whatman filter paper # 42 and made 50 mL volume by adding distilled water.

Metal analysis

Determination of metals in digested samples was done by using Atomic Absorption Spectrophotometer (AA-6300 Shimadzu Japan). Standard calibration curve was drawn for each metal. The metal under investigated was manganese (Mn), nickel (Ni), lead (Pb) and zinc (Zn). Instrument operating conditions for these metals were given in Table 1.

Table 1: Instrument operating conditions for determination of Ni, Mn, Pb, and Zn

Element	Mn	Ni	Pb	Zn
Wave length (nm)	238.3	316.8	198.8	314.9
Slit width (nm)	0.2	0.7	0.6	0.7
Lamp current low (mA)	12	6	8	8
Air flow rate (L/min)	15	15	15	15
Acetylene flow rate (L/min)	2.2	1.8	1.8	2
Burner height (mm)	9	7	7	7
Oxidant (Fuel)	Air, C_2H_2	Air, C_2H_2	Air, C_2H_2	Air, C_2H_2

Statistical analysis

One-way analysis of variance was applied for water, soil and wheat grains by using SPSS package SPSS-20. Correlation between soil and wheat grains with respect to each metal was also worked out. The differences between mean values of each soil and grain metals were determined using the Least Significance Difference (LSD) test at 0.05, 0.01 and 0.001 probability levels by following Steel & Torrie (1980).

Bioconcentration factor

To evaluate the transfer of metals from soil to edible parts of plants, the bioconcentration factor (BCF) was determined by following equation described by Cui *et al.* (2004).

BCF= metal level (mg/kg) in wheat grains **/** metal level (mg/kg) in soil

RESULTS

Morphological parameters

Industrial wastewater had a great effect on morphological characters i.e. plant height, leaf area per plant, leaf length, shoot and spike length of the wheat plant. The results showed that in all treatments non-significant effect (p>0.05) were seen on plant height, leaf area, leaf length, shoot and spike length of wastewater irrigated wheat plants. The highest values for morphological parameters were seen when T-II was applied which consisted of (70% groundwater and 30% industrial wastewater) while minimum values were obtained in T-IV, it consisted of 90% industrial wastewater and 10% groundwater (Table 2).

Table 2: Mean values of morphological parameters of *Triticum aestivum*

Treat ment	Plant height (cm)	Spike length (cm)	Shoot length (cm)	Leaf area (cm^2)	Leaf length (cm)
T-I	4.533± 0.66	8.100± 0.88	4.300± 0.56	6.057± 0.33	1.263± 0.17
T-II	5.066± 0.99	1.030± 0.55	4.866± 0.77	7.227± 0.89	1.243± 0.55
T-III	3.733± 0.09	8.600± 0.66	3.666± 0.16	3.037± 0.16	1.0900± 0.18
T-IV	3.433± 0.77	6.500± 0.99	3.133± 0.08	2.875± 0.99	1.316± 0.12

Results of water, soil and wheat grains

The results from the analysis of variance of the data exhibited non-significant effect (p>0.05) of treatments on Zn and Pb in water, Mn and Zn in soil and Mn and Ni in grains, while significant effect (p<0.05) was observed for Ni, and Mn in water, Ni and Pb in soil and Pb and Zn in grains (Table 3).

Table 3: Analysis of variance of heavy metals in water, soil and wheat grains

Metal	Mean Square			
	Mn	Ni	Pb	Zn
Water	0.076^{ns}	0.007^{*}	0.009^{*}	0.066^{ns}
Soil	0.060^{ns}	0.001^{***}	0.237^{***}	0.056^{ns}
Grains	0.032^{ns}	0.131^{ns}	0.003^{***0}	0.080^{***}

*****, *******: Significant at 0.05, 0.001 level, ns: non–significant

Physicochemical parameters of water

In water, EC ranged from 5.1 to 8.2 with mean concentration of: T-I - 7.0, T-II - 5.14, T-III - 6.2, T-IV - 8.2 dS/m. The value of Ca^{+2} and Mg^{+2} among four treatments were 7.8, 7.6, 7.9, 8.2 mg/L, respectively. The value of Cl^- varied from 1.11 to 1.176 mg/L with mean concentrations of T-I - 1.11, T-II - 1.26, T-III - 1.22, T-IV - 1.76 mg/L, respectively (Table 4).

Table 4: Physico-chemical parameters of irrigation water

Treatment	Physico-chemical parameters			
	EC (dS/m)	Ca^{+2}+Mg^{+2} (mg/L)	Na^{+} (mg/L)	Cl^{-} (mg/L)
T-I	7.96	7.88	0.16	1.11
T-II	5.14	7.66	0.14	1.26
T-III	6.21	7.93	0.15	1.22
T-IV	8.20	8.25	0.24	1.76
Standards limits	5.1[a]	200[a], 150[a]	900[b]	–

Sources: [a]MWE (2005), [b]FAO (1985)

Physico-chemical parameters of soil

The soil in four treatments was clay loam. The mean values of pH ranged from 8.0-8.1. The range of EC in four treatments was found between 1.50-1.80 dS/m with a mean concentration of T-I - 1.80, T-II -1.58, T-III - 1.58 and T-IV - 1.80 dS/m. The percentage of organic matter among four treatments ranged from 1.11 to 1.35. The mean values of available P were 2.91, 1.72, 1.71 and 1.90 mg/kg in T-I, T-II, T-III and T-IV, respectively. The values of available K in all treatments were 5.22, 4.90, 3.40 and 5.90 mg/kg in T-I, T-II, T-III and T-IV, respectively (Table 5).

Table 5: **Physico-chemical parameters of soil**

Treat-ment	Physico-chemical parameter					
	pH	EC (dS/m)	Organic matter (%)	Available phosphorous (mg/kg)	Available potassium (mg/kg)	Tex-ture
T-I	8.1	1.80	1.25	2.91	5.22	Loam
T-II	8.2	1.58	1.11	1.72	4.90	Loam
T-III	8.3	1.58	1.18	1.71	3.40	Loam
T-IV	8.2	7.89	1.50	1.90	5.90	Loam

Heavy metal concentrations in water

The range of heavy metals in water of four treatments was: Mn (0.29-0.61), Ni (0.79-1.02), Pb (0.01-0.42) and Zn (0.61-0.80). The decreasing order of heavy metals at T-I, T-II and T-III was: Pb<Mn<Zn<Ni, while in T-IV it was found in the following sequence: Pb<Mn<Ni<Zn. (Table 6, Figure 1).

Table 6: Mean concentrations of heavy metals in irrigation water, soil, in grains of *Triticum aestivum*

Metal	T-I	T-II	T-III	T-IV	Permissi-ble limit
Water (mg/L)					
Mn	0.291±0.04	0.319±0.04	0.403±0.05	0.611±0.08	0.2[a]
Ni	0.856±0.04	0.893±0.05	1.020±0.06	0.793±0.05	0.20[b]
Pb	0.013±0.03	0.017±0.02	0.219±0.05	0.423±0.04	0.1[b]
Zn	0.678±0.05	0.66±0.04	0.615±0.07	0.803±0.07	2[a]
Soil (mg/kg)					
Mn	0.723±0.09	0.403±0.1	0.483±0.11	0.611±0.9	46.74[c]
Ni	1.137±0.08	1.140±0.1	1.118±0.12	1.138±0.11	9.06[c]
Pb	0.068±0.07	0.260±0.9	0.376±0.08	0.736±0.12	350[d]
Zn	0.393±0.05	0.180±0.08	0.139±0.09	0.396±0.08	44.19[c]
Grains (mg/kg)					
Mn	0.750±0.11	0.774±0.09	0.836±0.11	0.981±0.09	500[e]
Ni	0.346±0.09	0.321±0.08	0.641±0.1	0.736±0.1	67[e]
Pb	0.183±0.08	0.385±0.02	0.195±0.3	0.524±0.11	0.3[e]
Zn	0.183±0.07	0.385±0.03	0.195±0.07	0.524±0.4	99.4[e]

Sources: [a]WWF (2007),[b]FAO (1985), [c]Singh *et al.*, . (2010), [d]CSEPA (1995),[e]FAO/WHO (2001)

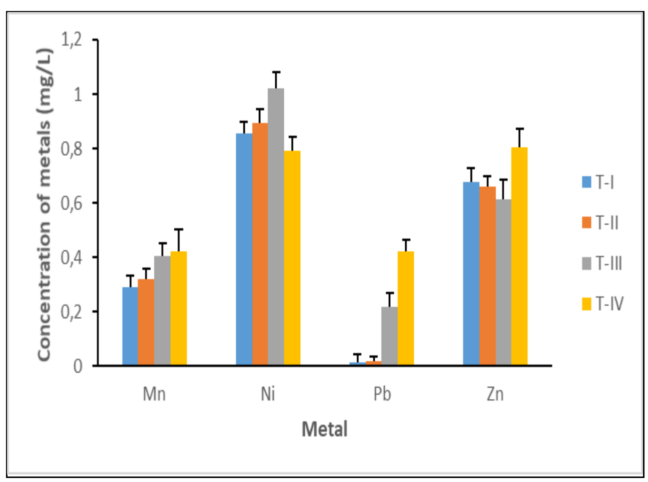

Fig. 1: The variation of heavy metals in irrigation water

Heavy metal concentrations in soil

The range of heavy metals noticed in soil was: Mn (0.40-0.72), Ni (1.11-1.14), Pb (0.06-0.73) and Zn (0.13-0.39) mg/kg respectively. The trend of heavy metals in T-I was Pb<Zn<Mn< Ni, T-II and T-III was Zn<Pb<Mn<Ni. While in T-IV it was found in the following sequence: Zn<Mn<Pb<Ni. Values of Zn was higher while Ni was lower in all treatments (Table 6, Figure 2).

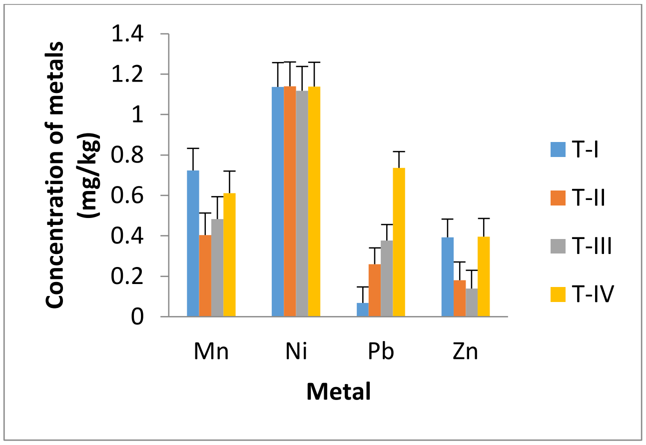

Fig. 2: The variation of heavy metals in soil

Heavy metal concentrations in grains

The range of heavy metals in all treatments was: for Mn 0.18-0.75, for Ni 0.32-0.77, for Pb 0.19-0.83, for Zn 0.52-0.98, respectively. The order of concentration of heavy metals in T-I - T-III and T-IV was: Pb, Zn<Ni<Mn. While in T-II it was found in increasing order of: Ni<Pb<Zn<Mn (Table 6, Figure 3).

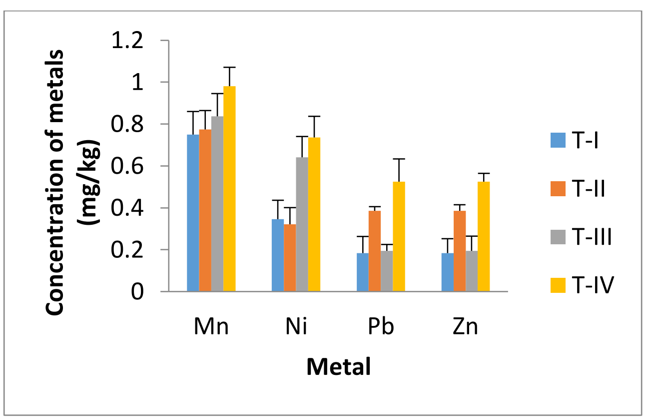

Fig. 3: The variation of heavy metals in grains of *Triticum aestivum*

Correlation

In present study, Mn, Ni and Zn showed positive and non-significant correlation between soil and grains of wheat (Table 7).

Table 7: Correlation between soil and grain metal concentrations

Metal	Soil-grains
Mn	0.364^{ns}
Ni	0.079^{ns}
Pb	-0.38^{ns}
Zn	0.19^{ns}

ns: non-significant

Bioconcentration factor

The order of BCF in T-I was: Pb>Mn>Ni>Zn, in T-II: Zn>Mn>Pb>Ni, in T-III: Mn>Zn>Ni>Pb, while at T-IV it was found in increasing order of: Mn>Zn>Pb>Ni (Table 8). Highest BCF was obtained for Zn and Mn.

Table 8: Bioconcentration factor for soil-plant

Treat-ment	Heavy metal			
	Mn	Ni	Pb	Zn
T-I	1.037344	0.30431	2.691176	0.465649
T-II	1.920596	0.281579	1.480769	2.138889
T-III	1.730849	0.573345	0.518617	1.402878
T-IV	1.605565	0.646749	0.711957	1.323232

DISCUSSION

Industrial wastewater had a great effect on morphological characters i.e. plant height, leaf area per plant, leaf length, shoot and spike length of the wheat plant. These morphological characters were higher in control treatment and decreased with increased concentration of industrial wastewater. A similar reduction in different morphological parameters was examined by Vijayaragavan *et al.* (2006). Present results for leaf area, leaf length, root and shoot lengths were found similar to the findings of Metwali *et al.* (2013). Growth and germination inhibited by higher concentration of waste water. Waste water is one of the major factors behind low productivity of crops as reported by Konwar & Jha (2010).

Ionic concentration of water is determined by calculating its electrical conductivity. The values of EC for Cl^-, Ca^{+2}, and Mg^{+2} in current investigation were lower than the findings of Nafees & Amin (2014). Alghobar *et al.,* (2014) reported higher values for these parameters as compared to present results. It was found that by increasing concentration of industrial wastewater to level of Ca^{+2}, Mg^{+2}, and Cl^- was also increased.

Soil pH decreased by application of industrial wastewater as compared to control. Similar results were found by Li *et al.,* (2001) who also reported decrease in soil pH due to wastewater irrigation. Decrease in soil pH may be due to the decomposition of organic matter present in soil (Vaseghi *et al.,* 2005). Wastewater irrigation considerably increases the EC, organic matter, available P, and available K. Our results are similar to the findings of Alghobar *et al.* (2014) and Verma *et al.* (2015).

According to FAO (1985) and WWF (2007) values of Pb, Mn, and Ni are above the permissible limit except for Zn, whose values fell within the permissible limit. Present concentrations of Pb and Zn were lower while Mn and Ni were higher than those recorded by Hassan *et al.* (2015). The concentration of heavy metal increased by increasing dose of sugar industrial effluent on agricultural land.

Present values for all metals were below the maximum permissible limits given by CSEPA (1995) and Singh *et al.* (2010). So the level of these metals in soil samples was found within safe limits.

In current results, concentrations of all metals were lower than the values reported by Alghobar *et al.* (2014). Khan *et al.* (2013) also reported higher values for these metals in their work. It revealed that these metals were not properly translocated to crop. Present values for all metals were below the maximum permissible limits given by FAO/WHO (2001) except for Pb. The permissible limit for lead is 0.3 mg/kg given by FAO/WHO (2001). The result obtained in this investigation was higher than the recommended level. Lead is a toxic heavy metal and causes physiological, hematological and neurological disorders (Sorme & Lagerkvist, 2002).

Pb showed negative and non-significant results between soil and wheat grains. In present investigation, Mn, Ni and Zn showed positive and non-significant relationship between soil and grains. Negative non-significant correlation for Pb indicated weak relationship between soil and wheat grains. Positive and non-significant correlation for various metals was also observed by Khan *et al.* (2013).

Bioconcentration factor is an important parameter to determine the extent of metal transfer from soil to eatable parts of plant. Present BCF value for all metals was higher as compared to BCF values for different metals like Mn, Ni, Pb, and Zn reported by Jaishree & Khan (2015). In present study, Pb and Zn showed higher BCF than those reported by Asdeo *et al.* (2014). The present concentration of Zn and Ni were also higher than those reported of Verma *et al.,* (2015). Bioconcentration factor was higher for Zn and Mn indicating that these metals had high tendency to move from soil to crop. Pb and Ni had low mobility as compared to other metals.

CONCLUSION

Use of industrial effluent and municipal wastewater in agriculture has increased due to the scarcity of fresh water resources. Industrial wastewater is contaminated with heavy metals and poses serious threats to the sustainability of ecosystem mainly human beings. Industrial wastewater irrigation considerably increased the EC for Ca^{+2}, Mg^{+2}, Cl^-, available P, and available K of soil. Variation of heavy metals concentration in grains samples showed the difference in uptake ability of these metals by wheat. Level of all heavy metals in our findings fell within tolerable range except for Pb whose concentration surpassed the acceptable limit recommended by FAO/WHO. Absorption of Pb by consumption of wheat grains poses serious threats to the lives of humankind. So, it is recommended that industrial wastewater should be treated properly before its application on agricultural land to reduce the extent of metal contamination in soil and wheat crop.

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