BIOLOGIA (PAKISTAN)
December, 2018, 64 (II), 257-264

PKISSN 0006 – 3096 (Print)
ISSN 2313 – 206X (On-Line)

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

ZAFAR IQBAL KHAN[1*], KAFEEL AHMAD[1], KHALID NAWAZ[2], MUHAMMAD NADEEM[3], ASMA ASHFAQ[1], BABAR MUNIR[1], HAFSA MEMOONA[3], MADIHA SANA[3], FARZANA SHAHEEN[1], NAUNAIN MEHMOOD[4], HIRA MUQADAS[4], MAHPARA SHEZADI[5], IJAZ RASOOL NOORKA[6], HUMAYUN BASHIR[1], MUDASRA MUNIR[1], ILKER UGULU[7] & YUNUS DOGAN[7]

[1]Department of Botany, University of Sargodha, Sargodha, Pakistan
[2]Department of Botany, University of Gujrat, Gujrat, Pakistan
[3]Institute of Food Science and Nutrition, University of Sargodha, Sargodha, Pakistan
[4]epartment of Zoology, Lahore College for Women University, Lahore, Pakistan
[6]Department of Zoology, University of Sargodha, Sargodha, Pakistan
[5]Department of Plant breeding and Genetics, Ghazi University, Dera Ghazi Khan, Pakistan
[6]Plant Breeding and Genetics, Agriculture College, University of Sargodha, Sargodha, Pakistan
[7]Buca Faculty of Education, Dokuz Eylul University, Izmir, Turkey

ARTICLE INFORMATION

Received: 11-09-2018
Received in revised form: 28-11-2018
Accepted: 17-01-2019

***Corresponding Author:**

Zafar Iqbal Khan: zikhan11@gmail.com

Original Research Article

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,

Author's Contribution: Z.I. & K.A., Designed and supervised study; **A.A. & B.M.,** Conducted experiment; **K.N. & M.N.,** Did statistical analysis; **H.M. & M.S.**, Compiled results; **F.S.**, wrote manuscript 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.

.

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)

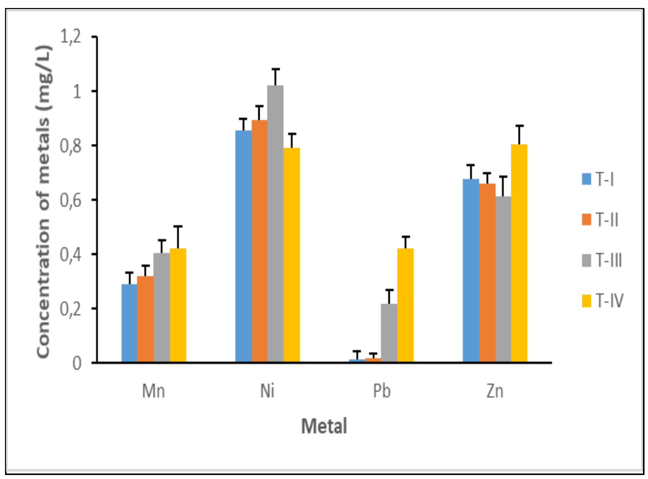

Fig. 1: The variation of heavy metals in irrigation water

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).

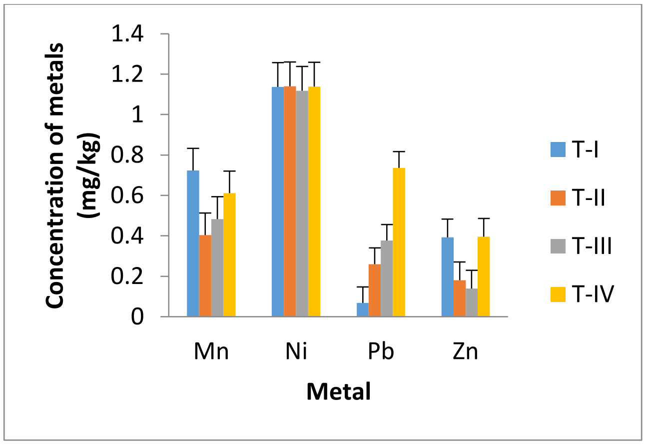

Fig. 2: The variation of heavy metals in soil

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).

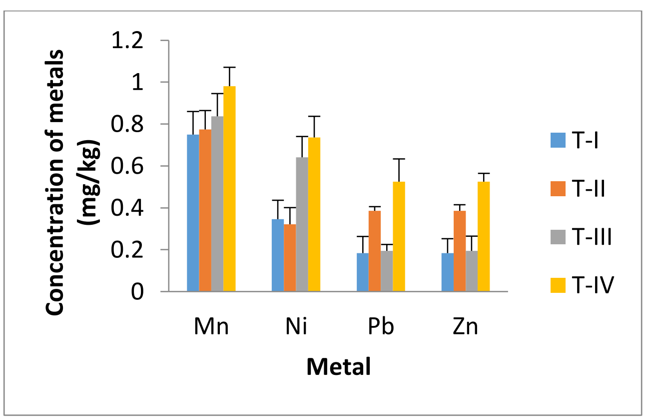

Fig. 3: The variation of heavy metals in grains of *Triticum aestivum*

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.

ACKNOWLEDGEMENT

The Higher Education Commission, Pakistan is acknowledged for providing the financial support through a research project # 2484/13. The authors acknowledge to Ramzan sugar mill Chiniot for providing effluent to irrigate wheat. The authors also thank all the colleague for suggestions and constructive comments for the improvement of this manuscript.

REFERENCES

Abdu, N., Abdulkadir, A., Agbenin, J.O. and Buerkert, A. (2011) Vertical distribution of heavy metals in waste water irrigated vegetable garden soils of three West African cities. *Nutrient Cycling and Agro eco system*, 89, 387-397.

Ahmad, K., Nawaz, K., Khan, Z.I. *et al.* 2018a). Effect of diverse regimes of irrigation on metals accumulation in wheat crop: An assessment-dire need of the day. *Fresenius Environmental Bulletin,* 27(2), 846-855.

Ahmad, K., Kokab, R., Khan, Z.I., Ashfaq, A., Bashir, H., Mudasra, M. *et al.* (2018b). Assessment of heavy metals in wheat variety ‘‘Chagi-2’’ under short-term wastewater irrigation. *Biologia (Pakistan),* 64(1), 15-25.

Alghobar, M.A., Ramachandra, L. and Suresha, S. (2014) Effect of sewage water irrigation on soil properties and evaluation of the accumulation of elements in grasscrop in Mysore city, Karnataka, India. *American Journal of Environmental Protection*, 3(5), 283-291.

Allen, S., Grimshaw, H. and Rowland, A. (1986) Chemical analysis. In: Moore, P.D. and Chapman, S.B. (Eds) Methods in Plant Ecology, *Blackwell Scientific Publications*, Oxford, pp. 285-344

APHA. (2005) Standard methods for the examination of water and wastewater. American Public Health Association. Washington, DC.

Asdeo, A. (2014) Toxic metal contamination of staple crops (wheat and millet) in peri-urban area of western Rajasthan*. International Refereed Journal of Engineering and Science*, 3(4), 8-18.

CSEPA (China State Environmental Protection Administration). (1995) GB 15618-1995 Environmental quality standard for soils. State Environmental Protection Administration of China, Beijing.

Cui, Y.J., Zhu, Y.G., Zhai, R.H., Chen, D.Y., Huang, Y.Z., Qiu, Y. and Liang, J.Z. (2004) Transfer of metals from soil to vegetables in an area near a smelter in Nanning, China. *Environment International*, 30(6), 785-791.

Dogan, Y., Baslar, S. and Ugulu, I. (2014) A study on detecting heavy metal accumulation through biomonitoring: Content of trace elements in plants at Mount Kazdagi in Turkey. *Applied Ecology and Environmental Research*, 12(3), 627-636.

FAO. (1985) Water Quality for Agriculture, FAO irrigation and drainage paper No. 29. Food and Agriculture Organization of the United Nations, Rome.

FAO/WHO. (2001) Report on the 32nd Session of the Codex Committee on Food Additives and Contaminants ALINORM 01/12, Beijing. China. 20-24 March 2000. Joint FAO/WHO Food Standard Programme. Codex Alimentarius Commission. 24th Session. 2-7 July. Geneva, Switzerland.

Hassan, M., Mirza, A.T.M., Rahman, T., Saha, B. and Kamal, K.I. (2015) Status of heavy metals in water and sediment of the Meghna River, Bangladesh. *American Journal of Environmental Sciences*, 11(6), 427-439.

Jaishree and Khan, T.I. (2015) Assessment of heavy metals risk on human health via dietary intake of cereals and vegetables from effluent irrigated land Jaipur district, Rajasthan*.* International Journal of Innovative Research in Science, *Engineering and Technology*, 4(7), 5142-5148.

Khan, N.H., Nafees, M. and Bashir, A. (2016) Study of heavy metals in soil and wheat crop and their transfer to food chain. *Sarhad Journal of Agriculture*, 32(2), 70-79.

Khan, Z.I., Ahmad, K., Ashraf, M., Akram, N.A., Rizwan, Y., Shaheen, M. and Arshad, F. (2013) Assessment of potential toxicological risk for public health of heavy metals in wheat crop irrigated with wastewater: a case study in Sargodha, *Pakistan. Asian Journal of Chemistry*, 25(17), 9704-9706.

Konwar, D. and Jha, D.K. (2010) Response of rice (*Oryza sativa* l.) to contamination of soil with refinery effluents under natural conditions. *Biological and Environmental Sciences*, 5(1), 14-22.

Li, X., Poon, C. and Liu, P.S. (2001) Heavy metal contamination of urban soils and street dusts in Hong Kong. *Applied Geochemistry*, 16(11-12), 1361-1368.

Lin, H.J., Sunge, T., Cheng, C.Y. and Guo, H.R. (2013) Arsenic levels in drinking water and mortality of liver cancer in Taiwan. *Journal of Hazardous Materials*, 262, 1132-1138.

Mahmood, T. (2010) Review Phytoextraction of heavy metals. The process and scope for remediation of contaminated soils. *Soil and Environment*, 29(2), 91-109.

Manzoor, S., Shah, M.H., Shaheen, N., Khalique A. and Jaffar, M. (2006) Multivariate analysis of trace metals in textile effluents in relation to soil and groundwater*. Journal of Hazardous Materials*, 137(1), 31-37.

Mapanda, F., Mangwayana, E.N., Nyamangara, J. and Giller, K.E. (2007) Uptake of heavy metals by vegetables irrigated using wastewater and the subsequent risks in Harare, Zimbabwe. *Physics and Chemistry of the Earth*, 32(15-18), 1399-1405.

Mclean, E.O. (1982) Soil pH and lime requirement. In: Page, A.L., Miller, R.H. and Keeney, D.R. (Eds.) Methods of soil analysis. Part 2. Chemical and microbiological properties.

American Society of Agronomy, Madison, WI, pp. 199-223.

Metwali, E.M.R., Gowayed, S. M.H., Al-Maghrabi, O.A. and Mosleh, Y.Y. (2013) Evaluation of toxic effect of copper and cadmium on growth, physiological traits and protein profile of wheat (*Triticum aestivum* L.), maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* L.). *World Applied Sciences Journal*, 21(3), 301-314.

MWE. (2005) National Wastewater Regulations, Section III–2.2 and 3.2.3. Ministry of Water and Electricity, Riyadh.

Nafees, M. and Amin, A. (2014) Evaluation of heavy metals accumulation in different parts of wheat plant grown on soil amended with sediment collected from Kabul river canal. *Journal of Agriculture Research*, 52(3), 383-394.

Nafees, M., Ahmad, T. and Arshad, M. (2011) A review of Kabul river uses and its impact on fish and fisherman. *Journal of Humanities and Social Sciences*, 19(2), 73-84.

Olsen, S.R. and Sommers, LE. (1982) Phosphorous. In: Page, A.L., Miller, R.H. and Keeney, D.R. (Eds.) Methods of soil analysis. Part 2. Chemical and microbiological properties. American Society of Agronomy, Madison, WI, pp. 403-430.

Otokunefor, J.V. and Obiukwu C. (2005) Impact of refinery effluent on the physicochemical properties of a water body in the Niger delta. *Applied Ecology and Environmental Research*, 3(1), 61-72.

Page, A.L. (1982) Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties. Agron. Monogr. 9.2. Agronomy Society of America, Soil Science Society of America, Madison, WI.

Richard, L.A. (1954) Diagnosis and improvement of saline and alkali soils. Agriculture Handbook Vol. 60*.* United States Department of Agriculture, Washington, DC.

Singh, A., Sharma, R.K. and Agarwal, M. (2010) Risk assessment of heavy metal toxicity through contaminated vegetables from wastewater irrigated area of Varanasi. *Tropical Ecology*, 51, 375-87.

Sorme, L. and Lagerkvist, R. (2002) Sources of heavy metals in urban wastewater in Stockholm. *Science of the Total Environment*, 298(1), 131-145.

Steel, R.G.D and Torrie, J.H. (1980) Principles and procedures of statistics. A Biometrical Approach, 2. McGraw-Hill, New York.

Ugulu, I., Unver, M.C. and Dogan, Y. (2016) Determination and comparison of heavy metal accumulatıon level of *Ficus carica* bark and leaf samples in Artvin, Turkey. *Oxidation Communications*, 39(1), 765–775.

USEPA. (2000) Supplementary guidance for conducting health risk assessment of chemical mixtures,Risk Assessment Forum Technical Panel.

Vaseghi, S., Afyuni, M., Shariatmadari, H. and Mobli, M. (2005) Effect of sewage sludge on some nutrients concentration and soil chemical properties. *Journal of Isfahan Water and Wastewater*, 53, 15-19.

Verma, S., Yadav, S., Yadav, S.K., Kadyan, P.S., Singh I. and Singh,D. (2015) Heavy metals in wheat grains of Haryana (India) and their health implications. *Journal of Chemical and Pharmacutical Research*, 10, 342-357.

Vijayaraghavan, K., Palanivelu, K. and Velan, M. (2006) Biosorption of copper (II) and cobalt (II) from aqueous solutions by crab shell particles. *Bioresource Technology,* 97, 1411-1419.

WWF (Waste Water Forum). (2007) Report on national surface water classification criteria, irrigation water quality guidelines for Pakistan, February-2007. Waste Water Forum, Pakistan.