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Monitoring and health risk assessment of selected trace metals in wheat rice and soil samples

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Abstract

Wheat and rice constitute important cereal crops and any contamination may pose potential adverse impacts on human health. The study was conducted to determine eight trace metals, i.e. cadmium (Cd), copper (Cu), cobalt (Co), chromium (Cr), zinc (Zn), lead (Pb), nickel(Ni), manganese (Mn), in wheat, rice and soil samples collected from different cities of Punjab. Results showed general trend of trace element accumulation in samples as Cd >Cu>Zn>Co>Mn. All wheat and nine rice samples exceeded permissible limit of Cd. Copper in eight wheat and rice samples and Zn levels in three wheat and one rice samples were in excess. While only one soil sample of Multan exceeded the WHO 2007 and EU 2000 maximum allowable limit for Cu. Manganese and Co concentration were within allowable limit, while Ni, Cr and Pb were not detected in any sample. Health Risk Index was higher than 1 for Cu, Cd, Co and Mn thereby showing potential health risks to consumers.

Keywords: trace metals; wheat; rice; health risk index.

Practical Application: Wheat and rice are widely consumed source of food worldwide. Therefore, this food group is important in agriculture and any contamination of this commodity with unwanted chemicals is a matter of grave concern. The increasing apprehension regarding food safety stimulated the research regarding pollution of trace metals in wheat and rice is an issue and must be monitored by the relevant authorities.

1 Introduction

Agriculture sector of Pakistan plays a dominant role in the economy with significant contribution (18.9 percent) to Gross Domestic Product (GDP). The sector provides employment to about 42.3 percent of total labor force and is most important in terms of foreign exchange earnings (Government of Pakistan, 2017).

Wheat and rice are amongst the most important cereal crop grown and consumed around the world. Among the wheat producing countries of the world, Pakistan is ranked 8th in terms of area under wheat cultivation, production and yield per hectare (Riazuddin et al., 2011). With an area of 8,972 thousand hectares and a total production of 25.492 million tonnes during 2017-18, it contributed to 1.7% of GDP and 9.1% to the total value added in agriculture. Similarly, rice added to about 1.3-1.6% of GDP (Pakistan Economic survey, 2017).

Cereal crops are major source of nutrients and the most commonly cultivated food in Pakistan. Therefore, this food group is important in agriculture and contamination of this commodity with unwanted chemicals is viewed with concern. The increasing concern regarding food safety stimulated research regarding contamination of food stuffs with different toxins such as pesticides and trace metals (Riazuddin et al., 2011).

Contamination of different food stuffs like meat, milk, vegetables, sea weeds etc. is highly prevalent and often widely reported around the world (Khan et al., 2018; Wang et al., 2019; Liang et al., 2018; Roleda et al., 2019). Trace metals (TMs) constitute one of the major contaminants of food commodities, the sources of which can be traced to the application of contaminated irrigation water, use of fertilizers and pesticides and industrial activities etc.

Studies have shown high concentration of TMs in different areas in Pakistan, including wheat samples analyzed from Abbottabad, Khyber Pakhtunkhwa (Hassan et al., 2013). A similar study carried out to evaluate the role of sewage sludge in increased concentrations of TMs showed that wheat grown on soil amended with sludge had high concentrations of lead (Pb) and Cd (Jamali et al., 2009).These metals not only persist in the environment but are also toxic to humans and animals since they cause cancers, mutations, teratogenicity, disruption of hormones, skin, eyes irritation, headache, nausea, dizziness, poisoning, coma, endocrine disruption, respiratory illness, convulsions, liver damage, tremors, decreased fertility, effects on central nervous system, kidneys, abdominal pain and loss of muscle coordination (Jaishankar et al., 2014; Roberts & Relgart, 2013). A study conducted in Sialkot and Gujranwala districts to determine HRI of TMs in wheat and some vegetables depicted that maximum HRI was observed in wheat samples of both areas with values up to 8.9 and 9.9, respectively. Hence, the population of study areas may experience serious health risks especially because of consumption of wheat (Khan et al., 2014).

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The present study focused on determination of TMs; Cd, Cr, Zn, Mn, Cu, Co, Pb and Ni in wheat, rice and soil samples and subsequent risk to human health.

2 Materials and methods

Wheat (W; n=15), rice (R; n=11) and soil (S; n=13) samples were collected from different areas including Kala Shah Kaku (KSK W1, KSK R1), Multan (MW1, MW2, MW3, MS1 MS2, MS3), Lahore (LW1, LW2, LW3, LW4,LR1, LR2, LR3, LR4, LS1, LS2, LS3, LS4), Minchinabad (MAW1, MAW2, MA R1, MAR2, MAS1, MAS2) Sargodha (SR W1, SRW2, SR R1, SRS1, SRS2), Eminabad (AMN W1, AMN R1) and Sheikhupura (SKPW1, SKPW2, SKP R1, SKP R2,SKPS1, SKPS2) (Figure 1). These areas were selected on the basis of their rich agricultural productivity. The TMs analyzed included Ni, Cd, Mn, Zn, Cu, Co and Pb. Stock solutions of 100 mg L^{-1} were prepared by diluting the respective metals' CRM with distilled water. From this, working solutions were prepared using calibrated glass ware.

2.1 Preparation of samples

100 - 200 g of sample was collected by coning and quartering and whole sample was mixed well followed by grinding in clean & oven dried pestle and mortar and sieved through a 20 mesh sieve. The ground sample was stored in air-tight jar before further use.

Estimation of trace metals in wheat, rice and soil samples

Extraction of TMs

Standard USEPA method 3015A, with some modifications, was followed for sample preparation for metal analysis (United States Environmental Protection Agency, 2007a). Two solutions were made using hydrogen peroxide and nitric acid. The solution A contained 30 mL hydrogen peroxide and 70 mL distilled water. Solution B contained 50 mL nitric acid and 50 mL distilled water. Weighed 2 g each of respective sample i.e. wheat/rice/soil and transferred into round bottom flask in which 5 mL of solution A, 5 mL of solution B and 10 mL distilled water were added. This round bottom flask was placed in a beaker containing silica gel. To keep the flask intact in its position thermopol sheet was cut and placed at the neck of the flask and as a cork on top of it.

Once the sample solution was made and initial reaction subsides, the beaker containing silica gel and round bottom flask was placed into microwave for 40 seconds for digestion of the sample. However, after every 10 second the beaker was taken out and allowed to cool. After digestion, the round bottom flask contents was filtered using Whatman filter paper 41 and the filtrate was diluted up to the mark in 100 mL volumetric/ flask with distilled water.

Same procedure was followed for rice and soil samples.

Metal analysis using AAS

Metals were determined using AAS (model 210VGP) as per standard USEPA method number 7000B (United States Environmental Protection Agency, 2007b). Graph between the concentration and the absorbance of standard solutions was drawn. The standards for each metal ion were prepared as 0.01ppm, 0.05ppm, 0.1ppm, 0.5ppm, 1ppm, 2ppm, 5ppm, 10ppm, 20ppm, 50ppm and 100 ppm. Concentration of the metals was determined using calibration curves. The correlation coefficients R2 (calibration curve), were in the range of 0.9817 and 0.9970. The instrumental limit of detection (LOD) and limit of quantification (LOQ) were determined by using certified reference materials of the respective metals supplied by VWR, which are given in Table 1.

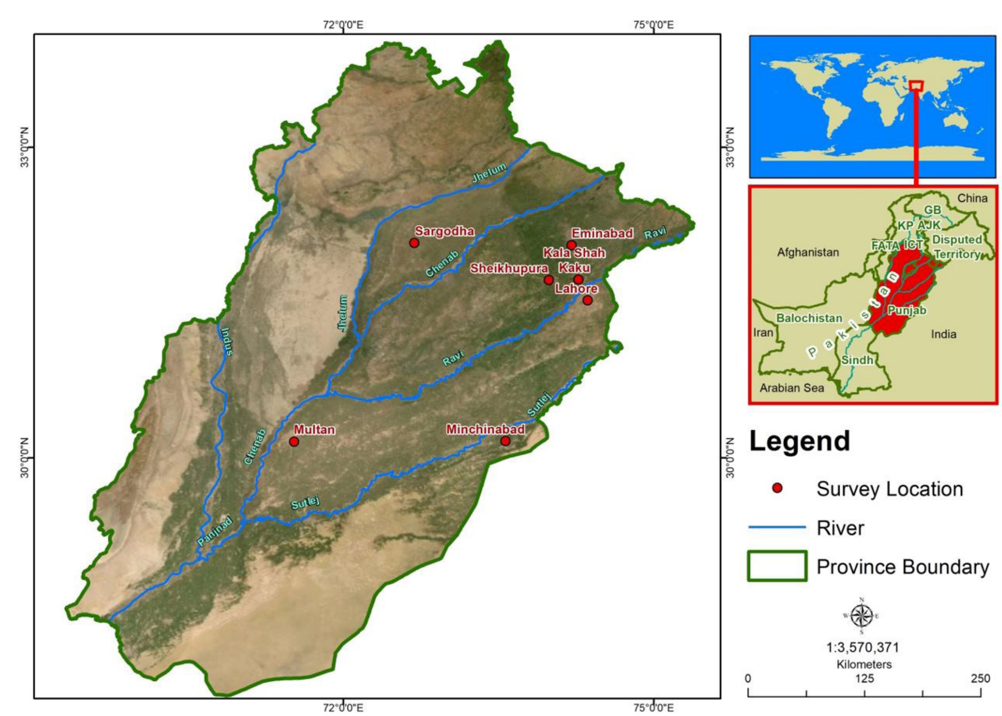

Figure1. Location map of study areas.

2.2 Determination of Health Risk Index (HRI)

Health risk index was calculated using the following formula (Equation 1)

$$HRI = \frac{DIM}{RfD} \quad (1)$$

Here RfD is reference oral dose and according to EPA, its values are 0.14, 0.3, 0.04, 0.001, 0.043 mg $kg^{-1}day^{-1}$for Mn, Zn, Cu, Cd, and Co, respectively (United States Environmental Protection Agency, 2010). DIM is Daily intake of Metals and it was calculated using formula (Equation 2)

$$DIM = \frac{Conc.\ of\ Metal \times Daily\ food\ intake}{Average\ body\ weight} \quad (2)$$

Taking average body weight in Pakistan 72 kg, average rice consumption per capita 0.15 kg day^{-1} (JCR-VIS Credit Rating Company Limited, 2017) and average wheat consumption per capita 0.34 kg day^{-1} (Global International Information Network, 2017).

An HRI index value of more than 1 is considered unsafe for human health (United States Environmental Protection Agency, 2010).

2.3 Target Hazard Quotient (THQ)

THQ of trace metals was calculated using following Equation 3 (Zhuang et al., 2009);

$$THQ = \frac{EFr \times ED \times FI \times MC}{RfD \times BW \times AT} \times 10^{-3} \quad (3)$$

Here, EFr is exposure frequency (365 days per year), ED is exposure duration which is basically the life expectancy (66.5 years for Pakistan as reported by WHO), FI is food ingestion rate (kg $person^{-1}day^{-1}$), MC is metal concentration in mg kg^{-1}, RfD is reference dose, BW is body weight (72 kg), and AT is average exposure time (365 days $year^{-1}$ × number of exposure years, assuming 66.5 years.

3 Results and discussion

Eight metals Ni, Mn, Zn, Co, Cd, Co, Cr, Pb were investigated in wheat, rice and soil samples. Metal concentration varies in wheat and rice depending upon the soil, water used for irrigation and the climate of the cultivated area whereas for soil the metal concentration is mostly higher when compared to the crops grown on that soil.

Out of all metals analyzed, Pb, Ni and Cr were not detected in any sample whereas the concentrations of other five metals are mentioned in Table 2-4.

Table 1. Instrumental Limit of detection (LOD) and limit of quantification (LOQ) of the trace metals.

Metals	Cd	Co	Cr	Ni	Zn	Mn	Cu
LOD mg/l	0.01	0.05	0.04	0.05	0.005	0.03	0.005
LOQ mg/l	0.03	0.15	0.12	0.151	0.015	0.09	0.015

Table 2. Concentrations of trace metals in wheat samples.

Sample code	Trace metals concentration (mg kg^{-1})				
	Mn	Zn	Cu	Cd	Co
AMNW1	34	43.75	**88**	**0.25**	**37.5**
KSKW1	36.25	**57.75**	**44**	**0.25**	**58.25**
LW1	48.25	**58.5**	**16.5**	**0.25**	**25**
LW2	50	34.75	**51.07**	**0.25**	**25**
LW3	0.75	26	2.5	**1.25**	ND
LW4	31.75	44	2.25	**0.25**	**25**
MAW1	38.75	45	**31.89**	**0.25**	**12.5**
MAW2	31.75	26.5	1.75	**0.25**	**50**
MW1	50	42.75	2.5	**14**	**54.25**
MW2	38.5	**50**	2.0	**2.5**	**2.5**
MW3	50	28	2.25	**0.25**	**12.5**
SKPW1	20.75	37.75	**44.75**	**14**	**50**
SKPW2	ND	45	**20.8**	**1.25**	**54.25**
SRW1	50	31.75	**40.5**	**0.25**	**12.5**
SRW2	ND	16.25	ND	**0.25**	ND
Max. concentration	50	58.5	88	14	58.25
Min. concentration	0.75	16.25	1.75	0.25	2.5
Standards (World Health Organization, 2004)	500	50	3.0	0.20/0.1	-

Table 3. Concentrations of trace metals in rice samples.

Sample Code	Concentration of Trace Metals in mg kg-1				
	Mn	Zn	Cu	Cd	Co
AMN R1	ND	29	ND	**0.25**	2.5
KSKR1	7.5	25	ND	**0.25**	12.5
LR1	25	46	**65.25**	ND	12.5
LR2	110	19.25	**352.75**	**17.5**	2.5
LR3	ND	12.5	ND	**1.25**	ND
LR4	25	**90**	**51.07**	**12.5**	ND
MAR1	20	26.25	**432.25**	**17.5**	0.25
MAR2	18.75	26.25	**277.5**	**17.5**	12.5
SKPR1	18.75	27.5	**68.5**	ND	37.5
SKPR2	1.25	33.75	**57.6**	**5.0**	ND
SRR1	20	40	**54.25**	**17.5**	2.5
Max. concentration	110	90	432.25	17.5	37.5
Min. concentration	1.25	12.5	51.07	0.25	0.25
Standards (European Union, 2002; World Health Organization, 2004)	500	50	3.0	0.20/0.1	-

Table 4. Concentration of trace metals in soil samples.

Sample code	Trace metals concentration (mg kg-1)				
	Mn	Zn	Cu	Cd	Co
LS1	120	44	20.8	**16**	2.5
LS2	392.5	34	31	**13**	12.5
LS3	303.75	47	36.25	**50**	0.25
LS4	83.25	40.75	27.5	**66**	12.5
MAS1	58.75	41.75	41.5	**17**	0.25
MAS2	172	53.5	28.5	**10**	0.25
MS1	170.5	30	**262.75**	**73.43**	12.5
MS2	132.5	28.5	20.8	**25**	02.5
MS3	254.25	28	27.5	**33**	2.5
SKPS1	144	37.5	27.5	ND	2.5
SKPS2	250	50	29.25	**63**	ND
SRS1	259.5	35.25	93.5	**35**	0.25
SRS2	179.75	31	32	**17**	0.25
Max. concentration	392.5	53.5	262.75	73.43	12.5
Min. concentration	58.75	28	20.8	10	0.25
Standards (Awashthi, 2000; European Union, 2002)	-	300-600	100/140	3-6	60

Three (20%) wheat samples; M W2 (50 mg kg^{-1}), L W1 (58.5 mg kg^{-1}) and KSK W1 (57.75mg kg^{-1}) exceeded the maximum allowable limit (MAL) of 50 mg kg^{-1} (World Health Organization, 2004) for Zn with highest being 17% more than the standard. In similar studies conducted earlier, Zn was reported to be as low as 35.5 mg kg^{-1} (Hassan et al., 2013) and between 108-133 mg kg^{-1}in wheat grains in Pakistan (Jamali et al., 2009).

One rice sample L R4 (90 mg kg^{-1}) exceeded the permissible limit being almost double the standard value. Zinc contaminated food crops can adversely affect human health. High zinc concentration in food crops can damage pancreas and hinder the protein functioning in a human body (Jaishankar et al., 2014). The MAL of Zn in soil, according to Indian standards 2000, is 300-600 mg kg^{-1}. None of the soil sample exceeded the MAL.

The permissible level of Cu in wheat and rice is 3 mg kg^{-1} (World Health Organization, 2004). The results showed that 53% wheat samples (LW1, LW2, KSK W1, SKP W1, SKP W2, MA W1, SR W1 and AMN W1) exceeded the MAL (average value of 25.1±26.1) while rest of the analyzed samples showed levels between the range of 1.75-2.5 mg kg^{-1}. Other studies from Pakistan reported Cu levels in the range of 3.1-5.1 mg kg^{-1} (Hassan et al., 2013) and 11.8 mg kg^{-1}in wheat samples (Jamali et al., 2009). Elsewhere, Bermudez et al. (2011) reported Zn (range 7.8–56.4 mg kg^{-1}) and Cu levels (range 1.25-6.93 mg kg^{-1}) in wheat grains to be higher than the standard limits set by FAO/WHO.

Copper concentrations were very high in eight (72%) rice samples. In another study conducted in China, 38 rice cultivars were examined for Cu, Cd and Hg levels. However, all rice samples had Cu concentration lower or close to the maximum safe intake level with range of 1.81-6.54 mg kg^{-1} (He et al., 2013). Copper is important for plant growth and is available in soil and sediments. It is also an essential element for human health which is naturally present in wide range of food varieties e.g. beef liver, beans, nuts etc. but exceeding levels may result in anemia, damage liver and kidney and cause intestinal irritation. Even though acute and chronic poisoning of Cu is not common in humans a prolonged exposure to excessive Cu levels may result in adverse reactions on liver (Jaishankar et al., 2014; Oosthuizen, 2012).

In the present study, though all soil samples, except M S1 (262.75mg kg^{-1}, with almost twice the standard value), had Cu levels less than the permissible limit (European Union, 2002) yet these were higher than reported by other similar study in Pakistan which was in the range of 3.1-5.1 mg kg^{-1}(Hassan et al., 2013). Cu concentration varies in various soil samples depending upon the soil type and pollution source. Fungicide sprays that are used on farm are also laden with Cu that may result in contamination. The soil to plant transfer of Cu determines the level of human exposure to copper.

All wheat samples exceeded the MAL (0.2 mg kg^{-1}) of Cd with maximum concentration (14 mg kg^{-1}) of Cd in M W1 & SKPW1which is much higher than reported earlier who also identified major reasons for higher levels of Cd to be wastewater irrigation or soil amendment by sewage sludge which changes the pH of soil. Nevertheless, most samples had Cd content similar as reported (Hassan et al., 2013). Other studies in Pakistan also reported higher Cd concentration in wastewater used for agriculture. One such study showed Cd levels in the range 0.18-0.37 mg kg^{-1}in Lahore (Mahmood & Malik, 2014). Whereas, in Iran, Co and Cd levels in wheat were found to be below detection limit (0.015μg g^{-1} & 0.0047 μg g^{-1} respectively) in all samples, whereas mean concentration of Cu was 0.501 μg g^{-1} (Esmaili et al., 2017).

Likewise, 81% of rice samples exceeded MAL (0.20 mg kg^{-1}) for Cd level with a maximum concentration of 17.5 mg kg^{-1} and minimum of 0.25 mg kg^{-1}. Similar study was conducted in Iran to determine the level of arsenic, Cd and lead (Pb) in rice samples, imported from Pakistan and India. Concentration of Pb and Cd were considerably higher than the safe limit by FAO/WHO (Roya & Ali, 2017). In another study carried out in Saudi Arabia, Cd concentration measured in 37 brands of imported rice showed higher levels than the standard limits. However, it has been demonstrated that soaking or rinsing rice grains with water helped decrease Cd to acceptable levels. Huo et al. (2016) found that Cd in rice could be reduced by > 95% by washing with citric acid, due to breaking of bond between Cd and rice protein (Al-Saleh & Abduljabbar, 2017). Amir et al. (2019) has also reported the potential of citric acid to effectively reduce metal content from vegetables.

As expected, all the analyzed soil samples also showed Cd values higher than the MAL compared to allowable concentration of 3-6 mg kg^{-1} (Awashthi, 2000).

Cadmium is one of the most toxic heavy metals because it is highly mobile in soil. It can damage human health at each level from cell formation to body functioning and acute or chronic intoxication. Application of fertilizer and sewage sludge on farm leads to soil contamination and increased uptake by plants especially at low pH. Moreover, farmers in developing countries like Pakistan and India use effluent for irrigation which becomes a source of many toxic metals (Chandra et al., 2009; Abbas et al., 2007). Studies have also shown that Cd can remain in soil for several decades and is slowly taken up by plants, accumulated and then concentrated along the food chain, reaching human body. Prolonged exposure of Cd to human body leads to kidney failure, liver failure and damage to central and peripheral nervous system. (Al-Saleh & Abduljabbar, 2017; Jaishankar et al., 2014; Oosthuizen, 2012; Malik et al., 2010).

The mean concentration of Co in wheat and rice samples was 32.3±14.3 and 10.34±12.2 mg kg^{-1}respectively. Whereas none of the soil samples exceeded the limit (60mg kg^{-1}) given by Indian regulations 2000 (Awashthi, 2000). When a high Co level is readily available, in polluted soil in particular, it can seriously affect plant growth and metabolic functions. The Co concentration of 25 and 50 ppm in soil is toxic to rice plants (Pendias & Pendias, 2001).

Manganese is important for growth, skeletal formation and reproductive functioning in humans and animals and its deficiency can lead to diabetes, bone disorder, stunted growth in infants and children and nervous system instability, whereas, in adults it can lead to arthritis (Rehman et al., 2017). It is also considered essential for the metabolic processes in plants. The Mn content in wheat samples was within limits and consistent with mean concentration in wheat grains and wheat flour reported in similar studies in Pakistan as 4.9 mg kg^{-1}(Hassan et al., 2013) and 38.1mg kg^{-1} (Hussain et al., 2011). In soil samples, Mn concentration were much higher than reported in India where Mn levels in soil and grain (wheat) samples were reported as 154-194 mg $kg^{-1}$and 11.19-40.57 mg kg^{-1}respectively (Vanita et al., 2014).

Highest concentrations of TMs were detected mostly in the samples of Eminabad, Sheikhupura and Lahore.

3.1 Health risk index

Health risk index calculation showed potential for health risk due to presence of Cu, Cd and Co in analyzed samples (Table 5).

Calculation of HRI of Cu indicates that 57% wheat samples while all rice samples had HRI value greater than 1 indicating potential high risk to human health. The HRI of Cd for all wheat samples was higher than 1 (average value 11.7) whereas, 78% of rice samples had HRI greater than 1. A total of 92% of the wheat samples while only 1 rice sample i.e. SKP R1 (1.81) had HRI value higher than 1 for Co. Whereas, for Mn 85% of wheat samples had HRI >1 with an average of 1.24 but only one rice sample had HRI greater than 1.

Similar study conducted in Swat shows HRI for Cd to be much higher as compared to Cr, Cu, Mn, Ni and Zn. Cadmium concentration was higher in all of the samples and it was observed that cereal grains were highly contaminated relative to fruits and vegetables. However, the risk assessment revealed that there were no health risks in the area for all TMS except Cd, which showed HRI > 1 (Khan et al.,

Table 5. HRI and THQ of trace metals in wheat and rice samples.

Values	HRI					THQ				
	Co	Cd	Cu	Zn	Mn	Co	Cd	Cu	Zn	Mn
Wheat										
Minimum value	0.25	1.18	0.20	0.25	0.025	0	0	0	0	0
Maximum value	6.39	66.1	10.3	0.92	1.68	0	0.06	0	0	0.02
Mean	3.53	11.7	2.94	0.61	1.24	0	0.01	0	0	0.001
Rice										
Minimum value	0.01	0.52	2.65	0.08	0.018	0	0	0	0	0
Maximum value	1.81	36.4	22.5	0.62	1.63	0	0.03	0.02	0	0.02
Mean	0.49	20.62	8.82	0.23	0.40	0	0.016	0.005	0	0.0002

2013). In a comparable study conducted in Sialkot and Gujranwala the values of HRI of TMs through consumption of wheat were found in the order of: Pb> Cd > Ni >Mn> Fe > Zn > Cu > Cr in Sialkot and Pb> Cd >Mn> Ni > Fe > Zn > Cu > Cr in Gujranwala. It was concluded that the population of these areas may experience a health risk because of the high consumption rate of wheat (Khan et al., 2014).

4 Conclusion

Trace metal accumulation is a rising concern in this era. These pollutants not only accumulate in the environment but also cause health hazards to humans. Among the studied metals, results show a substantial concentration of TMs in the samples. The general trend observed is in decreasing order Cd>Cu>Zn>Co>Mn. It is suggested that a comprehensive study must be conducted to monitor and evaluate irrigation water quality, crops contamination and the prevalent health risk in these and other food growing/ agriculture areas.

The HRI was found higher (>1) for Cd, Cu, Co, and Mn indicating potential health risk to consumers.

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