Environmental Science and Pollution Research (2020) 27:29567–29579
https://doi.org/10.1007/s11356-020-09256-4

RESEARCH ARTICLE

Human health risk assessment of heavy metals in raw milk of buffalo feeding at wastewater-irrigated agricultural farms in Pakistan

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Received: 14 November 2019 / Accepted: 11 May 2020 / Published online: 22 May 2020

Abstract
Wastewater irrigation to grow fodder for animals and cattle farming is common practice in Pakistan. Hence, this study was conducted in Multan, Pakistan, to assess heavy metal pollution, human health risk and the total target health quotient (TTHQ) of heavy metals in raw milk of buffalo feeding at different agricultural farms and to identify sources of toxicity in milk. Samples of raw milk ($n = 60$) were analyzed for Cd, Cr, Cu, Mn, Ni, and Pb by ICP-OES, Perkin Elmer, USA. The TTHQ values of heavy metals ranged from 6.92 to 42.44 in raw milk of buffalo, highest at wastewater-irrigated agricultural farms and lowest at tube well water site, indicating high carcinogenic health risk to exposed population. The multivariate statistical analysis revealed that contaminated fodder like Maize and Brassica plants grown with wastewater and contaminated soil are common sources contributing the heavy metal contamination in raw milk. It invites attention of government to remediate the situation to avoid the potential risks to public health from resulting food chain contamination.

Keywords Heavy metals · Animal fodder · Milk contamination · Carcinogenic health risk · Wastewater-irrigated farms

Introduction

Wastewater is a combination of effluents from domestic, commercial establishments including hospitals and institutions, industries, storm water, urban runoff, and agricultural activities (Corcoran et al. 2010). Wastewater irrigation is worldwide commonly practiced in urban and peri-urban areas of all cities and it is increasing in developing countries facing water scarcity. About 7% of world land is under wastewater irrigation in fifty countries and the unreported area may be even more as no legal harmonized system is available at a global and national level for systematic collection of the data. The use of wastewater in agricultural land is a primary source of soil and food contamination and can be detrimental to the health of food consumers. Such practice is likely to increase to meet the increasing food needs as the urban population is projected to be double by 2050 (UN Water 2014; UN Water 2015). The use of even treated wastewater in agriculture causes augmentation of metals in soil and crops (Qadir et al. 2010). In developing countries, farmers use wastewater in agriculture farming due to its fertility enrichment and the controlling authorities view it as cheap mode of disposal (Corcoran et al. 2010).

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The intensified food production to meet the needs of a growing population is putting pressure on water resources and contaminated wastewater is being used in productive ecosystem causing food chain contamination (Corcoran et al. 2010). The use of contaminated wastewater in agriculture is restricting development and increasing poverty due to increase in health care costs and low labor productivity (Corcoran et al. 2010). In Pakistan, about 350,000 ha land is irrigated with wastewater in 75 cities directly and about 550,000 ha land is irrigated indirectly with wastewater (Van der Hoek 2004). The continuous application of wastewater in arid regions to irrigate the crops as exclusive source is likely to impose unwanted effects on the quality of soil and crop and lead to accumulate the nutrients, heavy metals, and salts in crops exceeding the permissible limits (Rusan et al. 2007).

In Pakistan, major cities like Karachi, Lahore, Faisalabad, Rawalpindi, Multan, Peshawar, Hyderabad, Quetta, Gujranwala, and Sargodha discharge untreated wastewater into water surface bodies and such wastewater is used to grow food crops (Hernández et al. 1991; PCRWR 2006). Approximately 26% vegetables are grown with wastewater in urban and peri-urban agricultural areas of these cities. The quantity of direct wastewater being used in agriculture was estimated at 2,400,000 m^3/day and quantity of 400,000 m^3/day was estimated being discharged into irrigation canals without any treatment. This practice of direct use of wastewater as irrigation is likely to increase in the future in Pakistan (Ensink et al. 2004).

Soil is considered an integral part of ecosystem and source of essential nutrients and water requirements for growth of agricultural produce to meet the food needs of humans and animals, and on the other hand, soil has become a sink of heavy metals due to application/disposal of treated and untreated wastewater (Alloway 1995). Some heavy metals are considered imperative for the metabolism of living organisms at low contents but they tend to be toxic beyond the typical concentration. Heavy metals like Cd, Cr, and Pb have a wide range of toxicity (Nemati et al. 2011; Sun et al. 2016), accumulate easily in the soil, and pose a serious threat to human and animal health via skin contact, dust digestion, and food chain contamination (Kabata-Pendias and Pendias 2001). Hence, the researchers have diverted special attention to focus soil contamination and its resulting food chain contamination in recent years (Liu et al. 2016; Tedoldi et al. 2017). Increased production of wastewater due to rapid urbanization is inviting the farmers to irrigate the agricultural land with contaminated wastewater for more food production at low input cost (Nabulo 2009). The wastewater irrigation is a major source of urban agricultural contamination and agricultural produce (Qadir et al. 2000). The industrial wastewater containing heavy metals are major contributing agents to soil and food crop contamination (Mapanda et al. 2005). The heavy metal joins human organs via intake of polluted water, food stuff, or through digestion of contaminated soil dust (Cambra et al. 1999; Chen et al. 1997). Long-term consumption of contaminated food stuff can lead the heavy metals to accumulate in the liver and kidneys of the human population exposed to such a scenario and can result in disorder of biochemical processes like kidney, liver, cardiovascular, bone, and nervous system (WHO 2006). Pakistan was abundant in water and now has been converted into a water stressed country. Five thousand cubic meters per capita water available in 1947 has been shrunk to one thousand in 2012 and it has been projected to shrink to eight hundred by the year 2025, and such a situation may increase the pressure on the use of wastewater in agricultural produce (CCD 2013).

The accumulation of heavy metals in dairy animal organs excrete in milk (Burger and Elbin 2015). The consumption of contaminated milk by the population is resulting in various health implications (Singh et al. 2010). In Pakistan, buffalo are the main source of milk and are known as black gold and their milk is considered a major source of nutrition in all age groups of the population (Younus et al. 2016). To irrigate agricultural land with industrial and sewage wastewater is the main source of induction of heavy metals in animal produce like milk (Awasthi et al. 2012). The consumption of contaminated milk of buffalo and cattle feeding at polluted sites results in sever human health implications (Kar et al. 2015). Food stuff grown with wastewater or on contaminated soil accumulates heavy metals and is the main source of metal transmission to animal and human bodies (Ward and Savage 1994). The animals feeding on contaminated fodder accumulate residues of heavy metals in tissues and milk, and it is a matter of public health concern (Chitmanat and Traichaiyaporn 2010). The metals like Cd, Cr, Cu, and Pb are proven carcinogenic in nature (ATSDR 2017). Such metals pose toxic effects on human health even at low contents (Mahaffey 1977; Santhi et al. 2008).

There may be multidiscipline sources of heavy metals contamination in raw milk and hence multivariate statistical analyses (Pearson correlation matrix (PCM), hierarchical cluster analysis (HCA) and principal component analysis (PCA)) are mostly used to differentiate anthropogenic activities and natural source in contamination study (Ma and Gui 2017; Ma et al. 2016; Nethaji et al. 2017).

The above scenario reflects the sensitivity of the threat being posed by heavy metals to general public health and invites immediate attention of food, environmental, and health regulatory authorities for remediation. There are rare studies to assess the carcinogenic health risk of heavy metals in raw milk of buffalo. Therefore, this study was conducted to (i) investigate the concentration of Cadmium (Cd), Chromium (Cr), Copper (Cu), Manganese (Mn), Nickel (Ni), and Lead (Pb) in raw milk of buffalo feeding at different agricultural farms being irrigated with different qualities of wastewater, canal, and tube well water; (ii) assess the total target health quotient of heavy metals; and (iii) identify the sources of heavy metal contamination in raw milk using multivariate statistical analysis. The overall goal was to provide comprehensive information and baseline data to decision-makers and planners for formulating policies and legislation to remediate the situation to avoid the potential risks to public health from its resulting food chain contamination.

Materials and methods

Introduction to study area

Multan is the 5th largest city of province Punjab and the 6th major city of Pakistan. It is one of the oldest and central city of Pakistan. It is surrounded by Multan branch canal on the east-south side, Shuja abad canal on the west side, Sidhnai canal on the north side, and the Chenab River on the west side. District Multan is spread over an area of 3721 km^2 in Punjab, Pakistan. Multan was recognized as city District in 2005 keeping in view its urban and strategic importance (NESPAK 2017).

Multan city, which is the concern area of this research study, is the capital of district Multan with a current population of 1.8 million. Multan city is facing multi environmental problems specifically related to disposal of untreated wastewater into water surface bodies and its direct use to irrigate agricultural fields in peri-urban areas of Multan city. Hence, Multan city has been selected as a study area.

Climate of the study area

The climate of Multan is extreme hot in summer (29 to 42 °C) and cold in winter (4.5 to 21 °C). The annual rainfall is about 186 mm each year (Abbas 2013; Abbas et al. 2014).

Introduction to six selected sites under different qualities of wastewater irrigation

Six irrigation sites were selected in peri-urban areas of Multan City which are the main producer of agricultural produce like vegetables, wheat, fruits, fodder, milk, and meat which is supplied to the public in the study area and adjoining districts. One site under untreated industrial effluents (site A), two sites under untreated urban wastewater irrigation (site B and site D), one site under mixed water (canal water + urban untreated wastewater) irrigation (site C), one under canal water (site E), and one site under tube well water (site F) were selected. Keeping in view the importance of public health, six main irrigation sites were selected as representative sites.

Wastewater and soil sampling, preparation, and analysis

Samples of composite wastewater/water used for irrigation at six sites. Samples of composite surface soil (0–15 cm) and groundwater were collected. Samples were assigned proper identity with inventory and were analyzed for statistical analysis according to standard methods (data not shown).

Sampling of fodder plants (Brassica and Maize plants)

Samples of Brassica and Maize plants being used as fodder for animals across six sites were taken for analysis of same heavy metals for multivariate statistical analysis according to standard methods (data not shown).

Sampling of raw milk across six sites

To assess the transmission of heavy metals in raw milk via feeding of fodder, samples of raw milk of buffalos feeding at each site were collected. A total of 60 samples (each 500 ml) were collected from 6 selected sites (10 samples from each site) in sterilized glass bottles. Each bottle was properly labeled and sealed and placed in low temperature in ice boxes and was frozen till heavy metal analysis.

Analysis of samples

The samples of wastewater, soil, groundwater, Brassica, and Maize plants and raw milk were analyzed in the Pakistan Council of Scientific and Industrial Research (PCSIR), Center for Environmental Protection Studies (CEPS) laboratories Complex, Lahore, accredited for ISO/IEC 17025 using ICP-OES Perkin Elmer, USA, Optima DV 5300 for heavy metals Cd, Cr, Cu, Mn, Ni, and Pb using standard methods and guidelines (APHA 3030-1 for wastewater, ASTM 2007 for soil, AOAC 2012 for fodder plants and raw milk metal analysis).

Assessment of human health risk exposure

Several interactive and iterative steps are required for complete assessment of health risk to human population exposed to heavy metal pollution. Determination or estimation of level of exposure is one of the basic steps for risk assessment of any chemical (Weber et al. 2006). The assessment of exposure indicates the pathways, magnitude, duration, and frequency of toxicants to which humans are potentially exposed (Lee et al. 2005). In wastewater irrigation, four major pathways of exposure are anticipated (Qishlaqi et al. 2008). However, in this study, only intake of raw milk has been considered for assessment of human health risk and total target health quotient of selected heavy metals.

Daily intake of heavy metals via raw milk intake

The estimated daily intake of examined heavy metals via raw milk intake was calculated by the equation given below. The average body weight of infants, children, and adults (male) with reference to their age group and daily milk intake was adopted according to average consumption in the study area (Ismail et al. 2015; Yu et al. 2015).

$$\mathrm{DIM} = \frac{\mathrm{Mc} \times \mathrm{Di}}{\mathrm{Bw}}$$

where Mc is mean concentration of heavy metals in raw milk (mg/kg), Di is the daily intake of milk (kg), and Bw is body weight (average).

Human health risk index (HRI)

HRI can be defined as the ratio between daily intakes of metals in the food stuff to oral reference dose (RfD) values and was computed with Eq. (1) (Balkhair and Ashraf 2015):

$$\mathrm{HRI} = \frac{\mathrm{DIM}}{\mathrm{RfD}} \tag{1}$$

An HRI > 1.0 for any single metal indicates that the health of consumer population is at risk and value of HRI < 1.0 indicates that the metal is risk free. The RfD is an estimated daily oral reference dose prescribed by USEPA and is considered safe and free of risk of adverse health effects during a life time (Balkhair and Ashraf 2015). The oral reference dose values for Cd, Cr, Cu, Mn, Ni, and Pb were taken from integrated risk information system (IRIS US EPA) as, respectively, 0.001, 0.003, 0.04, 0.014, 0.02, and 0.0035 mg/kg BW per day (Randhawa et al. 2014; Likuku and Obuseng 2015).

Total target health quotient (TTHQ)

The consumption or intake of two or more contaminants via single food stuff may result in negative effect on health of exposed population. *TTHQ* was used to assess the overall non-carcinogenic and carcinogenic impacts of single or individual food stuff containing multiple heavy metals (US EPA 1986; Yu et al. 2015) and was computed by Eq. (2):

$$\mathrm{TTHQ} = \sum_{i=1}^{n} \mathrm{HRI}i \tag{2}$$

where HRIi is the HRI value of element i.

If the sum of calculated (∑ HRIi) is less than 1.0, the food stuff is considered non-carcinogenic or its impact on health is negligible. In case of TTHQ is more than 1.0, the food stuff is considered carcinogenic or harmful for human health (Lee et al. 2005). The TTHQ was calculated and used to assess the non-carcinogenic and carcinogenic health risk caused by multiple heavy metals intake (MHMI) via single food stuff by a specific receptor (US EPA 1986).

Statistical analyses

Descriptive analysis (DA) includes determining mean (DM), minimum and maximum values, standard deviation (SD), and coefficient of variation (CoV). Multivariate statistical analysis (MVSA) was conducted to examine the source of the heavy metals. Statistical software SPSS 21 and Minitab 16 were used for descriptive and multivariate statistical analysis including (i) ANOVA, (ii) Pearson correlation matrix (PCM), (iii) hierarchical cluster analysis (HCA), and (iv) principal component analysis (PCA) in selected objects across six sites.

Results and discussion

Heavy metal analysis in wastewater, soil, *Brassica campestris* (field mustard), Maize (*Zea mays*) plants, and ground water across six sites

The contents of heavy metals in wastewater/water, soil, *Brassica campestris* (Field mustard), Maize (Zea mays) plants, and ground water across six sites including mean and standard deviation (SD) were tabulated for multivariate statistical analysis (data not shown).

Heavy metals analysis in raw milk of buffalo across six irrigation sites

The results (Table 1) indicated that mean contents of Cd, Cr, Cu, Mn, and Pb exceeded permissible limits at sites A, B, C, and D while that of Ni remained below limits across six sites. Mean contents of Cr slightly exceeded the limit at site E while that of Cu and Mn also slightly exceeded the limits at sites E and F. The total metal contents were 2.9 mg/kg, 4.25 mg/kg, 2.97 mg/kg, and 2.97 mg/kg at sites A, B, C, and D, respectively, exceeding 3.4 to 5 times the permissible total metal contents while that at sites E and F were 0.76 and 0.57 mg/kg and were below permissible limits. The results showed that the milk of buffalos feeding at wastewater-irrigated sites was 3.4 to 5 times more contaminated with heavy metals with reference to permissible limits and that at canal water and tube well water irrigation sites was within permissible limits.

Assessment of human health risk of heavy metals in raw milk across six sites

The daily intakes of metals (DIM) for infants, children, and adults via intake of raw milk across six sites is given in Table 2. The HRI and TTHQ values of heavy metals in raw milk are given in Table 3. The data is described as follows:

Table 1 Mean concentrations of heavy metals (mg/kg) in raw milk of buffalos feeding in peri-urban areas of Multan city

Name of site		Cd	Cr	Cu	Mn	Ni	Pb	Total metals contents
Site A	Mean	0.104*	1.4*	0.91*	0.11*	0.178	0.2*	2.9
	± SD	0.0007	0.0707	0.0071	0.0071	0.0007	0.0071	
	Min	0.103	1.3	0.9	0.1	0.177	0.19	
	Max	0.105	1.5	0.92	0.12	0.179	0.21	
Site B	Mean	0.102*	0.05	1.57*	2*	0.162	0.37*	4.254
	± SD	0.0007	0.0071	0.0071	0.0212	0.0007	0.0071	
	Min	0.101	0.04	1.56	1.97	0.161	0.36	
	Max	0.103	0.06	1.58	2.03	0.163	0.38	
Site C	Mean	0.092*	1.02*	0.62*	0.95*	0.126	0.162*	2.97
	± SD	0.0007	0.0071	0.0071	0.0071	0.0007	0.0007	
	Min	0.091	1.01	0.61	0.94	0.125	0.161	
	Max	0.093	1.03	0.63	0.96	0.127	0.163	
Site D	Mean	0.101*	1.28*	0.9*	0.32*	0.168	0.2*	2.97
	± SD	0.0007	0.0071	0.0707	0.0071	0.0007	0.0071	
	Min	0.1	1.27	0.8	0.31	0.167	0.19	
	Max	0.102	1.29	1	0.33	0.169	0.21	
Site E	Mean	0.0022	0.398*	0.0124*	0.1996*	0.1224	0.024*	0.76
	± SD	0.0003	0.0716	0.0026	0.1057	0.0079	0.0051	
	Min	0.0018	0.3	0.01	0.018	0.11	0.018	
	Max	0.0026	0.48	0.016	0.28	0.13	0.03	
Site F	Mean	0.0012	0.296	0.0106*	0.126*	0.122	0.02	0.57
	± SD	0.0004	0.06229	0.00445	0.05177	0.02864	0.0071	
	Min	0.0009	0.2	0.006	0.08	0.09	0.01	
	Max	0.0018	0.36	0.018	0.2	0.16	0.03	
Mean	0.067*	0.7406*	0.6705*	0.6176*	0.1464	0.1627*	2.40	
MRL, mg/kg	0.0026[a]	0.3[b]	0.01[a]	0.1[c]	0.43[d]	0.02[a]	0.8626	

[a] IDF (1979), Younus et al. (2016)

[b] Yu et al. (2015)

[c] Wenlock et al. (1979), Salah et al. (2013)

[d] FAO/WHO (2011)

*Exceeded permissible limits

TTHQ of heavy metals in raw milk across six sites

The TTHQ values were in the range 6.92 to 42.4 at sites A, B, C, and D for adults to infants respectively indicating highest carcinogenic health risk to exposed population of all age groups while that at site E ranged 2.71 to 10.2 and at site F, TTHQ values ranged 2.02 to 7.69 for adults to infants respectively indicating carcinogenic health risk to all age groups. The values of TTHQ of at sites E and F were 1/4th and 1/6th of that at sites A, B, C, and D indicating low health risk. TTHQ of heavy metals in raw milk at site F exhibited lowest health risk to exposed population across six sites.

Multivariate statistical analysis

PCM between metals within raw milk across six sites The results indicated that most of the metal pairs within raw milk have highly significant positive correlation across six sites such as Cd-Cr (r = 0.535), Cd-Cu (r = 0.874), Cd-Ni (r = 0.711), Cd-Pb (r = 0.859), Cu-Mn (r = 0.731), Cu-Ni (r = 0.703), Cu-Pb (r = 0.995), Mn-Pb (r = 0.786), and Ni-Pb (r = 0.650) at the level of 0.01, while Cd-Mn (r = 0.459) and Cr-Ni (r = 0.429) at the level of 0.05 indicating that same source is responsible for metal contamination in raw milk across six sites.

PCM between metals of raw milk and metals of wastewater across six sites The results indicated that most of the metal pairs in raw milk have highly significant positive correlation with metals in wastewater across six sites such as Cd-wwCd (r = 0.773), Cd-wwCu (r = 0.808), Cd-wwMn (r = 0.576), Cr-wwNi (r = 0.489), Cr-wwPb (r = 0.491), Cu-wwCd (r = 0.865), Cu-wwCu (r = 0.853), Cu-wwMn (r = 0.597), Mn-wwCd (r = 0.530), Mn-wwCu (r = 0.642), Ni-wwCd (r =

Table 2 Summary of daily intake of heavy metals (DIM) (mg/day) for infants, children and adults (male) for heavy metals intake via raw milk of buffalo feeding in peri-urban areas of Multan City

Name of site	Age group years	Body weight, kg	Daily milk intake, kg/day	DIM					
				Cd	Cr	Cu	Mn	Ni	Pb
Site A	Infants 0–1	8	0.5	0.0065	0.0875	0.0569	0.0069	0.01113	0.013
	Child 1–2	10	0.6	0.0062	0.084	0.0546	0.0066	0.01068	0.012
	Child 2–4	11	0.7	0.0066	0.089	0.0579	0.007	0.01132	0.013
	Child 4–10	18	0.8	0.0046	0.0622	0.0404	0.0049	0.0079	0.009
	Child 10–15	30	0.8	0.0028	0.0374	0.0243	0.0029	0.00475	0.005
	Adult ≥ 60	60	1	0.0017	0.0234	0.0152	0.0018	0.00297	0.003
Site B	Infants 0–1	8	0.5	0.0064	0.0031	0.0981	0.125	0.01013	0.023
	Child 1–2	10	0.6	0.0061	0.003	0.0942	0.12	0.00972	0.022
	Child 2–4	11	0.7	0.0065	0.0032	0.0999	0.1272	0.0103	0.024
	Child 4–10	18	0.8	0.0045	0.0022	0.0697	0.0888	0.00719	0.016
	Child 10–15	30	0.8	0.0027	0.0013	0.0419	0.0534	0.00433	0.01
	Adult ≥ 60	60	1	0.0017	0.0008	0.0262	0.0334	0.00271	0.006
Site C	Infants 0–1	8	0.5	0.0058	0.0638	0.0388	0.0594	0.00788	0.01
	Child 1–2	10	0.6	0.0055	0.0612	0.0372	0.057	0.00756	0.01
	Child 2–4	11	0.7	0.0059	0.0649	0.0394	0.0604	0.00801	0.01
	Child 4–10	18	0.8	0.0041	0.0453	0.0275	0.0422	0.00559	0.007
	Child 10–15	30	0.8	0.0025	0.0272	0.0166	0.0254	0.00336	0.004
	Adult ≥ 60	60	1	0.0015	0.017	0.0104	0.0159	0.0021	0.003
Site D	Infants 0–1	8	0.5	0.0063	0.08	0.0563	0.02	0.0105	0.013
	Child 1–2	10	0.6	0.0061	0.0768	0.054	0.0192	0.01008	0.012
	Child 2–4	11	0.7	0.0064	0.0814	0.0572	0.0204	0.01068	0.013
	Child 4–10	18	0.8	0.0045	0.0568	0.04	0.0142	0.00746	0.009
	Child 10–15	30	0.8	0.0027	0.0342	0.024	0.0085	0.00449	0.005
	Adult ≥ 60	60	1	0.0017	0.0214	0.015	0.0053	0.00281	0.003
Site E	Infants 0–1	8	0.5	0.0001	0.0249	0.0008	0.0125	0.00765	0.002
	Child 1–2	10	0.6	0.0001	0.0239	0.0007	0.012	0.00734	0.001
	Child 2–4	11	0.7	0.0001	0.0253	0.0008	0.0127	0.00778	0.002
	Child 4–10	18	0.8	1E− 04	0.0177	0.0006	0.0089	0.00543	0.001
	Child 10–15	30	0.8	6E− 05	0.0106	0.0003	0.0053	0.00327	6E− 04
	Adult ≥ 60	60	1	4E− 05	0.0066	0.0002	0.0033	0.00204	4E− 04
Site F	Infants 0–1	8	0.5	8E− 05	0.0185	0.0007	0.0079	0.00763	0.001
	Child 1–2	10	0.6	7E− 05	0.0178	0.0006	0.0076	0.00732	0.001
	Child 2–4	11	0.7	8E− 05	0.0188	0.0007	0.008	0.00776	0.001
	Child 4–10	18	0.8	6E− 05	0.0131	0.0005	0.0056	0.00542	9E− 04
	Child 10–15	30	0.8	3E− 05	0.0079	0.0003	0.0034	0.00326	5E− 04
	Adult ≥ 60	60	1	2E− 05	0.0049	0.0002	0.0021	0.00204	3E− 04
MRL, mg/kg				0.0026[a]	0.3[b]	0.01[a]	0.1[c]	0.43[d]	0.02[a]
RfD[e] values, mg/kg/day				0.001	0.003	0.04	0.014	0.02	0.004

[a] IDF (1979); Younus et al. (2016)

[b] Yu et al. (2015)

[c] Wenlock et al. (1979); Salah et al. (2013)

[d] FAO/WHO (2011)

[e] USEPA (2005)

Table 3 Health risk index (HRI) and TTHQ of heavy metals intake via raw milk of buffalo feeding in peri-urban areas of Multan City

Name of site	Age group years	Body weight kg	Daily milk intake kg/day	HRI						TTHQ
				Cd	Cr	Cu	Mn	Ni	Pb	
Site A	Infants 0–1	8	0.5	6.50	29.17	1.4219	0.49	0.56	3.57	41.7
	Child 1–2	10	0.6	6.24	28.00	1.3650	0.47	0.53	3.43	40
	Child 2–4	11	0.7	6.61	29.68	1.4469	0.50	0.57	3.63	42.4
	Child 4–10	18	0.8	4.62	20.72	1.0101	0.35	0.40	2.54	29.6
	Child 10–15	30	0.8	2.78	12.46	0.6074	0.21	0.24	1.53	17.8
	Adult ≥ 60	60	1	1.74	7.79	0.3799	0.13	0.15	0.95	11.1
Site B	Infants 0–1	8	0.5	6.38	1.04	2.4531	8.93	0.51	6.61	25.9
	Child 1–2	10	0.6	6.12	1.00	2.3550	8.57	0.49	6.34	24.9
	Child 2–4	11	0.7	6.49	1.06	2.4963	9.09	0.52	6.72	26.4
	Child 4–10	18	0.8	4.53	0.74	1.7427	6.34	0.36	4.69	18.4
	Child 10–15	30	0.8	2.72	0.45	1.0480	3.81	0.22	2.82	11.1
	Adult ≥ 60	60	1	1.70	0.28	0.6555	2.39	0.14	1.77	6.92
Site C	Infants 0–1	8	0.5	5.75	21.25	0.9688	4.24	0.39	2.89	35.5
	Child 1–2	10	0.6	5.52	20.40	0.9300	4.07	0.38	2.78	34.1
	Child 2–4	11	0.7	5.85	21.62	0.9858	4.32	0.40	2.94	36.1
	Child 4–10	18	0.8	4.08	15.10	0.6882	3.01	0.28	2.06	25.2
	Child 10–15	30	0.8	2.46	9.08	0.4139	1.81	0.17	1.24	15.2
	Adult ≥ 60	60	1	1.54	5.68	0.2589	1.13	0.11	0.77	9.48
Site D	Infants 0–1	8	0.5	6.31	26.67	1.4063	1.43	0.53	3.57	39.9
	Child 1–2	10	0.6	6.06	25.60	1.3500	1.37	0.50	3.43	38.3
	Child 2–4	11	0.7	6.42	27.14	1.4310	1.45	0.53	3.63	40.6
	Child 4–10	18	0.8	4.48	18.94	0.9990	1.01	0.37	2.54	28.4
	Child 10–15	30	0.8	2.70	11.39	0.6008	0.61	0.22	1.53	17
	Adult ≥ 60	60	1	1.69	7.13	0.3758	0.38	0.14	0.95	10.7
Site E	Infants 0–1	8	0.5	0.14	8.29	0.0194	0.89	0.38	0.43	10.2
	Child 1–2	10	0.6	0.13	7.96	0.0186	0.86	0.37	0.41	9.74
	Child 2–4	11	0.7	0.14	8.44	0.0197	0.91	0.39	0.44	10.3
	Child 4–10	18	0.8	0.10	5.89	0.0138	0.63	0.27	0.30	7.21
	Child 10–15	30	0.8	0.06	3.54	0.0083	0.38	0.16	0.18	4.34
	Adult ≥ 60	60	1	0.04	2.22	0.0052	0.24	0.10	0.11	2.71
Site F	Infants 0–1	8	0.5	0.08	6.17	0.0166	0.56	0.38	0.36	7.56
	Child 1–2	10	0.6	0.07	5.92	0.0159	0.54	0.37	0.34	7.26
	Child 2–4	11	0.7	0.08	6.28	0.0169	0.57	0.39	0.36	7.69
	Child 4–10	18	0.8	0.06	4.38	0.0118	0.40	0.27	0.25	5.37
	Child 10–15	30	0.8	0.03	2.63	0.0071	0.24	0.16	0.15	3.23
	Adult ≥ 60	60	1	0.02	1.65	0.0044	0.15	0.10	0.10	2.02
MRL, mg/kg				0.0026[a]	0.3[b]	0.01[a]	0.1[c]	0.43[d]	0.02[a]	
RfD[e] values, mg/kg/day				0.00	0.00	0.04	0.01	0.02	0.00	

[a] (IDF 1979; Younus et al. 2016)

[b] Yu et al. (2015)

[c] (Wenlock et al. 1979; Salah et al. 2013)

[d] FAO/WHO (2011)

[e] USEPA (2005)

0.718), Ni-wwCu (r = 0.594), Ni-wwMn (r = 0.758), Ni-wwNi (r = 0.649), and Ni-wwPb (r = 0.576) at the level of 0.01, while Cd-wwNi (r = 0.439) and Cd-wwPb (r = 0.414) at the level of 0.05 showing the contaminated wastewater used for irrigation is responsible for metal contamination in raw milk across six sites.

PCM between metals of raw milk and metals of surface soil across six sites The results indicated that most of the metal pairs in raw milk have highly significant positive correlation with metals of surface soil across six sites such as Cd-ssCu ($r = 0.782$), Cr-ssCd ($r = 0.560$), Cr-ssCr ($r = 0.601$), Cr-ssCu ($r = 0.467$), Cr-ssPb ($r = 0.590$), Cu-ssCu ($r = 0.652$), Mn-ssMn ($r = 0.777$), Ni-ssCd ($r = 0.555$), Ni-ssCr ($r = 0.570$), Ni-ssCu ($r = 0.595$), Ni-ssPb ($r = 0.563$), and Pb-ssCu ($r = 0.625$) at the level of 0.01, while Cd-ssCd ($r = 0.370$), Cd-ssCr ($r = 0.370$), Cr-ssPb ($r = 0.404$), Cu-ssMn ($r = 0.373$), and Pb-ssMn ($r = 0.430$) at the level of 0.05 revealing that contaminated soil is responsible for metal contamination in raw milk across six sites.

PCM between metals of raw milk and metals of Brassica, Maize plants, and ground water across six sites The results (Table 4) indicated that most of the metal pairs in raw milk have significant positive correlation with metals of Brassica, Maize plants, and ground water across six sites at the level of 0.01 and 0.05 which indicated that maize plants, Brassica, and ground water are contributing metal contamination in raw milk across six sites.

Hierarchical cluster analysis (HCA) of metals in raw milk across six sites

HCA was conducted using the compositions of selected heavy metals to examine their potential sources in selected objects and the similarities were shown with dendogram. Figure 1 illustrated that the heavy metals in raw milk made two major groups. Group 1 was composed of Cu, Pb, Cd, Ni, and Mn, while group 2 was composed of Cr. It indicated that the source of group 1 (Cu, Pb, Cd, Ni, Mn) was common and source of group 2 (Cr) was different.

Principal component analysis (PCA) of metals in raw milk across six sites

Two principal components having Eigen values more than 1.0 were extracted for metals in raw milk (Table 5). The PC 1 (Eigen value 3.86) explained about 64.4% of total variance in data analyzed while PC 2 (Eigen value 1.70) explained about 28.8% of total variance in data analyzed. The PC 1 showed positive loadings of Cd, Cu, and Pb, and low positive loadings of Cr and Ni. PC 2 showed high positive loading of

Table 4 PCM between heavy metals in raw milk, Brassica, Maize plants and ground water

	Cd milk	Cr milk	Cu milk	Mn milk	Ni milk	Pb milk
Cd maize	*0.960***	*0.685***	*0.777***	0.234	*0.776***	*0.736***
Cr maize	*0.826***	0.340	*0.703***	*0.558***	*0.394**	*0.713***
Cu maize	*0.945***	*0.464***	*0.898***	*0.415**	*0.816***	*0.867***
Mn maize	*0.869***	*0.712***	*0.671***	0.089	*0.767***	*0.622***
Ni maize	*0.499***	*0.321*	*0.438**	0.044	*0.488***	*0.398**
Pb maize	*0.838***	*0.732***	*0.648***	0.029	*0.800***	*0.593***
Cd water	*0.536***	0.187	*0.571***	0.315	*0.479***	*0.559***
Cr water	0.303	− 0.174	*0.462**	*0.447**	0.235	*0.489***
Cu water	*0.646***	0.172	*0.766***	*0.364**	*0.723***	*0.738***
Mn water	*0.682***	*0.744***	*0.384**	− 0.023	*0.448**	0.350
Ni water	*0.438**	*0.469***	*0.364**	− 0.108	*0.548***	0.315
Cd Brassica	*0.596***	*0.720***	0.355	− 0.202	*0.613***	0.301
Cr Brassica	*0.874***	*0.533***	*0.634***	*0.437**	*0.365**	*0.633***
Cu Brassica	*0.943***	*0.615***	*0.705***	*0.390**	*0.510***	*0.692***
Mn Brassica	*0.742***	*0.664***	*0.429**	0.150	0.347	*0.416**
Ni Brassica	0.266	0.254	− 0.016	0.225	− 0.329	0.020
Pb Brassica	*0.976***	*0.590***	*0.856***	0.336	*0.806***	*0.818***
Cd milk	1	*0.535***	*0.874***	*0.459**	*0.711***	*0.851***
Cr milk	*0.535***	1	0.097	*− 0.470***	*0.429**	0.0354
Cu milk	*0.874***	0.097	1	*0.731***	*0.703***	*0.995***
Mn milk	*0.459**	*− 0.470***	*0.731***	1	0.134	*0.786***
Ni milk	*0.711***	*0.429**	*0.703***	0.134	1	*0.650***
Pb milk	*0.851***	0.035	*0.995***	*0.786***	*0.650***	1

*Correlation is significant at the 0.05 level (2-tailed)

**Correlation is significant at the 0.01 level (2-tailed)

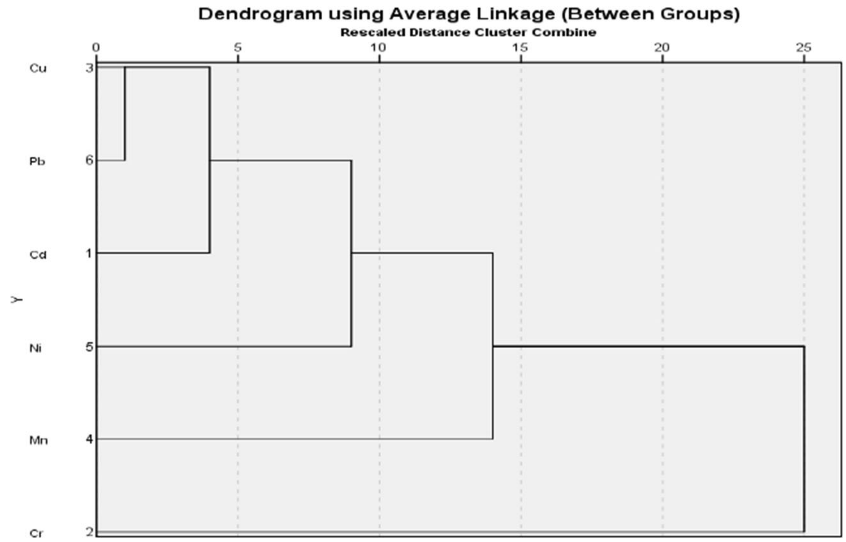

Fig. 1 HCA of heavy metals in raw milk of buffalo in peri-urban areas of Multan city

Mn than PC 1. All the positive loadings on PC 1 and PC 2 indicated the same/common source of contamination of selected metals in raw milk and indicated towards anthropogenic source like industrial, commercial, and domestic waste/sludge (Bourliva et al. 2016; Chen et al. 1997; Keshavarzi et al. 2015; Lv et al. 2013; Ma and Gui 2017; Xia et al. 2011). The PCA bi-plot of selected metals in raw milk (Fig. 2) indicated that the metals associated with PC 1 and PC 2 made two major groups. Majority of group 1 comprised of Mn, Pb, Cu, Cd, and Ni, and group 2 contained Cr which is similar behavior as illustrated by HCA dendrogram of raw milk (Fig. 1) which validated the findings of PCA.

The multivariate statistical analysis indicated that contaminated groundwater, contaminated fodder Maize plants and Brassica, wastewater used for irrigation, and contaminated soil are common sources contributing the heavy metal contamination in raw milk across six sites.

Table 5 Principal component loadings of selected heavy metals in raw milk samples

	Raw milk	
	PC1	PC2
Eigenvalues	3.86	1.70
% total variance	64.4	28.2
% cumulative variance	64.4	92.7
Cd	0.472	− 0.217
Cr	0.114	− 0.718
Cu	0.502	0.093
Mn	0.34	0.549
Ni	0.386	− 0.323
Pb	0.497	0.151

Discussion

The comparison of heavy metal concentration observed in raw milk of buffalos in this study with other studies is given in Table 6 and described as follows.

Ismail et al. (2015) conducted study on heavy metal contamination in raw milk of buffalos located within Multan city (study area) and reported the concentration of Cd, Cu, Ni, and Pb which are within the range observed in this study. Akhtar et al. (2015) collected raw milk samples from open market of Multan city (study area) and reported the concentration of Cd, Ni, and Pb higher than observed in this study. Younus et al. (2016) collected raw milk samples from cattle dairy farms located near wastewater drain and from open market in Jhang city, Punjab province, Pakistan. They reported the concentration of Cd, Cr, Cu, and Pb higher than observed in this study. Aslam et al. (2011) collected cow milk samples from cattle farms located on wastewater irrigation site near wastewater drain (carrying urban wastewater and industrial effluents) in peri-urban area of Faisalabad city, Punjab province, Pakistan, and reported the concentration of Cd, Cr, Ni, and Pb much higher than the concentration of the same metals observed in this study and were the highest in the literature reviewed. Najarnezhad and Akbarabadi (2013) reported the concentration of Cd and Pb in cow milk in Iran above the results of same metals observed in this study. Elsayed et al. (2011) reported the contents of Cu and Pb in raw milk samples collected from cattle farms located in industrial area of Shubra Egypt higher than the results of this study. Salah et al. (2013) reported the contents of Cd, Mn, and Pb in Egypt higher than contents of Cd and Pb observed in this study (Table 6). The mean contents of Cd, Cr, Cu, Mn, and Pb observed in this study exceeded permissible limits at wastewater-irrigated sites. The total metal contents in raw milk at four wastewater-irrigated fields were 3.4 to 5 times higher than

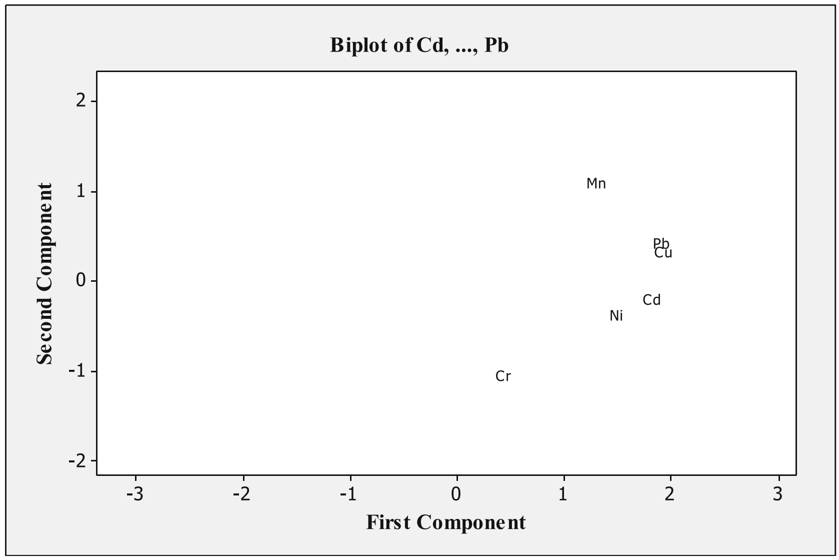

Fig. 2 Principal component analysis bi-plot of heavy metals in raw milk

total permissible metals contents while at canal water and tube well water-irrigated sites were below than the total permissible metal contents (Table 1). The results of this study and other studies within Pakistan and other countries showed that the raw milk of animals feeding at wastewater-irrigated sites contained higher contents of heavy metals than permissible limits. In this study, the TTHQ values of heavy metals in raw milk of buffalos were computed. TTHQ ranged 6.92 to 42.4 for adults to infants indicating highest carcinogenic health risk to exposed population at wastewater-irrigated agricultural fields. TTHQ at canal water irrigation site ranged 2.71 to 10.2 and at tube well water site ranged 2.02 to 7.69 for adults to infants, respectively, indicating carcinogenic health risk to all age groups. The values of TTHQ at Canal water and tube well water were 1/4th and 1/6th that of wastewater-irrigated fields indicating lower health risk. TTHQ of raw milk at tube well water irrigation site exhibited lowest health risk to the exposed population.

Review of literature indicates that long-term application of wastewater in agricultural fields and disposal of untreated wastewater in the study area and in Punjab has contaminated the soil, groundwater, crops, and canal water which have become source of food chain contamination.

Tariq et al. (2010) conducted a study on soil and groundwater contamination by wastewater in Multan city (study area), Pakistan; identified by PCM and PCA that wastewater is the main source of respective soil contamination and groundwater; and concluded that large fertile agricultural area has become nonproductive due to heavy metal contamination. Saleemi (1993) reported that large quantity of untreated wastewater is being discharged into water surface bodies in Pakistan for ultimate disposal. PCRWR (2006) conducted a study in Faisalabad, Pakistan, for assessment of impacts of industrial and sewage effluents on crops and reported that the crops grown with wastewater were contaminated with higher contents of heavy metals which joined the food chain causing toxicity to plants and humans.

The canal water irrigation site in this study is being irrigated by Multan Branch Canal taking off from Sidhnai Canal which is originating from River Chenab at Trimmu Barrage. PCRWR (2004) reported that the disposal of large volume of untreated industrial effluents and urban sewage has depleted the DO totally in various patches of the River Chenab. The River Chenab is extremely being polluted up stream of Multan city. The BOD level was 4.2 mg/l downstream of Faisalabad and the concentration of Cr (16.00 mg/l) was recorded in River Chenab. These may be sources of Multan Branch Canal contamination with heavy metals. River Chenab located on west side, Sidhnai Canal on the north side, and Multan Branch Canal on the south side of Multan City are the main sources of groundwater recharge in the study area. WASA Multan is discharging 34.88 m^3/s untreated wastewater in to canals and the River Chenab. The tube well water irrigation site selected in this study is located in between the wastewater irrigation site D and River Chenab and its contamination with heavy metals may be due to recharge sources (Iqbal et al. 2019).

The multivariate statistical analysis in this study indicated that contaminated groundwater, contaminated fodder (Maize plants and Brassica) wastewater and water used for irrigation, and contaminated soils are common sources contributing the heavy metal contamination in raw milk.

The results of this study invite the attention of policy- and decision-makers to formulate regulations and standards for treatment of wastewater before its use in agricultural fields and disposal in water surface bodies to grow crops like fodder for animal to remediate the situation which is causing food chain contamination and intake of contaminated raw milk

Table 6 Comparison of heavy metals concentrations (mg/kg) in raw milk of this study with other studies

Location	Type of milk	Cd	Cr	Cu	Mn	Ni	Pb	HRI	TTHQ	Reference
Pakistan Multan city	Raw milk buffalo	0.0023*	–	1.054**	–	0.069*	0.034*	–	–	Ismail et al. (2015)
Pakistan Multan city	Raw milk from market	0.102**	–	0.159*	0.356*	0.176**	0.2**	–	–	Akhtar et al. (2015)
Pakistan Jhang city	Raw milk from dairy farms near wastewater drain	0.092**	12.4**	0.938**	–	–	1.25**	–	–	Younus et al. (2016)
	Open market	0.092**	8.4**	1.354**	–	–	2**	–	–	
Pakistan Faisalabad city	Milk of cow near sewerage drain	0.145**	1.277**	–	–	20.421**	43.414**	–	–	Aslam et al. (2011)
Iran	Cow milk	0.3**	–	–	–	–	12.9**	–	–	Najarnezhad and Akbarabadi (2013)
Egypt Shubra	Raw milk from industrial area	0.018*	0.35*	1.194**	–	–	0.577**	–	–	Elsayed et al. (2011)
Egypt	Milk powder	0.322**	–	–	0.497*	–	0.791**	–	–	Salah et al. (2013)
Poland	Raw milk cow	0.004*	–	0.045*	0.02*	–	0.0412*	–	–	Pilarczyk et al. (2013)
Pakistan Multan city	Raw milk of buffalo feeding at wastewater irrigation sites	0.067	0.74	0.67	0.617	0.1464	0.162	Yes	Yes	This study

*Less concentration than observed in this study

**Higher concentration than observed in this study

resulting in carcinogenic health risk to the exposed human population.

Conclusions

1. The raw milk of buffalo feeding at agricultural farms irrigated with wastewater, canal water, and tube well water exhibited *carcinogenic health risk* to the exposed population of all age groups (infants, children, and adults). The carcinogenic health risk of milk was highest at wastewater-irrigated agricultural farms and lowest at tube well water irrigation site.
2. The carcinogenic health risk of raw milk at canal water-irrigated site was 1/4th and at tube well water-irrigated site was 1/6th than that of wastewater-irrigated sites exhibiting *less health risk* than the milk at wastewater-irrigated sites.
3. The milk at tube well water irrigation site was of better quality than that at canal water irrigation site. However, the milk at both sites posed carcinogenic health risk to exposed population of all age groups.
4. The multivariate statistical analysis indicated that contaminated groundwater, contaminated fodder like Maize plants and Brassica, wastewater used for irrigation, and contaminated soil are common sources contributing the heavy metal contamination in raw milk.

Recommendation

The results of this study indicated that the wastewater containing heavy metals are not suitable to irrigate agricultural fields to grow fodder for animals. The government should make legislation for the proper treatment of wastewater like SACB method (Ahmad et al. 2019), before its use in agricultural fields to avoid the food chain contamination to save the public health from carcinogenic health risk being caused by intake of contaminated raw milk of buffalo feeding at wastewater-irrigated agricultural fields.

Acknowledgments The authors acknowledge the cooperation of Farzana Bashir and Rauf Ahmed from the Pakistan Council for Science and Industrial Research lab Lahore, Punjab, Pakistan, for help during sample analysis.

Funding information The authors declare that there is no source of funding in this study.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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