

Determination of heavy metals in cow's and buffalo's fresh raw milk from different areas of Pakistan

Heavy metals
in milk of cows
and buffalos

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Abstract

Purpose – The presence of heavy metals in milk causes many acute and chronic physiological dysfunctions in human organs. The present study aims to investigate the heavy metals in cow's and buffalo's milk of two major cities, Karachi and Gujranwala, Pakistan to estimate metal intake by humans from this source.

Design/methodology/approach – In total, 48 milk samples from 2 cities were drawn from animals' udder to avoid contamination. Each sample was digested with nitric acid at 105 °C (degree Celsius) on a pre-heated electric hot plate to investigate the metals by atomic absorption spectroscopy (flame type). Air-acetylene technique analyzed chromium, cadmium and lead, and the hydride method analyzed arsenic in the milk samples.

Findings – The results revealed the highest mean lead concentration (19.65 ± 43.86 ppb) in the milk samples, followed by chromium (2.10 ± 2.33 ppb) and arsenic (0.48 ± 0.73 ppb). Cadmium was not detected in any sample, assuming cadmium's occurrence was below the detection level. The concentrations of all the metals in the samples of the two cities do not differ statistically. Lead concentrations in the buffalo's milk were higher than in cow's milk ($p < 0.05$). However, the concentrations of arsenic and chromium between buffalo's and cow's milk do not differ statistically. The present study reveals a lower level of metals in the milk than those conducted elsewhere. The mean concentrations of all the metals met the World Health Organization's (WHO) safety guidelines (1993).

Research limitations/implications – Although cadmium causes toxicity in the human body, cadmium could not be measured because cadmium's concentration was below the detection level, which is 1 ppb.

Practical implications – This study will help reduce the toxic metals in our environment, and the sources of heavy metals, particularly from the industrial sector could be identified. The feed and water consumed by the milking animals could be carefully used for feeding them.

Social implications – This study will help reduce the diseases and malfunction of human organs and organ systems since these heavy metals cause toxicity and carcinogenicity in humans. Arsenic and chromium cause cancer while lead causes encephalopathy (a brain disease).

Originality/value – The study reports heavy metal concentrations in the two attributes of four independent variables of raw milk samples that were scarcely reported from Pakistan.

Keywords Milk, Cow, Buffalo, Toxic metals, Pakistan

Paper type Research paper

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Compliance with ethical standards:

Ethical approval: This article does not contain any studies with human participants or animals performed by the authors.

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



1. Introduction

The significance of milk for its dietary worth has been widely recognized (Akhtar *et al.*, 2017; Batool *et al.*, 2016; Abdulkhaliq, Swaileh, Hussein, & Matani, 2012) as a valuable source of both micronutrients and macronutrients. Humans essentially require these nutrients for nourishment and regulatory processes Sanz Ceballos *et al.* (2009). Buffaloes are the main source of milk in Pakistan and are considered as black gold since they fulfill the nutritional requirements of all age groups (Iqbal *et al.*, 2020).

On the contrary, contaminated milk contains some hazardous metals, such as arsenic, cadmium, lead and mercury, which are potentially harmful and cause many physiological dysfunctions in humans (Sager & Hoesch, 2005; Li, McCrory, Powell, Saam, & Jackson-Smith, 2005; WHO, 1996). The presence of these metals has been extensively explored in the animal organs used for milk consumption as these metals emerged in the milk (Burger & Elbin, 2015). Heavy metals may enter the food chain via soil, water and feed, which then find their ways to milk (Zhou, Zheng, Su, Wang, & Soyeurt, 2019). The adverse effects of these contaminants in diets consumed worldwide have threatened human health (Zergui, Boudalia, & Joseph, 2023). These metals are not essential nutrients and not required for any biological function in humans, but are harmful even at low concentrations (Boudebbouz *et al.*, 2020; Varol & Sünbül, 2020). Young children, particularly infants, are at high health risk due to the hazardous metals in the milk because they take it daily for their growth and development. Metal-contaminated milk has harmful consequences on human health even if it has metals in low concentrations (Abu-Darwish, Abu-Dieyeh, Mufeed, Al-Tawaha, & Al-Dalain, 2009; Zheng *et al.*, 2007; Islam, Yang, He, & Mahmood, 2007).

Consequently, many studies demonstrated the occurrence of heavy metals in milk across the world (Boudebbouz *et al.*, 2020; Iftikhar, Arif, Siddiqui, & Khattak, 2014; Abdulkhaliq *et al.*, 2012; Javed *et al.*, 2009; Kazi *et al.*, 2009; Tajkarimi *et al.*, 2008; Caggiano *et al.*, 2005; Licata *et al.*, 2004). US Environmental Protection Agency and Agency for Toxic Substances and Disease Registry listed arsenic, lead and cadmium as the 20 most hazardous and toxic substances (Hameed, Akhtar, Amjada, Naeema, & Tariqa, 2019). The current study was carried out to analyze the concentrations of four hazardous metals, namely arsenic, chromium, lead and cadmium in the milk samples of buffaloes (*Bubalus bubalis*, L.) and cows (*Bos taurus*, L.), which were from Karachi and Gujranwala. 12 buffaloes (6 from Karachi and 6 from Gujranwala) and 12 cows (6 from Karachi and 6 from Gujranwala) were milked twice daily to collect 24 samples each morning and evening. Further, the effects of the feeding mode on the metal concentrations of the milk were investigated since limited studies are available on this aspect. The study will help reduce the harmful heavy metals in the milk to decrease human diseases and physiological malfunctioning and make it more nourishing, particularly for infants and young children.

2. Material and methods

Two attributes of each of the four independent variables of the milk samples were selected to investigate the statistical difference of the aforesaid toxic metals among them.

- (1) 24 samples each from Gujranwala and Karachi were studied to divulge the consistency or the difference in the quantity of various metals in their milk. The two cities are 1200 km away from each other (Figure 1). Karachi is the most populous city and economic hub of the country, located in Sindh Province along the Arabian Sea. Gujranwala is the 7th most populous city, located in the Punjab Province of Pakistan. Both cities have different climates and geography.
- (2) 24 samples each in the Morning and Evening were taken to show the temporal consistency or the difference in the amount of different metals.



Source(s): Kanza Abid drew this map with the help of Google Earth

Figure 1.
Location of Karachi
and Gujranwala in
Pakistan

- (3) 24 samples each of cows and buffalos were taken to reveal the consistency or the difference in the quantity of different metals (Ahmad *et al.*, 2008; Fundora *et al.*, 2001)
- (4) 24 samples of each of the control and uncontrol feeding modes of the animals to demonstrate the consistency or the difference in the quantity of different metals. The animals of dairy farms were 'Controlled Feeding' while the roaming buffaloes and cows that feed on debris and municipal solid waste were 'Uncontrol Feeding'.

A total of 48 milk samples were analyzed to determine whether the toxic metals differ or not significantly between each of the above variables of the samples. In addition, the milk samples from animals were extracted twice daily (morning & evening) to determine the temporal difference in their metal concentrations (Supplementary data).

2.1 Sample collection

The milk samples of both animal species were directly drawn from their udder to avoid contamination during transportation and retailing because retail shops generally vend adulterated milk. The milk is usually mixed and diluted with water in the retail shop or during its supply chain (Arif *et al.*, 2020).

The milk of both the animal species of the controlled environment was extracted twice daily after serving them fodder in the morning and evening hours. However, the milk of the animals in the uncontrolled environment is extracted in the morning without serving them fodder. After taking out milk, they are set free for food from municipal debris and other wastes, which commonly exist under bridges and along the city roadsides. However, the animals' milk in the uncontrolled environment is extracted in the evening after serving them as fodder on the dairy farms. In the present study, 48 samples were from twelve dairy farms, six samples each from Karachi and Gujranwala, located in different areas. Each milk sample has three replicates.

2.2 Sample digestion

The milk samples were taken in transparent plastic bottles, which were instantly labeled and then placed into the freezer. Approximately 50 ml of milk was taken as a sample from each animal for the study.

Each milk sample was digested by a low-priced digestion method (AOAC, 2000). This method was selected to keep the digestion process less contaminated and simple. Analytical-reagent-grade of 65% concentrated nitric acid in its highest purity (E. Merck, Darmstadt, Germany) was used in this experiment. 10 ml of the sample was added to 5 ml of concentrated HNO₃ and then heated at 105 °C on a pre-heated electric hot plate until the solution become transparent. The sample solution was then filtered with Whatman filter paper # 40. The filtered digested sample was made 100 ml by adding deionized water.

2.3 Toxicological analysis

The filtered samples were investigated by PG instrument (UK) AA500 atomic absorption spectroscopy (flame type) in the laboratory of Space and Upper Atmosphere Research Commission (SUPARCO). The instrument, equipped with flame atomizer, air-acetylene (standard configuration), computer and AA Win software, analyzed chromium, cadmium and lead. Calibration was done by 3 points, with one duplicate sample and standards used for calibration were bought from Perkin Elmer, USA. Hydride method analyzed arsenic in the milk samples. The lead was kept at 217.0 nm wavelength and cadmium, chromium and arsenic at 228.8 nm, 375.9 nm and 193.7 nm wavelength, respectively. The slit was kept at 0.5 nm during the analysis of all four metals. It is worth mentioning that the concentration of the standard solution of 0.1 ppb, 1.0 ppb and 10 ppb was for arsenic, while for the other three metals, the concentrations were 0.1 ppm, 1.0 ppm and 10 ppm.

LOD was calculated from following equation:

$$\text{LOD} = \text{LOB} + 1.645 (\text{SD of low concentration sample})$$

Whereas,

$$\text{LOB} = \text{mean of blank} + 1.645 (\text{SD of blank})$$

2.4 Statistical analysis

Kruskal–Wallis *H* test was applied to determine the statistical difference among concentrations of three metals in the milk samples and each metal among four variables. Mann–Whitney *U* test was performed to determine the statistical difference between two attributes of each of the four variables (Table 2).

3. Results

Four metals, namely arsenic, cadmium, chromium and lead were investigated in the forty-eight milk samples. The present study demonstrated zero cadmium in all the milk samples. Its concentration is assumed to be below the detection level, so excluded from further analysis. The detection level of the Atomic Absorption Spectroscopy was one ppb. Karachi's samples contained the highest mean chromium concentration (2.40 ± 2.61 ppb). Gujranwala's samples divulged the highest mean arsenic concentration (0.59 ± 0.88 ppb). The buffaloes' milk showed the highest mean lead concentration (28.43 ± 59.98 ppb) (Table 1). The lead levels were significantly higher (*p* < 0.01) than the levels of arsenic and chromium.

The arsenic concentrations in the milk samples of the two cities do not differ significantly. Similarly, non-significant differences were divulged between two attributes of arsenic levels for the other three independent variables, that is, between the milk samples of buffaloes and

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Pollutants	Attributes	Cities		Animal species		Feeding mode		Milking time	
		Karachi	Gujranwala	Cow	Buffalo	Controlled feed	Uncontrolled feed	Morning	Evening
Arsenic	Q1	0.15	0	0	0.17	0.15	0	0	0.14
	Median	0.25	0.22	0.17	0.3	0.27	0.18	0.22	0.26
	Q3	0.40	0.69	0.53	0.63	0.45	0.63	0.53	0.45
	Mean ± SD	0.40 ±0.55	0.59 ±0.88	0.48 ±0.84	0.57 ±0.65	0.48 ±0.79	0.51 ±0.69	0.41 ±0.51	0.58 ±0.90
Lead	Q1	6.19	0	0	7.56	5.66	0	3.53	1.21
	Median	11.04	7.53	6.19	11.66	9.33	10.2	9.87	10.01
	Q3	21.25	17.91	16.37	22.87	21.31	17.91	27.47	12.94
	Mean ± SD	16.64 ±20.64	21.46 ±58.98	9.67 ±11.65	28.43 ±59.98	13.24 ±11.64	24.86 ±60.92	28.03 ±60.54	10.07 ±9.11
Chromium	Q1	0	0	0	0.83	0	1.2	0	0
	Median	1.50	1.20	1.15	1.60	0	1.60	1.20	1.40
	Q3	3.30	2.48	2.33	3.23	3.45	2.55	2.35	3.23
	Mean ± SD	2.40 ±2.61	1.77 ±2.05	1.86 ±2.44	2.31 ±2.28	1.97 ±2.79	2.20 ±1.85	1.78 ±1.87	2.39 ±2.75

Source(s): Table by authors

Table 1.
Descriptive statistics of
toxic metal levels (in
ppb) in the milk of
buffaloes and cows

cows, the milk samples of controlled and uncontrolled feeds and between the arsenic levels of the milk samples of morning and evening. The chromium demonstrated the same results. However, the lead concentrations in the cow's and buffalo's milk samples differ significantly ($p = 0.02$), while the two attributes of lead concentrations did not differ significantly in the other three independent variables (Table 2).

3.1 Box-and-whisker plot

The concentrations of different metals in the milk of different categories are plotted in the box-and-whisker diagram, which is non-parametric visual descriptive statistics. Figure 2 illustrates the box plots of chromium levels in the milk samples of eight groups. The box reveals an outlier of chromium concentration in the milk of Gujranwala while two outliers each of chromium concentrations are found in the milk of cows and both the animal species that took uncontrolled feed.

Figure 3 demonstrates the box plot of arsenic levels in the samples of two different attributes and four variables. All the attributes and variables show outlier values of arsenic in the samples. The animals of Gujranwala and the animals that took uncontrolled feed reveal one outlier value for each arsenic in their milk samples. Other categories exhibit a higher number of outliers of arsenic in the samples.

Table 2.
P-values demonstrating statistical difference between attributes and variables

Variables	Attributes		Lead	Pollutants	
	Karachi	Gujranwala		Arsenic	Chromium
	Cow	Buffalo	0.03**	0.28*	0.24*
	Morning	Evening	0.43*	0.79*	0.57*
	Controlled	Uncontrolled	0.58*	0.29*	0.09*

Note(s): *Non-significant, **Significant
Source(s): Table by authors

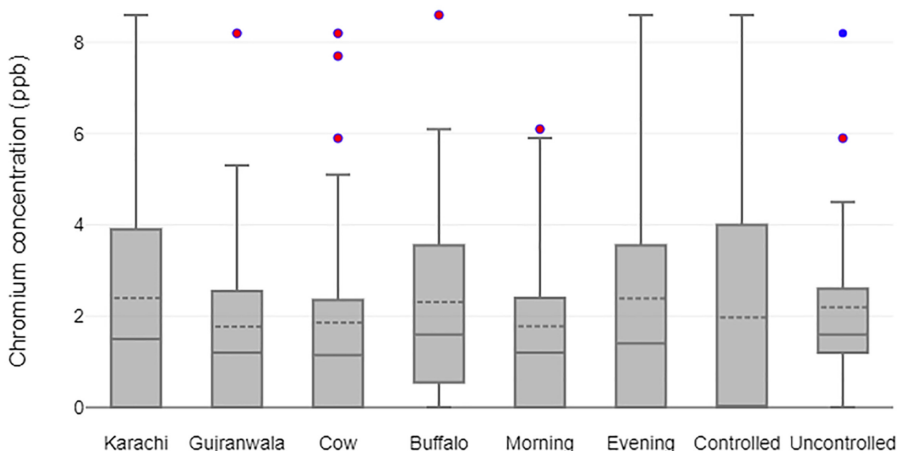
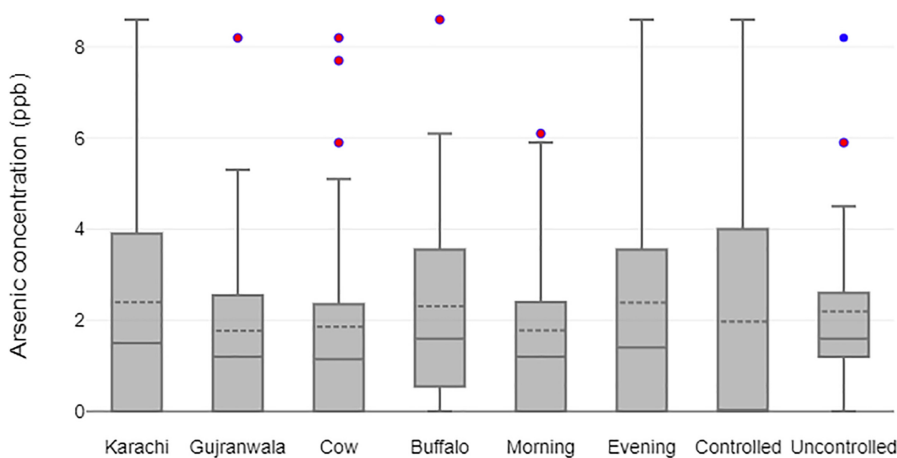


Figure 2.
Box-and-whisker plot of chromium concentration in the milk of different categories

Note(s): The continuous line is the median and broken line in the box is mean. The upper quartile is the upper end and the lower quartile is the lower end of box. Minimum and maximum values are shown by thin bars. The dots above the maximum values are outliers
Source(s): Figure by authors



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Figure 3.
Box-and-whisker plot
of arsenic
concentration in the
milk of different
categories

Note(s): The continuous line is the median and broken line in the box is mean. The upper quartile is the upper end and the lower quartile is the lower end of the box. Minimum and maximum values are shown by thin bars. The dots above the maximum values are outliers

Source(s): Figure by authors

Figure 4 presents the box plot of lead levels in the milk samples of various attributes and variables. The box plot shows one outlier concentration each of lead in the milk samples of Karachi and Gujranwala whereas two outlier values each are found in the milk of buffaloes, in the milk of the animals that took the uncontrolled feed and in the milk samples that were extracted in the morning session.

In addition, all three box-and-whisker plots reveal the means, quartiles and standard deviations of the concentrations of all the attributes and variables.

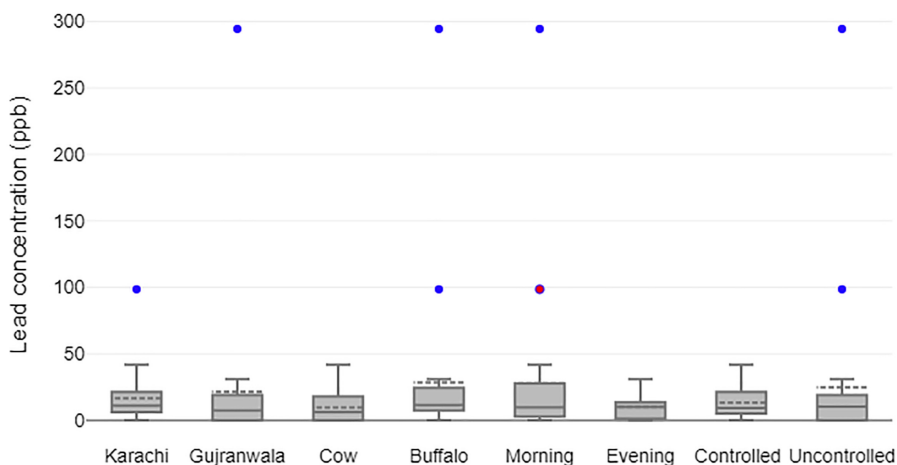


Figure 4.
Box-and-whisker plot
of lead concentration in
the milk of different
categories

Note(s): The continuous line is the median and broken line in the box is mean. The upper quartile is the upper end and the lower quartile is the lower end of box. Minimum and maximum values are shown by thin bars. The dots above the maximum values are outliers

Source(s): Figure by authors

4. Discussion

The results divulge that all three metals do not significantly differ between their two different attributes for three variables, namely Cities, Time of Milking and Mode of Feed. The arsenic levels in the milk samples of Gujranwala and Karachi did not differ significantly. Similarly, the lead concentrations in the milk samples of the two cities did not differ significantly. The chromium levels in the milk samples from the two conurbations also did not differ statistically.

Similarly, all the heavy metals concentrations in the milk samples demonstrated no statistical differences between controlled and uncontrolled feeds and between the milk samples collected in the morning and evening. However, the lead concentrations differ significantly between the milk samples of cow and buffalo (Table 2). It is worth mentioning that cadmium remained undetected in milk samples of both cities. It may exist below the detectable level in the samples. In the current study, the minimum detection level was one ppb, an extremely low concentration. It concluded that the milk is not tainted with cadmium.

However, the contamination of cadmium in the milk samples is widely detected. For instance, Hossain, Tarafder, Hasan, Kabir, and Azad (2017) reported that the milk of roaming animals in Dhaka, Bangladesh contains 7.52 ppm cadmium on average. The animals feed on municipal garbage that has lower cadmium concentrations. The leaves and the dust in the urban environment are contaminated with lead that might have entered into animals' fodder (Farid, Shams, & Khan, 2017; Shams & Beg, 2000). Moreover, cadmium was found higher in their feces. This could be due to the accumulation of metals in their body. Kazi *et al.* (2009) found cadmium in both the processed and raw milk of Hyderabad, Pakistan. Iftikhar *et al.* (2014) detected greater cadmium concentration in the animals' milk of the rural expanses of Peshawar and compared their findings with results, which were in the reports of the Joint Expert Committee of Food and Agriculture Organization (FAO)/World Health Organization (WHO) (1989). Arif *et al.* (2020), reported 0.021 ± 0.006 ppm as the mean cadmium concentration in raw milk samples of animals collected from different urban districts of Lahore. Whey, watery, or liquid fraction of milk generally contains cadmium. It does not attach to fat and caseins. Unlike other toxic metals, cadmium attaches to objects of smaller molecular weight, like citrates, lactose and whey proteins (Kazi *et al.*, 2009). Su *et al.* (2021) revealed a higher concentration of chromium and arsenic in the milk of the cows that were reared and fed in the industrial areas of China compared to those in the non-industrial areas.

The present study demonstrates considerable variations in the lead levels in the milk. The lead levels in 48 milk samples ranged from 0.0 to 294.37 ppb. The median lead levels in the milk samples of the eight groups were between 6.19 to 11.66 ppb. The median lead level of cows' milk was 6.19 ppb, while that of buffaloes was 11.66 ppb. The median lead levels of the other six categories were within these values. Likewise, mean lead levels in the samples of eight different groups were between 9.67 ± 11.65 ppb to 28.43 ± 60.92 ppb. A significantly higher lead concentration was found in the buffalo's milk compared to that of cow's milk (Table 2). Khan *et al.* (2014) also found higher lead levels in buffaloes' milk than the cows' milk. These studies suggest that the milk of cows is less contaminated with lead than that of buffaloes. However, no significant difference was noted between the attributes of different variables.

The environmental lead goes into the food chain through the water and fodder on which the animals are raised. Environmental lead is an important factor in escalating the lead level in animals' milk (Kazi *et al.*, 2009; Sikirić, Brajenović, Pavlović, Havranek, & Plavljančić, 2003). Its accumulation rate depends on the size and age of the animal. According to Croatian Legislation, 100 ppb is the tolerance limit of lead (Narodne Novine, 1994). Of 48 milk samples, the lead level in one milk sample exceeded 100 ppb. The lead of other milk samples was within 100 ppb. The joint FAO/WHO Committee in 1993 on Food Additives and Contaminants recommended 20 ppb lead as the maximum permissible limit for raw milk. Khan, Ullah,

Wahid, Sultana, and Rahim (2021) reported a mean level of 1.12 ppm in raw milk samples in Peshawar, which exceeds the WHO maximum permissible lead limit for raw milk. Arif *et al.* (2020) reported 0.024 ± 0.005 ppm lead in the raw milk samples collected from urban areas of Lahore city.

However, Javed *et al.* (2009) found a higher lead level (18.87 ppm) in the animals' milk from Faisalabad. They suggested that the industries in the city are responsible for environmental lead, which contaminates the animals' milk. Khan *et al.* (2014) explored the lead levels in the cattle's milk from Swat and found that the fodder and water contaminated the cattle's milk. Iftikhar *et al.* (2014) noted 2.24 ppm lead residues in the animals' milk of Peshawar City, which was higher than that of the current study. They have not found any difference in the lead levels of milk in suburban and urban areas of the city. Arif *et al.* (2020) reported that the concentrations of lead and cadmium exceeded the maximum permissible limit recommended by the International Dairy Federation. However, Shar, Pirhot, Shar, and Channa (2021) reported that the mean lead concentrations in the milk samples of cows and buffalos met the safety guidelines. They found 3.1 ppb and 2.8 ppb lead in the milk samples of cows and buffalos of Hyderabad, Pakistan.

The arsenic levels in the milk samples of the present study were significantly lower compared to those of lead and chromium. The arsenic concentrations in 48 milk samples ranged from 0.0 to 3.70 ppb. The median arsenic concentrations in the samples of eight different categories were calculated between 0.17 to 0.30 ppb. The median arsenic concentration of cows' milk was 0.17 ppb, while that of buffaloes was 0.30 ppb. The median arsenic levels of the other six categories were within these concentrations. Similarly, mean arsenic concentrations in the milk samples of eight different groups ranged between 0.40 ± 0.55 ppb to 0.59 ± 0.88 ppb. Unlike lead, Gujranwala shows higher arsenic concentrations (0.59 ± 0.88 ppb) in their milk samples than the arsenic level (0.40 ± 0.55 ppb) in the milk from Karachi. This may be due to the arsenic in the groundwater or soil of some areas of the Punjab Province. However, no significant difference was noted between the attributes of three variables.

Chromium concentration in the milk samples was lower than lead concentration and higher than the arsenic levels. Chromium concentrations in 48 milk samples ranged from 0.0 to 8.60 ppb. The median chromium in the samples of eight groups ranged between 0.0 to 1.60 ppb. The mean chromium levels in the milk of eight different categories were between 1.77 ± 2.05 ppb to 2.40 ± 2.61 ppb. Unlike arsenic, Karachi reveals higher chromium concentrations (2.40 ± 2.61 ppb) in their milk samples than the chromium level (2.40 ± 2.61 ppb) in the milk from Gujranwala. Both the values meet the WHO guidelines of maximum residual concentration (25 ppb) for chromium. Karachi is an industrial city that accommodates many chromium-releasing industries, particularly chrome tanning industries, which could be a possible source of chromium contamination. Nonetheless, the attributes of different variables do not differ significantly. Javed *et al.* (2009) found greater mean concentrations (1.07 ± 0.07 ppm) of chromium in the animals' milk of Faisalabad City.

It is observed that the fodders of the animals are also irrigated with industrial wastewater that sometimes contains a very high level of toxic metal. This suggests that the fodders of controlled feed are contaminated with toxic, which may go into the food chain and find their way into the milk of the animals. In the uncontrolled feed category, both animal species take their food from other sources. Alam, Kabir, Sakib, Salahuddin, and Azad (2016) and Hossain *et al.* (2017) investigated the toxic metals in the milk of animals nourished on municipal solid waste. Hossain *et al.* (2017) found very high chromium concentrations (10.99 ppm) in the milk of roaming animals in Dhaka, Bangladesh. These animals take uncontrolled feed. However, very few studies exist to establish any relationship between the different toxic metals in the milk due to diverse feeding modes.

In the current study, all the metals in the buffaloes' milk were higher than those of cows. It may be because of the tendency of the metals to accumulate in the lipid content of the milk

since buffalo milk contains higher lipids than that of cows. [Jan et al. \(2011\)](#) reported that buffalo milk has a higher tendency toward harmful metal accumulation than the milk of other animals. This may be due to the lipophilic properties of heavy metals, which form lipophilic complexes in the milk. [Khan et al. \(2013\)](#) and [Khan, Malik, Muhammad, Ullah, and Qadir \(2015\)](#) reported an upsurge of the toxic metals in the animals' milk of Swat, Pakistan. They recorded 68 ppb of lead, 455 ppb of chromium and 98 ppb of cadmium in the milk, fruits and grains, which were higher than their earlier report.

Worldwide, the milk of cows and buffaloes are vital source of nutrients for nourishing human health, particularly the growth and development of infants and young children ([Tripathi, Raghunath, Sastry, & Krishnamoorthy, 1999](#)). However, the dangerous metals in the milk samples lower their health benefits and elevate their toxicity. The infants and the children are the high-risk sections of the population since they generally take milk daily as their regular feed. The intake of contaminated milk may cause health effects, particularly in growing children, namely dysfunctions of the brain, kidneys and liver. For instance, young ones develop encephalopathy when exposed to lead. The increase in the heavy metal concentration in human blood may cause disturbances in cardiovascular systems ([Salma, Maenhaut, Dubtsov, Zemplen-Papp, & Zaray, 2000](#)). For that reason, it suggests minimizing and controlling the load of toxic metals in dairy milk at the national level.

5. Conclusion

The present study suggests that fresh raw milk of Karachi and Gujranwala contains lower concentrations of lead, chromium and arsenic than the studies conducted elsewhere, which may be because the milk samples were extracted directly from animals' udder. In other studies, the milk samples were from retail shops or tetra packs, which could have been contaminated during handling and supplies from dairy farms to retail markets. Moreover, it could have been contaminated through dilution with tap water, which is usually added to expand the volume of the milk for shady earnings ([Arif et al., 2020](#)). The addition of contaminated water in the milk for increasing its volume is a primary aspect of metal contamination in the milk. A non-significant difference was found between the two attributes of three independent variables. The metal concentrations of the milk from Karachi do not differ significantly from those from Gujranwala. Likewise, the metal concentrations of the milk extracted during the morning were similar to those extracted in the evening. In the same way, no significant difference was found between the milk of controlled-fed and uncontrolled-fed animals. However, lead concentrations in buffalo milk were significantly ($p = 0.02$) higher from those in cow milk.

6. Policy recommendation

The present study demonstrates that the concentrations of lead, chromium, cadmium and arsenic met the maximum permissible limit recommended by the WHO. However, it suggests that the milk during the supply chain, i.e. from farmland to retail shops, should be checked for heavy metal contamination. This study could be used as a reference for heavy metal contamination during handling and supplies.

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Further reading

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S.#	City	Animal	Milking time	Type of feed	Concentration of metals (ppb)		
					Lead (Pb)	Arsenic (As)	Chromium (Cr)
43	Gujranwala	Buffalo	Evening	Controlled	11.56	0.42	0
44	Gujranwala	Buffalo	Evening	Controlled	1.21	0.13	2.9
45	Gujranwala	Buffalo	Evening	Controlled	9.95	0.26	5.3
31	Gujranwala	Cow	Evening	Controlled	6.34	3.7	2.4
32	Gujranwala	Cow	Evening	Controlled	0	0	0
33	Gujranwala	Cow	Evening	Controlled	21.65	0.62	0
37	Gujranwala	Buffalo	Morning	Controlled	11.03	0.41	1.2
38	Gujranwala	Buffalo	Morning	Controlled	8.71	0.15	1.6
39	Gujranwala	Buffalo	Morning	Controlled	6.11	0	0
25	Gujranwala	Cow	Morning	Controlled	0	0	0
26	Gujranwala	Cow	Morning	Controlled	4.29	1.8	0
27	Gujranwala	Cow	Morning	Controlled	28.28	0.18	0
46	Gujranwala	Buffalo	Evening	Uncontrolled	27.32	0.36	0
47	Gujranwala	Buffalo	Evening	Uncontrolled	12.33	0.16	1.3
48	Gujranwala	Buffalo	Evening	Uncontrolled	21.31	1.8	4.2
34	Gujranwala	Cow	Evening	Uncontrolled	0	0	8.2
35	Gujranwala	Cow	Evening	Uncontrolled	0	0	2.7
36	Gujranwala	Cow	Evening	Uncontrolled	2.63	1.6	1.2
40	Gujranwala	Buffalo	Morning	Uncontrolled	294.37	0.91	1.2
41	Gujranwala	Buffalo	Morning	Uncontrolled	16.77	0.53	4.5
42	Gujranwala	Buffalo	Morning	Uncontrolled	31.09	1.1	1.9
28	Gujranwala	Cow	Morning	Uncontrolled	0	0	1.08
29	Gujranwala	Cow	Morning	Uncontrolled	0	0	1.7
30	Gujranwala	Cow	Morning	Uncontrolled	0	0	1.1
19	Karachi	Buffalo	Evening	Controlled	31.10	0.33	8.6
20	Karachi	Buffalo	Evening	Controlled	0	0	0
21	Karachi	Buffalo	Evening	Controlled	7.66	0.17	5.3
7	Karachi	Cow	Evening	Controlled	7.12	0.36	7.7
8	Karachi	Cow	Evening	Controlled	21.20	0.53	0
9	Karachi	Cow	Evening	Controlled	14.76	0.16	0
13	Karachi	Buffalo	Morning	Controlled	1.22	0	0
14	Karachi	Buffalo	Morning	Controlled	7.24	0.25	1.1
15	Karachi	Buffalo	Morning	Controlled	27.29	0.27	6.1
1	Karachi	Cow	Morning	Controlled	41.86	0.9	0
2	Karachi	Cow	Morning	Controlled	21.20	0.53	0
3	Karachi	Cow	Morning	Controlled	28.01	0.27	5.1
22	Karachi	Buffalo	Evening	Uncontrolled	1.20	0.25	1.6
23	Karachi	Buffalo	Evening	Uncontrolled	11.75	2.4	1.8
24	Karachi	Buffalo	Evening	Uncontrolled	10.32	0.39	0
10	Karachi	Cow	Evening	Uncontrolled	0	0	1.3
11	Karachi	Cow	Evening	Uncontrolled	10.07	0.17	1.4
12	Karachi	Cow	Evening	Uncontrolled	12.22	0.14	1.4
16	Karachi	Buffalo	Morning	Uncontrolled	12.78	0.11	1.6
17	Karachi	Buffalo	Morning	Uncontrolled	21.40	1.6	2.5
18	Karachi	Buffalo	Morning	Uncontrolled	98.59	0.18	2.7
4	Karachi	Cow	Morning	Uncontrolled	0	0	5.9
5	Karachi	Cow	Morning	Uncontrolled	6.19	0.41	2.3
6	Karachi	Cow	Morning	Uncontrolled	6.19	0.17	1.1

Table S1.

Source(s): Table by authors