

Assessing the risk of heavy metals contamination in milk from Pakistan

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Abstract

Milk is an essential component of human food, and natural source of many important elements. Besides essential elements it is also became a source of toxic metals due to heavy environmental pollution. In order to assess the essential metals (calcium) and toxic metals (cadmium and lead) in milk, sixty different fresh milk samples were analyzed by using atomic absorption spectroscopy (AAS). Accuracy and precession were checked by external standard addition method. Calcium was found in all samples with highest concentration (24µg/L) in camel milk. Lead was found in all milk samples with mean level 3.14µg/L and highest concentration found in packed milk sample 6.7µg/L. Cadmium was detected in 33% of total samples analyzed with range of (1.1-3.1µg/L). Results of this study will be helpful in setting the standards in one of the most consumed commodities in Pakistan.

Keywords: Toxic metals; Milk; Flame atomic absorption spectroscopy; Acid digestion

1. Introduction

Milk is an indispensable commodity of the human diet; provide essential nutrients needed for growth and development of the body. Milk is considered as a rich source of many micro and macro nutrients; but at the same time it has become a source of many toxic metals due to environmental pollution [1]. Occurrence of the toxic metals in milk could be considered as an important indicator of the hygienic quality of the milk [2, 3]. Milk originated from different animals, have different nutrients profile so it is imperative to assess the essential and toxic heavy metals in milk originated from the different animal sources.

Milk is the most varied natural foodstuff in terms of its composition and contain more than twenty different essential trace elements needed for many physiological functions [4]. Deficiencies of these trace elements lead to many diseases; however, if present at higher levels, they may have a negative effect on human or animal health. Presence of these elements in milk depend on many factors including the stage of lactation, nutritional status of the animal, environmental and genetic factors, characteristic of the manufacturing practices and possible contamination from the equipment during processing [5].

Milk is considered as a rich source of calcium and essential for bone health along with potassium (K) and magnesium (Mg). Optimal Ca intake positively affects bone mass, ensuring adequate bone development in childhood and youth. Calcium is an essential for preventing osteoporosis[6, 7], It is also crucial for nerve conduction, muscles contraction, heart beat and blood coagulation, production of energy and maintenance of immune function. Calcium decreases the odds of getting colon and breast cancer, improves weight control and reduces the risk of developing kidney stones [8-11].

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Reports of milk contamination with heavy metals have received much attention recently. Heavy metal is a general term applied for metals and metalloids having density greater than 6 g/cm^3 [12]. One of the most problems associated with heavy metal is their ability to bio accumulate and poses greater risk when reached at the end of food chain [13-16]. Heavy metals gain entry into the milk mainly through cattle feed and drinking water (besides air) which in turn are contaminated from sewage sludge, artificial fertilizers, metals used in fungicidal agents, agricultural chemicals and wastewater from industries.[17]. The occurrence of Lead inhibits ferrochelatase, which leads to decrease red blood cell survival, decrease in the rate of globin synthesis and an inhibition of the heme synthesis [18, 19]. Cadmium has been implicated in osteoporosis with impaired general health, lung and kidney damage [20]. Long term chronic exposure to cadmium has been associated with anemia, osteomalacia and cardiovascular diseases especially hypertension [21-23]. Due to their toxicity, various countries have set up maximum regulatory limits for food products. see Table 1.

Pakistan is facing water shortage and use of sewage and industrial wastages for the growth of plants is the most common practice, which increase heavy metal accumulation in plants [24]. Heavy metals accumulation in plants leaves and vegetables have been reported from Pakistan [25-30]. These studies have suggested that the fodders grown with such soil and water will have higher heavy metals, and animal's rear on contaminated fodders will accumulate heavy metals in animal tissues and their milk.

Hyderabad city is located at the end of river Indus and most of the sewage water and industrial wastes directly goes into river water. Fodders grown on contaminated water used for rearing animal in this region likely to have higher heavy metal contamination [31, 32]. Therefore, the present study was undertaken to assess the heavy metal residues in different milk samples. The result of this study will help to determine the potential toxic effects of heavy metals.

Table 1 Recommended levels/ safe intake of heavy metal

Heavy metals	Allowable limit	Reference
Cadmium	15 -50 $\mu\text{g/day}$ adults	[33]
	2 -25 $\mu\text{g/day}$ children	
Lead	20 - 280 $\mu\text{g/day}$ adults	[33]
	10 - 275 $\mu\text{g/day}$ children	
Calcium	1000 mg/L	[34]

2. Material and methods

2.1. Chemical and reagents

Standard solutions for different metals were purchased from Fisher Scientific (Bishop Meadow Road Loughborough, UK), and working standard solutions were prepared by simple dilution with distilled water. Sulfuric acid, perchloric acid and nitric acid were all of Analytical grade (BDH, England). All glassware's were of borosilicate and purchased from British Glass (Churchill way, Sheffield, UK).

2.2. Instrumentation

Perkin Elmer flame atomic absorption spectrometer (A Analyst 200) equipped with hollow cathode lamp was used for metals determination. The operating parameters were set as recommended by the manufacturer, slit width, 0.5 nm; current, 10 mA each with different wavelength as shown in table 2. The flame composition was air (flow rate, 10 L/min) and acetylene flame (flow rate, 2.5 L/ min). The calibration curves were drawn for each element by plotting absorbance versus concentration of each element. Weight measurement was done by using Kern Analytical balance (Kern GmbH, Germany).

2.3. Sample collection and sample preparation

Nearly five hundred milliliter of each milk sample was collected in sterilized plastic bottles from Hyderabad and its adjacent areas. Milk of different animal's which include camel (9), sheep (10), goat (10) cow (10) and buffalo (11) and packed milk (10) was collected. Before injecting into AAS each sample was subjected to acid digestion as described by Javed et al 2009 [35]. Briefly, one gram of milk was added to ten millilitre of concentrated nitric acid and heated for twenty minutes. Finally five milliliter of perchloric acid was added and heated vigorously until white fumes appeared

and volume reduced 2-3 ml. Final volume was adjusted to fifty milliliter by adding distilled water in it. Calcium, lead and cadmium concentration in milk was determined by AAS as described by Licata et al 2004 [2].

2.4. Statistical analysis

The data was analyzed by using excel data sheets and SPSS 18. The simple percentage plus mean values + SD of the heavy metals were calculated.

3. Results and discussion

3.1. Method validation

Calibration curves were developed by plotting absorbance versus concentration of each element, and coefficient of determination for each element was determined and presented in table 2

Table 2 Parameters and method characteristic used for different metals determination

Metal	Wavelength	Slit width	Calibration equation	Coefficient of determination	Flame	LOD ¹ (µg/L)	LOQ ² (µg/L)
Ca	422.7nm	0.5	$y = 0.003x - 0.024$	0.986	Oxy-acetylene	10	30.3
Cd	228.8nm	0.5	$y = 0.001x - 0.008$	0.987	Oxy-acetylene	1.5	15
Pb	217.3 nm	0.5	$y = 0.25x + 1.5$	0.976	Oxy-acetylene	1.5	15

$$\text{Limit of detection (LOD)} = 3.3 \times \frac{\sigma}{\text{Slope}}$$

$$\text{Limit of quantification LOQ} = 10 \times \frac{\sigma}{\text{Slope}}$$

Accuracy and precision for each element was determined by external standard addition method and found to be in the range of 95-98 % (Results are not given here). Limit of detection and limit of quantification was determined as described by Shrivastava et al 2011 [36] and given in table 2.

3.2. Surveillance results

Calcium, lead and cadmium concentration in milk samples after acid digestion were analyzed by using flame atomic absorption spectroscopy. The mean concentration of metals in milk sample collected from Hyderabad is presented in Table 3.

Nearly all samples were found to be contaminated with lead having mean concentration of 4.3, 3.1, 2.8, 3.0, 2.5, 6.7 µg/L in goat, cow, buffalo, camel, sheep and packed milk respectively (table 3). Higher concentration of lead was found in packed liquid samples (6.7 µg/L). This higher value of lead might be attributed to extra process of packaging which may contribute to its higher value. Figure 1 shows the box and whisker plot of different metals in milk samples. From figure it is clear that next higher concentration is found in goat milk samples 4.3 µg/L. This higher concentration of lead is due to goat feeding interest. Goat is generally keep grazing in open filed and mostly eat smaller shots and buds which usually contains higher concentration of heavy metals as described by Mahmood et al 2010 [37]

Cadmium was found in twenty samples out of sixty with mean concentration level 1.9 µg/L. The same trend of cadmium is also observed for liquid packed milk.

Calcium being as an essential element was found in all samples and highest concentration was found in the camel milk 24 µg/L (Table 3). Calcium content in milk is dependent upon the feeding and animal species, and camel milk have highest amount of calcium as reported by Said Zibae et al 2015 [38].

Table 3 Metals concentration in milk samples of different animals determined by using AAS

Metal	Goat (n=10)		Cow (n=10)		Buffalo (n=11)		Camel (n=9)		Sheep(n=10)		Packed (n=10)	
	Range	Mean (µg/L)	Range	Mean (µg/L)	Range	Mean (µg/L)	Range	Mean (µg/L)	Range	Mean (µg/L)	Range	Mean (µg/L)
Cadmium	1.1-3	1.9±0.1	1.1-2.8	1.5±0.1	2.1-3.1	2.2±0.1	1.1-1.8	1.3±0.1	1.1-2.1	1.6±0.1	1.2-2.8	2.0±0.1
Lead	2.9-7.1	4.3±0.2	2.5-5.1	3.1±0.2	1.1-4.2	2.8±0.2	2.1-4.2	3.0±0.2	1.1-5.2	2.5±0.2	5.2-8.1	6.7±0.2
Calcium	9-15	12±0.1	10-17	13.3±0.1	10-24	18±0.1	17-28	24±0.1	8-12	10±0.1	9-16	12±0.1

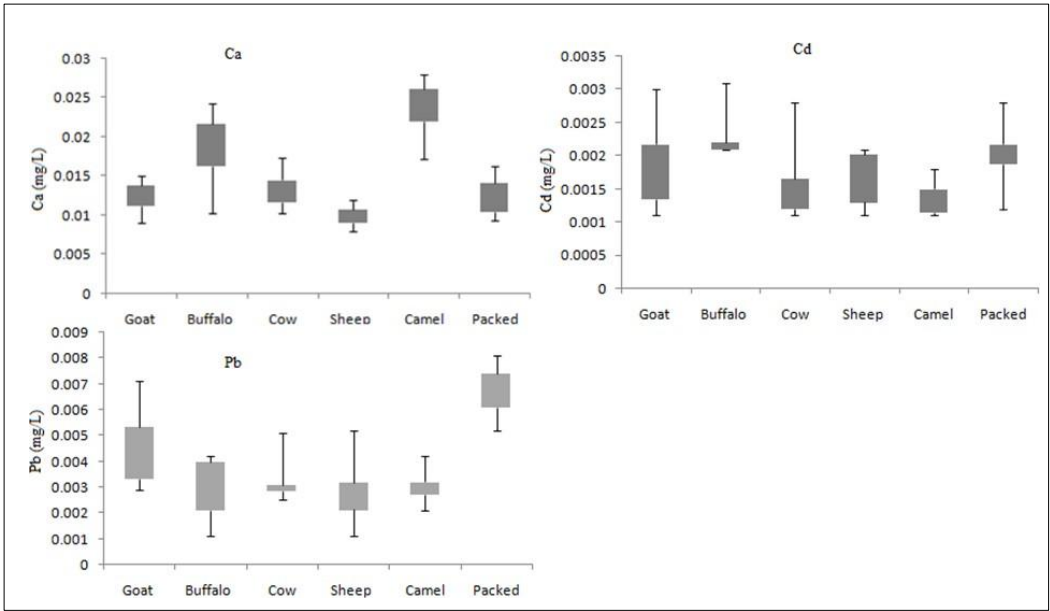


Figure 1 Box and Whisker plot of calcium, cadmium, and lead in different milk samples

Heavy metals contamination in milk available in Pakistan is much lower, when compared with the allowable limits in milk (Table 1). There is a wide variation in the published data for the elemental concentrations of milk in different countries (Table 4). Most probably level of concentration is dependent upon the sampling point. Some of the results are recorded in table 4 for comparison with the present values. The concentrations of the present study are comparable with the published data. This shows that the milk consumed in Hyderabad region is poor source of Cd, Pb and good source of calcium.

Table 4 Comparative study of metal concentration reported in milk

Country	Heavy metals reported in milk		Reference
	Pb (µg/L)	Cd (µg/L)	
Turkey	6.83	0.257	[39]
Taiwan	2.03	0.044	[40]
Italy	1.32	0.02	[2]
Croatia	42.11	5.31	[41]
California	91	6.0	[42]
Argentina	25	1.47	[43]
Brazil	40	1000	[44]
Libya	3.43	1.24	[45]
Present	3.14	1.9	

4. Conclusion

Different milk samples commonly consumed in Hyderabad were analyzed by AAS after simple acid digestion. Camel milk was found to be rich source of calcium, while lead and cadmium (potentially toxic elements) were found in lower quantities than allowable limit of EU and Pakistani standard specification. This indicates that area which investigated in this study is not at risk of environmental pollution. But still strict control of trace and toxic element levels in these foods is therefore advisable.

Compliance with ethical standards

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No potential conflict of interest was reported by the authors.

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