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Original Research

Concentrations of Cadmium, Lead and Mercury in Raw Bovine, Ovine, Caprine, Buffalo and Camel Milk

Pouya Parsaei[1,2], Ebrahim Rahimi[1,2]*, Amir Shakerian[1,2]

[1]Department of Food Hygiene, Faculty of Veterinary Medicine, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran.
[2]Research Center of Nutrition and Organic Products, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran.

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Abstract

Toxic heavy metals and especially lead (Pb), cadmium (Cd) and mercury (Hg) can easily transmit to humans through consumption of contaminated milk. The present research was done to study the concentrations of Pb, Cd and Hg in different types of milk samples. In total, 1100 bovine, ovine, caprine, buffalo and camel raw milk samples were collected in different regions of Iran and analyzed to determine concentrations of Pb, Cd and Hg by a graphite furnace atomic absorption spectrometric method. The mean recoveries of the analytical method were 88%, 93%, and 96%, for Hg, Cd and Pb, respectively. The mean Cd, Pb and Hg contents obtained from 1100 samples were 3.62±0.35 ppb (range: 0.06-14.03 ppb), 11.73±1.09 ppb (range: 0.12-33.62 ppb) and 4.35±0.42 ppb (range: 1.03-10.38 ppb), respectively. The highest concentrations of Cd, Pb and Hg were found in raw bovine samples (4.05±0.38, 12.36±1.21 and 5.76±0.53 ppb, respectively), while raw camel milk harbored the lowest concentrations of heavy metals. Milk of 5≤ year-old animals had the highest concentrations of Cd, Pb and Hg. Milk samples of all studied animals collected in spring had the highest concentrations of Cd, Pb and Hg heavy metals ($P<0.05$). The mean concentrations of Cd and Hg heavy metals were lower than the allowed limits announced by the standard organizations, while those of Pb were higher. These results highlight the importance of periodically monitoring levels of Cd, Pb and Hg heavy metals in milk of bovine, ovine, caprine, buffalo and camel – especially in spring and summer seasons and also in 5≤ year-old animals in Iran.

Keywords: cadmium, lead. mercury, raw milk, seasons, age

*e-mail: Ebrahimrahimi55@yahoo.com

Introduction

Dairy products, and in particular milk, contain diversity of important nutrients such as proteins (casein, lactoglobulin, lactoalbumin), carbohydrates (lactose, glucose and galactose), fats (linoleic, linolenic, mystiric, palmitic, and oleic acids), minerals (calcium, phosphate, magnesium, sodium, potassium, citrate and chlorine), and vitamins (A, B, D, E, K) which are crucial to maintaining a healthy life of every individual [1]. There is evidence that milk and other dairy products might contain varying amounts of different toxic contaminants and especially heavy metals [2]. Heavy metals are defined as metallic elements that have a relatively high density compared to water [2-4]. Heavy metal pollution is a result of increasing industrialization throughout the world, which has penetrated into all sectors of the food industry. Industrial, agricultural, domestic and technological processes have resulted in an increased concentration of heavy metals in air, water, soil and subsequently, these metals are taken by plants or animals and find their ways into food chain [2-4]. Milk of animal species can become contaminated with toxic heavy metals and especially cadmium (Cd), lead (Pb) and mercury (Hg) due to their feeding with contaminated forage and water and also their grazing in contaminated areas [2-4]. Chronic and even acute exposure to mercury, lead and cadmium is responsible for the occurrence of several types of cancers, tumors, autoimmune diseases, oxidative stress, enzymatic disorders, iron deficiency and hepatic and renal toxicities [5-8]. From a toxicological view, Iran is one of the main regions with boosted toxic heavy metals content [7]. Several studies have reported the high contents of Cd, Pb and Hg elements in different types of food samples in Iran [7]. From a public health prospective, it is important to assess the concentrations of Pb, Cd and Hg toxic heavy metals in milk. Thus, the present investigation was done to study the concentrations of Pb, Cd and Hg in raw bovine, ovine, caprine, buffalo and camel milk samples collected from different parts of Iran.

Materials and Methods

Samples and Study Population

The protocol for collection of milk samples was designed to minimize any outer contamination. From January 2017 to January 2018, a total of 1100 raw milk samples including bovine (n = 300), ovine (n = 250), caprine (n = 250), buffalo (n = 150) and camel (n = 150) (2-5-year-old animals) were randomly collected from the dairy farms of Isfahan Province, Iran, located at the center of Iran (32.6546°N, 51.6680°E). The province covers an area of approximately 107,027 km^2 and is situated in the center of the country. It has population of 4,500,000. All animals had similar lactation stages. Ovine and caprine milk samples were collected through the spring and summer seasons. Additionally, animals of the same species had similar breeds. Bovines whose their milk samples were collected for this study had routine feed of pellet and forage. Ovine and caprine, whose their milk samples were collected for this study, had free grazing and feed from natural forage, and plants grew on pastureland. Bovines whose milk samples were collected for this study had free grazing and feed from natural forage and thorns in the desert. Fifty ml of each milk sample was purchased and immediately transferred to a laboratory. In order to clear any remaining mineral elements from the laboratory tubes, dishes and glassware used in this study, all of them were immersed in 1% nitric acid (Merck, Germany) overnight and rinsed well with deionized water. Moreover, in order to clear additional remaining mineral elements we used microwave mineralizer (MLS 1200 mega). Samples were subsequently sub-sampled in aliquots of about 50mL in Falcon tubes (free polypropylene) and stored in a refrigerator at −20ºC until the moment of analysis.

Sample Preparation

Twenty-five g of each sample was weighed in ceramic crucibles and dried at 450°C by a heater. Then the crucibles were put on a flame and burnt. After that, crucibles containing the samples were put in an oven at 450ºC for 4 hours until the sample turned to ash. In the next step, 0.1 mol of nitric acid was added to the vessel containing the sample. Then it was flattened in a balloon and the volume was increased to 50 ml with nitric acid 0.1 molar. In the next step, 20 ml of each sample was transferred into a funnel decanter (separation), a few drops of Bromocresol were added to the detector, and eventually 4 ml of citric acid was added. pH was regulated at 5.4 by ammonia.

Analytical Procedure

Mercury was determined in all the digests using cold vapor atomic absorption spectrophotometry flow infection mercury/hydride analyzer (FIAS 4100, Perkin Elmer, Foster City, CA, USA), equipped with a weigh hollow cathode mercury lamp operated at a wavelength of 253.7 nm. Quartz absorption cell was used for mercury determination. The concentrations of cadmium and lead were determined by graphite furnace atomic absorption spectrophotometry (Perkin Elmer 4100), employing paralytic platform graphite tubes (Perkin Elmer, AS-40), ascorbic acid and palladium for matrix modification. Details of the analytical methods have been previously published [9]. The detection limits calculated as three times the SD were 0.11, 0.041 and 0.039 mg/L of milk for mercury, cadmium and lead, respectively. The accuracy of the analysis was checked by various methods, including the use of reference material (BCR No 150). The mean recoveries of

Table 1. Concentrations of toxic heavy metals in different types of raw milk samples.

Types of milk samples	No samples collected	Concentrations of heavy metals (ppb)					
		Cd		Pb		Hg	
		Mean±SD	Range	Mean±SD	Range	Mean±SD	Range
Bovine	300	4.05±0.38[a]	0.16-14.03	12.36±1.21[a]	0.80-33.62	5.76±0.53[a]	2.36-10.38
Ovine	250	3.41±0.31[b]	0.15-11.55	11.94±1.92[a]	0.60-29.96	5.44±5.37[a]	2.29-9.60
Caprine	250	3.22±0.31[b]	0.12-10.88	10.25±1.01[a]	0.53-27.48	5.10±0.50[a]	2.10-8.98
Camel	150	1.22±0.11[c]	0.06-4.25	3.14±0.26[b]	0.12-16.74	2.36±0.21[b]	1.03-5.61
Buffalo	150	3.73±0.34[b]	0.15-13.28	12.04±1.19[a]	0.71-29.77	5.52±0.44[a]	2.27-10.11
Total	1100	3.62±0.35	0.06-14.03	11.73±1.09	0.12-33.62	4.35±0.42	1.03-10.38

*Dissimilar small leathers in each column shows significant statistical difference ($P<0.05$).

mercury, cadmium, and lead were 88%, 93%, and 96%, respectively [9].

Statistical Analysis

Concentrations were expressed as mean±SD. Statistical analysis was done using the SPSS 21.0 statistical software (SPSS Inc., Chicago, IL, USA). A t-test analytical method was performed and differences were considered significant at $P<0.05$. For statistical analysis, values below the detection limit were set to half that level.

Results and Discussion

The increase in agricultural production efficiency causes using large quantities of chemical products not even in animal feed production, but also on farms with milk production. Due to non-compliance with the right

Table 2. Senile distribution of toxic heavy metals in different types of raw milk samples.

Types of milk samples	Age (Year)	Concentrations of heavy metals (Mean ± SD, ppb)		
		Cd	Pb	Hg
Bovine	≤2	1.07±0.09[d]	3.29±0.35[f]	2.98±0.14[bc]
	3	3.12±0.27[c]	9.82±0.84[e]	5.21±0.37[ab]
	4	6.19±0.58[b]	14.30±1.36[c]	6.10±0.60[ab]
	5≤	10.33±1.26[a]	23.72±2.29[b]	7.14±0.68[a]
Ovine	≤2	0.82±0.04[e]	2.51±0.23[f]	2.95±0.13[bc]
	3	2.23±0.18[d]	5.27±0.48[f]	4.40±0.36[b]
	4	4.91±0.46[c]	12.33±1.15[d]	6.21±0.58[ab]
	5≤	10.29±0.94[a]	20.85±1.90[b]	8.30±0.79[a]
Caprine	≤2	1.14±0.10[d]	1.90±0.12[f]	2.86±0.17[bc]
	3	2.48±0.26[c]	8.22±0.43[e]	3.98±0.33[b]
	4	7.71±0.65[b]	18.84±1.62[c]	7.19±0.61[a]
	5≤	9.23±0.87[a]	23.33±2.21[b]	8.08±0.69[a]
Camel	≤2	0.14±0.01[e]	0.92±0.08[f]	1.71±0.07[d]
	3	1.77±0.12[d]	4.15±0.31[e]	2.52±0.20[bc]
	4	3.10±0.25[c]	10.02±0.88[e]	4.95±0.41[b]
	5≤	4.01±0.21[c]	14.44±1.26[c]	5.23±0.47[b]
Buffalo	≤2	1.02±0.52[d]	1.26±0.11[f]	2.73±0.18[c]
	3	3.33±0.28[c]	5.21±0.49[e]	3.96±0.24[bc]
	4	8.25±0.76[b]	15.92±1.37[c]	5.51±0.45[b]
	5≤	12.02±1.04[a]	28.61±1.02[a]	8.24±0.65[a]

*Dissimilar small leathers in each column shows significant statistical difference ($P<0.05$).

Table 3. Seasonal distribution of toxic heavy metals in different types of raw milk samples.

Types of milk samples	Season	Concentrations of heavy metals (Mean±SD, ppb)		
		Cd	Pb	Hg
Bovine	Winter	2.12±0.17[b]	3.61±0.32[e]	2.99±0.21[c]
	Spring	10.17±1.00[a]	22.11±2.02[a]	7.03±0.55[a]
	Summer	9.01±0.65[a]	20.36±1.54[a]	6.80±0.59[a]
	Autumn	3.41±0.33[b]	8.52±0.73[d]	5.42±0.51[a]
Ovine	Spring	2.49±0.22[b]	7.83±0.45[d]	4.52±0.39[ab]
	Summer	7.05±0.61[a]	17.96±1.22[b]	7.17±0.67[a]
Caprine	Spring	3.55±0.28[b]	5.31±0.46[d]	3.56±0.29[bc]
	Summer	8.14±0.43[a]	20.20±1.55[a]	7.33±0.51[a]
Camel	Winter	0.72±0.05[c]	1.24±0.10[e]	1.09±0.08[c]
	Spring	3.98±0.25[b]	14.03±1.14[b]	5.18±0.46[a]
	Summer	3.03±0.21[b]	11.56±0.92[c]	4.85±0.39a[b]
	Autumn	1.63±0.18[c]	5.10±0.42[d]	2.96±0.21[c]
Buffalo	Winter	1.22±0.09[c]	1.20±0.14[e]	2.80±0.19[c]
	Spring	12.00±1.01[a]	25.59±2.12[a]	8.11±0.60[a]
	Summer	10.36±0.93[a]	17.08±1.62[b]	5.77±0.47[a]
	Autumn	5.09±0.47[b]	6.38±0.52[d]	4.04±0.31[ab]

*Dissimilar small leathers in each column shows significant statistical difference (*P*<0.05).

technologies, these substances are becoming a part of agricultural products, including milk from cows, ewes, goats, buffalo and camels. Additionally, the presence of toxic heavy metals in milk causes no special changes in color, odor, taste and consolidation, and is considered a silent agent of toxicity [10]. Therefore, it is important to assess the concentrations of toxic heavy metals and especially Cd, Pb and Hg in milk of animal species. Heavy metals are added to the environment in large quantities through atmospheric deposition, solid waste disposal, sludge application and wastewater irrigation. Animal feed, drinking water and environmental exposure, for example irrigation of agriculture land with sewage and industrial wastewater, might be sources of heavy metals in animal products such as milk. Consumption of milk from cattle and buffalo reared in the polluted sites leads to long exposure to these environmental heavy metals, which results in considerable human health hazards. Milk could also become contaminated during manufacturing and packaging. The sources of heavy metals are multiple and their entry into the dairy chain depends on biological variables such as the rate of absorption into the animal body. Table 1 represents the concentrations of toxic heavy metals in different types of raw milk samples. The mean concentrations of Cd, Pb and Hg in all studied samples were 3.62±0.35, 11.73±1.09 and 4.35±0.42 ppb, respectively. In total, concentrations of Cd, Pb and Hg had ranges of 0.06-14.03, 0.12-33.62 and 1.03-10.38 ppb, respectively. We found that bovine raw milk samples harbored the highest concentrations of Cd (4.05±0.38 ppb), Pb (12.36±1.21 ppb) and Hg (5.76±0.53 ppb). Statistically significant differences were seen as types of raw milk samples and concentrations of toxic heavy metals (*P*<0.05). Table 2 represents the senile distribution of toxic heavy metals in different types of raw milk samples. The highest mean concentrations of Cd, Pb and Hg were detected in 5≤ year-old buffalo (12.02±1.04 ppb), 5≤ year-old buffalo (28.61±1.02 ppb) and 5≤ year-old ovine (8.30±0.79 ppb) milk samples, respectively. We found that milk of 5≤ year-old animals had the highest concentrations of all studied toxic heavy metals (*P*<0.05). Table 3 represents the seasonal distribution of toxic heavy metals in different types of raw milk samples. The highest mean concentrations of Cd, Pb and Hg were detected in raw buffalo milk samples collected in spring (12.00±1.01, 25.59±2.12 and 8.11±0.60 ppb, respectively). Raw milk samples collected in spring had the highest concentrations of toxic heavy metals, while those collected in winter had the lowest (*P*<0.05). In keeping with this, there were no significant differences for the concentrations of all studied toxic heavy metals between spring and summer seasons (*P*>0.05). The presence of toxic heavy metals in milk causes no special changes in color, odor, taste and consolidation of milk and is considered a silent agent to cause toxicity [10]. Therefore, it is important to assess the concentrations of toxic heavy metals and especially Cd, Pb and Hg in milk of animal species. The present investigation showed that raw milk samples of bovine, ovine, caprine, camel and buffalo species harbored considerable amounts of Pb, Hg and Cd toxic heavy metals. The mean concentrations of Cd, Pb and Hg in bovine, ovine,

Table 4. Standard allowed limit concentrations of Cd, Pb and Hg in milk samples [13-21].

Toxic heavy metals	Standard allowed limit (ppb)
Cd	1000
Pb	0.01
Hg	0.5

caprine, camel and buffalo milk samples were 4.05±0.38, 12.36±1.21 and 5.76±0.53, 3.41±0.31, 11.94±1.92 and 5.44±5.37, 3.22±0.31, 10.25±1.01 and 5.10±0.50, 1.22±0.11, 3.14±0.26 and 2.36±0.21 and finally 3.73±0.34, 12.04±1.19 and 5.52±0.44 ppb, respectively. To the best of our knowledge, the present investigation is the first report of detection of Cd, Pb and Hg in raw camel and buffalo milk samples in Iran. Furthermore, rare studies have been conducted in this field on ovine and caprine milk samples in Iran. The allowed limit concentrations of Pb, Cd and Hg in milk were 1000 ppb [11], 0.01 ppb [11] and 0.5 ppb [12], respectively. Thus, the mean concentrations of Cd and Hg elements were lower than standard allowed limits, while those of Pb were higher [11, 12] (Table 4). Rahimi (2013) [13] described that the mean concentrations of Pb and Cd in raw bovine, buffalo, caprine and ovine milk samples were 90.88±4.75, 60.16±3.16, 70.37±3.18 and 120.1±5.63 and 9.92±0.77, 7.74±0.41, 20.33±1.23 and 30.31±1.53 ppb, respectively. Javed et al. (2009) [14] presented the mean concentrations of Cd in raw bovine and caprine milk samples as 7.07±0.14 and 8.08±0.03 ppb, respectively, while those of Pb were 180.87±160.91 and 420.68±40.05 ppb, respectively, which were much more than our findings. Najarnezhad et al. (2013) [15] showed that the mean concentrations of Pb, Cd and Hg in raw bovine milk samples were 12.90±6.00 ppb, 0.30±0.30 ppb and 3.10±0.30 ppb, respectively. They also showed that the mean concentrations of these trace elements in raw ovine milk samples were and 14.90±7.80 ppb, 1.60±1.20 ppb and 3.10±0.30 ppb, respectively, which all were lower than our findings. The mean concentrations of cow, buffalo, goat, sheep and camel milk samples in Pakistan [16] were of Cd in 76.07±6.06, 117.11±8.08, 74.34±3.52, 10.01±1.00 and 102.14±7.09 ppb, respectively. Mean concentrations of Pb and Cd in raw milk samples of cow, goat and sheep in Sudan [17] were 502.50, 74.48 and 30.23 and 0.60, 5.92 and 7.10 ppb, respectively. Camel milk samples harbored low concentrations of Pb (3.14±0.26 ppb), Cd (1.22±0.11 ppb) and Hg (2.36±0.21 ppb) toxic heavy metals. Konuspayeva et al. (2009) [18] reported that the concentrations of Pb in raw camel milk samples of Kazakhstan were 25.02±1.91 ppb. Contents of Pb and Cd in camel milk samples of another Iranian research were in the ranges of 2262±210.35 to 8280±800.93 ppb and 90.09±5.05 to 720.14±70.40 ppb, respectively [19]. Nnadozie et al. (2014) [20] reported that the amount of Cd in camel milk in Nigeria was 191.11 ppb. Damarany (2016) [21] reported that the overall mean of Pb and Cd contents in camel milk in Egypt were 1056±105.85 and 8.00±0.21 ppb, respectively. The obtained results agreed with those reported by Saini et al. (2007) [22], Meldebekova et al. (2008) [23] and El-Bagermi et al. (2014) [24]. To the best of our knowledge, there were no previously published data on detection of Hg in camel milk samples. High concentrations of lead in camel milk for the reason of industrial and feeding animals in those areas and the existence of roads full of haze [25]. Concentrations of Cd, Pb and Hg in buffalo milk samples of our research were 3.73±0.34, 12.04±1.19 and 5.52±0.44 ppb, respectively. Roy et al. (2009) [26] reported that the mean concentrations of Pb, Cd and Hg toxic heavy metals in Indian raw buffalo milk samples were 48.03 ppb, 6.06 ppb and 0.73 ppb, respectively. Higher concentrations of Pb in buffalo milk samples (0.75 ppm) were also reported by Dwivedi et al. (2001) [27]. Similar concentrations of toxic heavy metals in buffalo milk were reported previously by Ismail et al. (2017) [28], Gaafar (2008) 29] and El-Ansary and El-Leboudy (2015) [30]. Buffalo breeding is often done around cities. Concentrations of toxic heavy metals of milk samples were higher when the protein and fat content increased. The relationship between the fat and protein of milk of animal species with concentrations of toxic heavy metals is another probable reason for differences found in the concentrations of toxic heavy metals [13, 31, 32]. Milk from older than 5-year-old animals had higher concentrations of Cd, Pb and Hg ($P<0.05$). Rubio et al. (1998) [33] reported that the Pb and Cd concentrations were significantly lower in milk samples obtained from cows aged <5 years (17000 ppb and 370 ppb, respectively) compared to cows aged >5years (34000 ppb and 2087 ppb, respectively). The main reason for choosing the three metals Pb, Cd and Hg in the present study is because of the higher impact of these three trace elements on human health. Additionally, the possibilities of detection of these trace elements in raw milk of bovine, ovine, caprine and camel species is higher than other types of toxic heavy metals. Previous studies revealed that in animals, Cd accumulates in kidney and liver and Pb accumulates in bone and tissues, metal concentration increases with age [33, 34]. Additionally, older animals have the highest exposure to toxic heavy metals for a longer period of time. Therefore, the amounts of heavy metals shed in their milk samples were higher than in younger animals. Milk samples that were collected in the spring and summer had the highest concentrations of all studied heavy metals ($P<0.05$). High concentrations of Cd and Pb in milk samples in warm seasons have been reported from Pakistan [35], Iran [15] and Lithuania [36]. Higher concentrations of toxic heavy metals in spring milk may be due to the differences in feeding patterns of dairy animals in Iran, differences in the amount of soil ingested by animals and vegetation types in different seasons, differences in the amounts of rainfall in various seasons and finally using chemical fertilizers, pesticides and insecticides in agricultural farms in this season [13, 31, 32, 37, 38]. A similar seasonal distribution of heavy metals in milk have been reported previously [7, 14, 39, 40].

In keeping with the high chemical contamination of food stuffs, microbial contamination has a higher importance. Several investigations have been conducted in this field and all of them have been demonstrated the high microbial contamination of diverse types of food samples [41-52].

Conclusions

In conclusion, results of the current research showed that Cd, Pb and Hg concentrations in bovine, ovine, caprine, camel and buffalo milk samples were relatively high. Bovine and buffalo milk samples had the highest concentrations of Cd, Pb and Hg heavy metals, while camels had the lowest. The mean concentrations of Cd and Hg heavy metals were lower than the allowed limits announced by the standard organizations, while those of Pb were higher. Additionally, there were no previously published data about the concentrations of Hg in buffalo and camel milk samples. Moreover, our data revealed marked seasonal and senile distribution of heavy metals with higher concentrations of Cd, Pb, and Hg in 5≤ year-old animals and also in milk samples collected in spring and summer. These results highlight the importance of periodically monitoring levels of Cd, Pb and Hg heavy metals in milk of bovine, ovine, caprine, buffalo and camel and also dairy products in Iran.

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Conflict of Interest

The authors declare no conflict of interest.

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