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Lead and Cadmium Concentrations in Raw Milk and Dairy Products in Zanjan, Iran: A Study on Winter and Summer Variations

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ABSTRACT

Background: The contamination of food with heavy metals is a significant concern for humans. Among food products, milk and dairy products have received more attention due to their widespread consumption at all ages, particularly among children. The aim of this study is to determine the concentration of lead and cadmium in milk and dairy products in Zanjan.

Methods: 132 samples of milk, doogh, yogurt, and cream were collected from Zanjan city during the winter and summer of 2020-2021. The lead and cadmium levels in the samples were quantified using microwave digestion and graffiti furnace atomic absorption spectrophotometry.

Results: The average concentrations of lead and cadmium in traditional milk, industrial milk, doogh, full-fat yogurt, low-fat yogurt, and cream samples were 96.23, 89.91, 78.79, 49.79, 53.74, 106.08, and 2.76, 2.07, 2.59, 1.09, 1.34, 5.60 µg/kg, respectively. The mean lead concentrations in milk and dairy products during winter were significantly higher than those during summer, whereas the mean cadmium concentrations during summer were higher than those during winter for all dairy products.

Conclusion: The lead and cadmium concentrations in most of the samples exceeded the WHO-FAO standards of 20 and 2.6 µg/kg for lead and cadmium, respectively. Therefore, prompt action is necessary to address this issue.

1. Introduction

Milk and dairy products are an integral part of the daily diet, particularly for vulnerable groups such as children and elderly. Milk is considered a complete and nutritious food for humans, as it contains essential nutrients including protein, fat, and minerals (e.g., calcium, magnesium, zinc, iron, and copper) [1]. Moreover, milk consumption serves as a valuable health indicator in societies [2]. Studies have shown the importance of consuming milk during three critical periods of life [3]. Swedish researchers have reported that individuals who consume 1.5 glasses of milk per day are 35 % less likely to develop colon cancer [4]. The consumption of milk and dairy products varies significantly across different parts of the world [5]. Studies have demonstrated that an annual intake of 200 kg of milk and its products increases growth and health, learning abilities, and work capacity, and delays disability, ultimately increasing life span [6]. However, in recent decades, there has been a decrease in the per capita consumption of milk worldwide [7]. Metals with a density greater than 5 g/cm^3 are the most important chemical contaminants in food, and their presence in animal-derived foods increases the possibility of human exposure to their detrimental effects. These metals are accumulative poisons and can be toxic even at low concentrations [8]. Their metabolic functions in the body remain unknown, but upon their entry, they disrupt normal cellular processes and have the potential to accumulate in biological tissues through bioaccumulation [8]. Heavy metals are not excreted from the body and accumulate in tissues such as muscles, bones, fat, and joints which are associated with various diseases. Additionally, they can replace solutes and minerals

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necessary for normal body function. Arsenic (As), cadmium (Cd), and zinc (Zn) are classified as carcinogenic metals by the International Agency for Research on Cancer (IARC 2016), while lead (Pb) is considered a possible carcinogen [9]. Iran is one of the leading producers of Pb and Zn in Asia, following China, Kazakhstan, and India [10]. Zanjan province is one of the industrial centers in Iran, with many Pb and Zn mines. Over one hundred factories in Zanjan province are involved in this industry, producing a considerable amount of waste soil annually. Improper disposal of this waste has resulted in increased concentrations of these metals in the environment, with the potential for them to enter the livestock feed [11-14]. Therefore, livestock feeding in this region increases the possibility of heavy metals accumulation in the human body. This study aims to investigate the concentration of Pb and Cd in milk, doogh, yogurt, and cream produced during the summer and winter of 2020-2021 in Zanjan province.

2. Materials and Methods

Nitric acid (HNO_3) (65 %), hydrochloric acid, hydrogen peroxide (H_2O_2) (30 %), cadmium sulfate ($CdSO_4.xH_2O$), and lead acetate (Merck, Darmstadt, Germany) were analytical grades. Calibration solutions for Cd and Pb were prepared by diluting standard stock solutions (1000 mg/L) purchased from Merck Company. Microwave digester (SINEO model MSD-10, China), oven (Kavosh mega, Iran), Bain-marie (Iran), and heater (Shimifan, Iran) were used in this research. Moreover, a double distilled water machine (TKA-Smart 2 Pure, Germany), and an atomic absorption spectrophotometer (VARIAN-120, Australia) equipped with a graphite furnace were employed in this study.

2.1 Digestion method

In our previous study, we showed that the microwave digestion method is the optimal method for digesting milk and dairy products [15]. For this study, 132 samples of raw milk, low-fat and full-fat yogurt, doogh, and cream were randomly collected. The milk samples were obtained from traditional and industrial livestock farms before entering a milk and dairy production factory in Zanjan, Iran during the winter and summer of 2020-2021. The samples were transported to the laboratory at refrigerator temperature, homogenized, digested, and stored in the refrigerator until analysis. Following the instructions of the digestion device, 2 g of raw milk, full-fat yogurt, low-fat yogurt, and doogh were transferred into the digestion vessel of the microwave digester. Next, 8 mL of HNO_3 and 1 mL of H_2O_2 were added, and the lid was closed. The samples were placed on the rotating plate of the microwave digester and digested based on the device's method and temperature program outlined in Table 1. After digestion, the dishes were allowed to cool for 20 min in a ventilated area, and the residue was transferred to a volumetric flask and reached 25 mL with 3 % HNO_3. For the digestion of cream samples, 10 mL of HNO_3 was added to 1g of the sample, and after 30 min, 3 mL of HNO_3 and 1 mL of H_2O_2 were added and digested using the microwave method.

Table 1. Digester temperature programs for milk samples and dairy products

Digestion of milk, yogurt, doogh			Digestion of cream		
Step (N)	Temp (T) / ℃	Time (t) / min	Step (N)	Temp (T) / ℃	Time (t) / min
1	130	10	1	130	10
2	150	5	2	150	5
3	180	10	3	180	5
			4	200	10

We used an absorption spectrophotometer equipped with a graphite furnace (GF-AAS) to analyze the samples. The device was equipped with an autosampler and high-purity argon gas (99.99 %) as the carrier gas. The samples were analyzed for Pb and Cd at wavelengths of 283.3 nm and 228.8 nm, respectively. The digestion and measurement steps were repeated three times for each sample.

2.2 Validation and statistical analysis

The RSD %, recovery percentage, LOD, and LOQ parameters were measured to validate the method and analysis. The statistical analysis was conducted using SPSS (version 25) software. The obtained data from the concentrations of dairy products were analyzed using the software to evaluate significant differences in the calculated variables at a significance level of $P < 0.05$.

3. Results and Discussion

In this study, samples of milk, doogh, yogurt, and cream were digested using the microwave digestion method and analyzed by GF-AAS. The LOD and LOQ values for Pb were determined to be 0.564 and 0.188 and for Cd 0.471 and 0.157 µg/l, respectively. In the statistical analysis, concentrations of samples that were below the LOD were considered to be 0.5 LOD in all calculations. The RSD % value for all measurements was less than 10 % and the recovery percentage was between 90 to 110 %. The mean concentrations of Pb and Cd in milk, doogh, yogurt, and cream during summer and winter are presented in Tables 2 to 5 and Figures 1 and 2. The mean concentrations of Pb and Cd in traditional milk, industrial milk, doogh, full-fat yogurt, low-fat yogurt, and cream samples were 96.23, 89.91, 78.79, 49.79, 53.74, 106.08, and 2.76, 2.07, 2.59, 1.09, 1.34, and 5.60 µg/kg, respectively. According to the Codex Standards (2019), the maximum acceptable concentration of Pb for milk is 20 µg/kg [16, 17]. Additionally, the International Dairy Federation (IDF) has reported the maximum acceptable Cd concentration to be 2.6 µg/kg [18]. It was found that in 75 % of the total tested samples, the Pb concentration exceeded the standard level.

Table 2. Mean concentration of Pb in milk during summer and winter (μg/kg)

Sample type	Season	Samples																Mean	SD
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16		
Industrial milk	Summer	20.1	15.1	67	76	23	14	0.1	0.1	20.5	47	14.5	34	88	63	90	76	40.4	31.5
	Winter	130	143	119	107	56	106	146	194	107	113	190	144	67	164	267	178	139.5	52
Traditional milk	Summer	66.6	53.2	0.1	0.1	58	80	48	0.1	56	58	-	-	-	-	-	-	42.02	30.16
	Winter	150	166	150	188	118	154	152	144	147	151	-	-	-	-	-	-	150.44	17.79

* All values less than the LOD were re placed with 0.5 LOD.

Table 3. Mean concentration of Pb in dairy products during summer and winter (μg/kg)

Sample type	Seasons	Samples										Mean	SD
		1	2	3	4	5	6	7	8	9	10		
Full-fat yogurt	Summer	0.1	0.1	0.1	43.3	0.1	31.6	0.1	0.1	18.7	50	14.4	20.1
	Winter	92	168	111	70.4	105	175	87.9	97	23.9	0.1	93.07	54.54
Low-fat yogurt	Summer	0.1	16.6	8.78	0.1	3.2	8.58	13.4	0.1	5.5	10	6.64	5.83
	Winter	106	100	76.7	143	110	78.7	108	116	108	0.1	92.94	40
Doogh	Summer	0.1	0.1	96.2	0.1	93.5	104	83.9	109	91	87.2	66.5	46.4
	Winter	95	130	91.4	128	104	187	0.1	0.1	175	0.1	91.08	70.03
Cream	Summer	67	128	45.6	60.3	111	64.8	61.3	173	62.3	136	90.95	42.79
	Winter	80	174	116	136	88.8	113	111	117	185	91	121.18	34.88

* All values less than the LOD were replaced with 0.5 LOD.

Table 4. Mean concentration of Cd in milk during summer and winter (μg/kg)

Sample type	Seasons	Samples																Mean	SD
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16		
Industrial milk	Summer	2.2	2.6	1.6	3.55	5.4	4.6	5.75	3.55	4.2	2.95	5.2	3.2	2.8	2.1	2.35	2.75	3.42	1.25
	Winter	1.2	0.08	0.08	0.08	0.08	2.95	0.08	1.25	1.85	2.2	1.45	0.08	0.08	0.08	0.08	0.08	0.73	0.95
Traditional milk	Summer	4.25	3	4.65	2.5	3.75	3.1	3.8	2.7	4.4	3.7	-	-	-	-	-	-	3.58	0.74
	Winter	0.08	4.15	0.08	3.1	1.6	2.1	2.3	1.9	2.5	1.7	-	-	-	-	-	-	1.95	1.24

* All values less than the LOD were replaced with 0.5 LOD.

Table 5. Mean concentration of Cd in dairy products during summer and winter (μg/kg)

Sample type	Seasons	Samples										Mean	SD
		1	2	3	4	5	6	7	8	9	10		
Full-fat yogurt	Summer	2.75	5.95	2	2.5	2.2	0.08	0.08	2.65	2.1	0.08	2.04	1.76
	Winter	1.15	0.08	0.08	0.85	1.2	0.08	2.65	0.08	0.08	0.08	0.63	0.8
low-fat yogurt	Summer	2.3	2	2.05	3.3	2.6	2.7	0.08	0.08	0.08	0.08	1.5	1.3
	Winter	0.08	1.05	0.08	1.45	0.9	1.4	0.79	0.55	0.08	0.08	0.6	0.6
Doogh	Summer	6.2	7.85	0.08	0.08	0.08	0.08	5.6	5.45	4.6	6.6	3.7	3.2
	Winter	1.2	2.5	0.08	0.08	0.08	0.08	2.6	2.4	2.6	3.6	1.5	1.4
Cream	Summer	10.1	10.9	11.1	2.4	2.6	5.1	4.9	4.5	4.9	8.3	6.5	3.3
	Winter	5.5	3.3	7.1	7.2	5.2	5.1	1.9	2.4	2	7.6	4.8	2.2

* All values less than the LOD were replaced with 0.5 LOD.

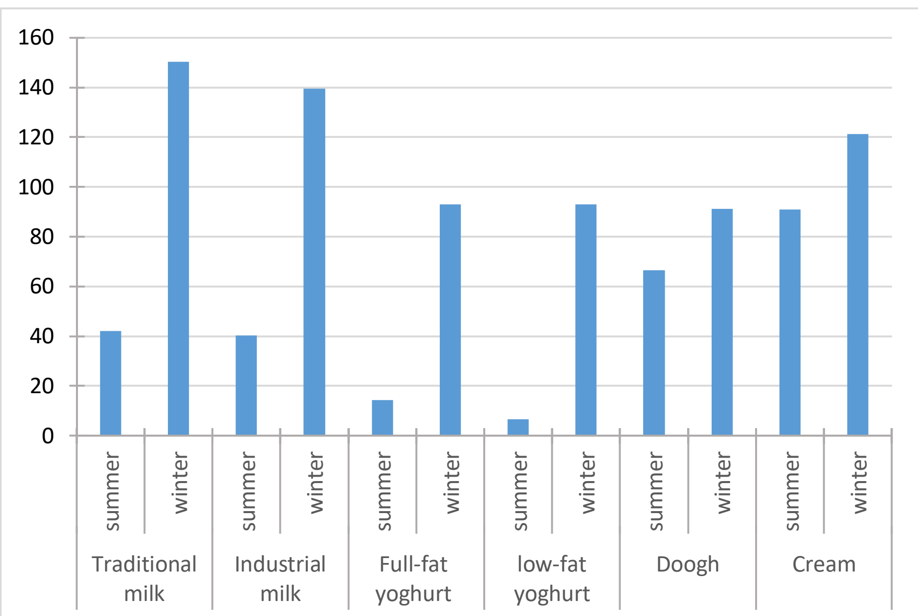

Figure 1. Comparison of Pb concentration in milk and dairy products during summer and winter (μg/kg)

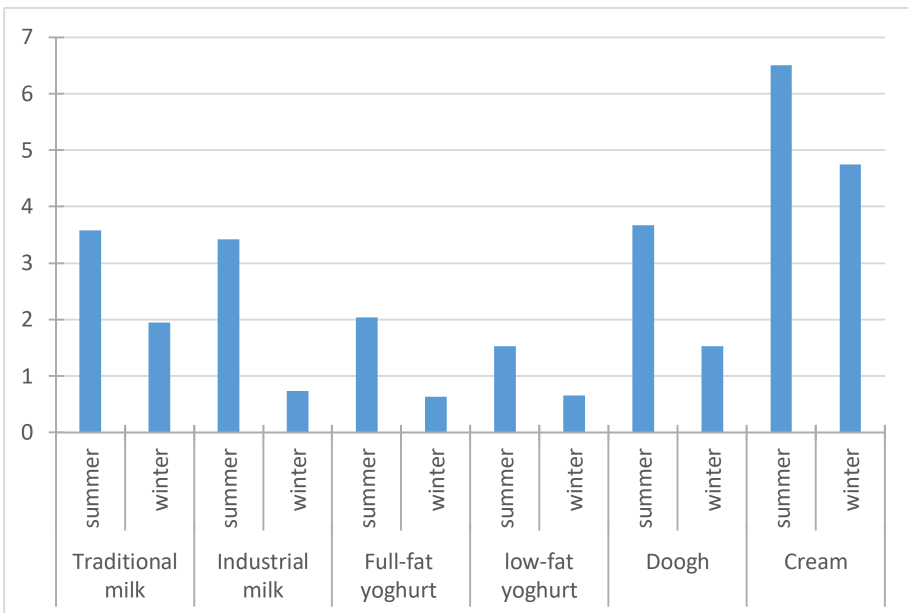

Figure 2. Comparison of Cd concentration in milk and dairy products during summer and winter (μg/kg)

In 75 % of the tested samples, the concentration of Pb exceeded the Codex standard. During the winter season, the concentration of Pb was significantly higher than in the summer ($p < 0.05$), resulting in 92.4 % of the harvested samples of milk and products being higher than the Codex standard. Statistical analysis showed no significant difference between the concentration of Pb and Cd in industrial and traditional milk ($p > 0.05$). The mean concentration of Pb in cream during the winter and summer seasons was 121.2 and 99.95 µg/kg, respectively, which was higher than the concentration of Pb in other milk and dairy products tested. The results of the statistical test demonstrated that the concentration of Pb in each of the tested dairy products and milk (separately) was higher in winter than in summer ($p < 0.001$). The concentration of Pb in cream was significantly different compared to both types of yogurt ($p < 0.05$), but no significant difference was observed with milk and doogh, although the average concentration of Pb in cream was106.08 µg/kg, and in doogh, traditional and industrial milk were 78.79, 96.25 and 89.91 µg/kg, respectively. Moreover, the average concentration of Pb in high-fat yogurt was 53.74 µg/kg, and in low-fat yogurt was 49.79 µg/kg. The concentration of Cd was higher than the IDF standards in 37 % of all the samples collected.

Table 6. Comparison of the present study with other research in other countries and Iran

Number	Study country	Sample type	Pb (µg/kg)	Cd (µg/kg)	Reference
1	Pakistan	Raw milk	47.6	44.2	[19]
		Pasteurized milk	50.6	54.2	
2	Croatia	Raw milk	41	5.31	
3	Egypt	Buffalo milk	84	118	[20]
		Cow milk	66	68	
		Cream	230	451	
		Butter	345	676	
		Yogurt	60	90	
4	Italy	Pasteurized milk	180	70	[21]
		Cream cheese	391	130	
		Fresh cheese	114	190	
		Old cheese	110	110	
5	Poland	Cow milk powder	189	99	[22]
6	Iran (Hamadan)	Cow milk	94	3	[23]
		Sheep milk	218	5	
		Goat milk	223	6	
		Yogurt	136	3	
		Cheese	325	7	
		Doogh	150	3	
7	Iran (Shahrkord) Baby Formula factory	Raw cow's milk	118.9	8.6	[24]
		Skim milk	101	10.5	
		Cream	133	1.2	
		Milk concentrate	143	14	
		Skim powder	515	9	
8	Sanandaj	Raw milk	252	76	[25]
		Yogurt	273	83	
		Cheese	546	107	
		Butter	663	62	
		Cream	612	56	
		Doogh	143	132	
9	This study	Traditional milk	96.23	2.76	
		Industrial milk	89.91	2.07	
		Doogh	78.79	2.59	
		Low-fat yogurt	49.79	1.09	
		Full-fat yogurt	53.74	1.34	
		Cream	106.08	5.6	

The statistical test showed a significant difference in the total samples and each of the registered products of Cd in the winter and summer seasons, and in the summer season, the Cd concentration in the test products was higher than in winter ($p < 0.05$), and in 59 % of all samples harvested in summer, the concentration of Cd was higher than the IDF standard. The concentration of Cd in cream was higher than in other products and a significant difference was observed ($p < 0.05$). The average concentration of Cd in cream was 5.6 µg/kg, while the average concentration of Cd in other dairy products was less than 2.7 µg/kg. Also, the concentration of Cd was 1.34 in high-fat yogurt and 1.09 µg/kg in low-fat yogurt. Industrial activities, automobiles, and mining are the main sources of Pb in the environment, and therefore, the main sources of the presence of Pb in dairy products are environmental sources such as atmospheric deposition transportation and industrial effluents. The main sources of Cd are either natural or anthropogenic origins such as fertilizers and industrial effluents. Cd and Pb can strongly bind to casein fractions in the milk. Polluted water, soil, and plants are the primary carriers of transferring Pb and Cd into the animal′s body. Cd is easily volatilized at the operating temperature in the industrial processes. Incineration of ferrous scrap and metallurgy processed are other sources of Cd in the environment. Zanjan is the main center of Pb and Zn mining and industrial activities in Iran, with more than 100 Zn and Pb factories active around the city. More than 10 million tons of tailing soil are dumped near the city, which can spread Zn, Cd, Pb, chromium (Cr), and other heavy metals into the environment. The spreading of soil particles, along with other industrial effluents, are the main sources of Pb and Cd in milk and dairy products. Using dry food (grass) in the winter and mixing livestock food with soil in the processing of food can increase Pb concentration. During the summer season, livestock are fed wet grass, and the use of phosphorous fertilizer can increase the concentration of Cd in the soil and plants, which could be a possible reason for increasing Pb in milk and dairy products during this season. Similarly, the increase in Pb during the winter season could be attributed to the use of dry grass and the mixing of livestock food with soil, which can elevate Pb concentration in the soil and plants. The results of the study indicated that the concentration of Cd and Pb was higher in fatty products. This could be explained by the binding of these metals to proteins and lipoprotein membranes of fat globules in milk, which is the main reason for the increase in Cd and Pb in cream and other fatty products. The results of the present study and other studies are shown in Table 6.

4. Conclusion

In general, most of the tested milk and dairy product samples had concentrations of Pb and Cd that exceeded the recommended standards. The average concentration of Pb was higher in the winter season than in summer, while the average concentration of Cd was higher in the summer season than in winter. The concentration of both metals was found to be higher in cream than in other products, and no significant difference was observed between the concentration of Pb and Cd in traditional and industrial milk. Given the harmful effects of these metals, it is imperative to monitor water and soil pollution in this area to prevent contamination of the food chain and ensure public health.

Authors' Contributions

Zohre Farahmandkia: Conceptualization and Writing-original draft. Rezgar Feizolahi: Data curation. Mohammad Reza Mehrasebi: Writing-review & editing. Mazyar Peyda: Validation.

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Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

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Ethical considerations

The authors have adhered to all ethical considerations, including avoiding plagiarism, double publication, data distortion, and data fabrication in this article. (Ethics no. IR.ZUMS.REC.1399.022).

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