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Heavy Metal Contamination in Raw and Pasteurized Cow's Milk in Shahrekord, Iran

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ABSTRACT

Background: Milk is a widely consumed food product, and contamination with heavy metals poses a significant public health concern due to their potential toxicity and bioaccumulation. This study assessed heavy metal contamination in raw and pasteurized cow's milk in Shahrekord, Iran.

Methods: Seventy nine bovine milk samples, including raw and pasteurized milk collected across warm and cold seasons were enrolled. Elemental concentrations of mercury (Hg), cadmium (Cd), lead (Pb), and arsenic (As) were quantified using inductively coupled plasma optical emission spectrometry (ICP-OES).

Results: Hg was the predominant contaminant detected in 10.13% of samples with concentrations ranging from 8.47 to 31.70 µg/L. Warm-season pasteurized milk exhibited the highest contamination (35%) with a mean concentration of 25.19±7.96 µg/L. Only 1.3% of samples contained detectable levels of Cd. Among the 39 pasteurized samples, 2.6% of samples were contaminated with Cd, corresponding to one sample collected during the warm season (1 out of 20; 5%). No Pb or As was detected in any of samples.

Conclusion: Hg represented the main heavy-metal risk in milk samples of Shahrekord, Iran particularly in pasteurized products during warmer months. The sporadic occurrence of Cd highlighted the potential localized contamination, while the absence of Pb and As indicated low environmental burden. These findings underscore the need for milk-specific regulatory limits, seasonally adaptive monitoring programs, and strict control of feed and processing inputs to ensure consumer safety.

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Introduction

Milk and dairy products are critical nutritional sources, particularly for children, due to their rich content of macro- and micronutrients, including vitamins and bioactive fatty acids with nutraceutical benefits (1-3). Despite their dietary importance, milk may pose health risks if contaminated by heavy metals, which have been frequently detected in dairy products worldwide (4). Heavy metals, defined by high atomic weight and density, include essential elements (e.g., Fe, Co, and Zn) necessary for biological functions and nonessential toxic metals such as arsenic (As), cadmium (Cd), lead (Pb), and mercury (Hg) that exhibit toxicity even at trace levels (5, 6). These toxic metals bioaccumulate and biomagnify through ecological systems, eventually entering bovine milk via environmental pollution, contaminated feed, milking equipment, and physiological factors (7, 8).

Environmental contamination including industrial emissions, sewage, and atmospheric deposition represents a major pathway for heavy metals like Pb and Cd to enter agricultural ecosystems and subsequently milk (8). Soil pollution transfers contaminants to vegetation and water, further facilitating bioaccumulation in livestock. Global epidemiological data from 2015 estimated that dietary exposure to As, methylmercury (MeHg), Pb, and Cd caused over one million morbidities, 56,000 deaths, and 9 million disability-adjusted life years (DALYs) lost (9, 10). Exposure to these metals is linked to neurological disorders, carcinogenicity (Pb and Cd are IARC Group 2A probable carcinogens), cardiovascular diseases, and developmental impairments, with children being particularly vulnerable due to their high dairy consumption and susceptibility to neurotoxicity and growth retardation (11, 12).

Intensified industrialization, urbanization, and mechanized agriculture have escalated heavy metal emissions, threatening livestock health and dairy safety (13). So this study quantitatively assessed concentrations of four toxic heavy metals in raw and pasteurized milk samples collected from Shahrekord, Iran.

Materials and Methods

This cross-sectional study analyzed a total of 79 bovine milk samples, comprising 40 raw and 39 pasteurized samples that were systematically collected during both cold and warm seasons in Shahrekord, Iran. Raw milk samples (n=20 per season) were obtained from traditional dairy shops supplying milk from non-industrial farms distributed across the city. To ensure geographic representativeness, the study area was stratified into four urban zones, from which five shops per zone were randomly selected using a computer-generated randomization list. Pasteurized milk samples were collected during the same periods, including 20 samples in the cold season and 19 samples in the warm season, and were sourced from 10 different commercial brands. Sampling across multiple brands and distinct batch numbers was conducted to capture batch-to-batch variability and to ensure representative coverage of industrial dairy production.

All milk samples were collected in pre-cleaned glass containers to minimize the risk of exogenous contamination. Samples were transported under refrigerated conditions (≤4°C) and stored at 4°C until laboratory analysis. The sampling design incorporated both seasonal variation and urban zone stratification to reduce potential environmental and geographic confounding. Although residual confounding cannot be entirely excluded, the applied sampling framework was designed to minimize its influence and enhance the representativeness of the collected samples. This study quantified the concentrations of toxic heavy metals, including Cd, Hg, Pb, and As in milk samples. Elemental quantification was performed using inductively coupled plasma-optical emission spectrometry (ICP-OES; Horiba Jobin-Yvon Ultima 2 CE, Germany) under optimized operating conditions (Table 1).

A 10 g milk sample was placed in a beaker, and 10 mL of a 1:1 (v/v) mixture of 65% HNO_3 (super purity quality; Romil Ltd., Cambridge, UK); while 35% H_2O_2 (suprapure quality; Merck, Darmstadt, Germany) was added. The solution was heated at

Table 1: The Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) of instrumental operating conditions.

Variable	Magnitude
RF generator power (W)	1400
Frequency of RF generator (MHz)	Resonance frequency: 27.12
Plasma gas flow rate (L/min)	13
Auxiliary gas flow rate (L/min)	0.8
Nebulization gas flow rate (L/min)	0.9
Sample uptake rate (mL/min)	1.5
Type of detector solid state	CCD
Injector tube diameter (mm)	0.3
Measurement replicates	3
Type of spray chamber cyclonic	Modified Lichte
Element (λ/nm)	As: 228.812 Cd: 228.802 Pb: 261.418 Hg: 194.227

Table 2: Limits of Detection (LOD) and Quantification (LOQ) for heavy metals determined by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES).

Metal	LOD (ppb)	LOQ (ppb)*
Mercury (Hg)	1.647	5.44
Cadmium (Cd)	0.410	1.35
Arsenic (As)	0.698	2.30
Lead (Pb)	1.871	6.17

* LOQ values were calculated theoretically as 3.3 times the corresponding LOD values.

100°C on a hot plate for 15 min. After evaporation, 2 mL of 70% $HClO_4$ (v/v) was added, followed by digestion at 150°C for 1 h until the solution became transparent. The digest was then diluted to 100 mL with deionized water (14). Blank digests were prepared identically. Prior to analysis, samples were filtered through a 0.45 µm nitrocellulose membrane filter to remove particulate matter and introduced into an ICP-OES system (Horiba Jobin-Yvon Ultima 2 CE, Germany) for quantification of heavy metals.

The inductively coupled plasma optical emission spectrometry (ICP-OES) method was employed for the quantification of heavy metals in milk samples and was fully validated according to international guidelines (ICH Q2(R1) and ISO/IEC 17025). Method validation was performed by determining the limits of detection (LOD) and limits of quantification (LOQ) for the analyzed heavy metals. The LOD values were obtained from the instrument detection limits as provided by the ICP-OES system under the selected analytical conditions. The LOQ values were calculated theoretically as 3.3 times the corresponding LOD values. The LOD and LOQ values for each metal were presented in Table 2. Data were analyzed using SPSS software (IBM Corp., Version 23, Chicago, IL, USA). Differences in metal concentrations between groups were assessed via one-way ANOVA followed by Tukey's HSD post-hoc test. Contamination frequency distributions were compared using Fisher's exact test. A p value less than 0.05 was considered statistically significant.

Results

The analysis of 79 bovine milk samples revealed detectable Hg contamination in 8 out of 79 samples (10.13%), with concentrations ranging from 8.47 to 31.70 µg/L. Among the 40 raw milk samples analyzed, Hg contamination was detected in only one sample (2.5%), which belonged to the warm season. None of the 20 raw milk samples collected during the cold season contained detectable concentrations of Hg. In total, just 5% of the warm-season raw milk samples (1 out of 20) were contaminated with the concentration of 8.47 µg/L. Among the 39 pasteurized milk samples examined, seven (17.95%) contained detectable level of Hg. While none of the 19 samples collected during the cold season exhibited measurable Hg levels, contamination was observed in the warm season, where 7 of 20 samples (35%) showed Hg concentrations ranging from 10.02 to 31.70 µg/L with a mean concentration of 25.19±7.96 µg/L. Only one of the 79 milk samples (1.3%) contained detectable levels of Cd. Among the 39 pasteurized milk samples, only a single sample (2.6%) was contaminated with Cd, which corresponded to one sample collected during the warm season (1 out of 20; 5%). No detectable levels of Pb or As were observed in any of the samples analyzed (Table 3).

Discussion

Milk and dairy products are widely consumed across various populations and represent a nutritionally important food group with relatively low cost. Due to their high consumption and bioaccumulative potential, these products are particularly susceptible to heavy metal contamination. The detection of heavy metals in milk is widely regarded as a

Table 3: The frequency of raw and pasteurized cow's milk samples contaminated with heavy metal marketed in Shahrekord, Iran in the cold and warm season.

Milk sample	Heavy metals					
	Season	No.	Hg	Pb	Cd	As
Raw	Cold	20	ND	ND	ND	ND
	Warm	20	1 (5%)	ND	ND	ND
	Total	40	1 (2.5%)	ND	ND	ND
Pasteurized	Cold	19	ND	ND	ND	ND
	Warm	20	7 (35.0%)	ND	1 (5.0%)	ND
	Total	39	7 (17.94%)	ND	1 (2.6%)	ND
Total		79	8 (10.13%)	ND	1 (1.3%)	ND

ND indicates the values below the detectable limit.

sensitive and integrative marker of environmental contamination, given that dairy animals readily incorporate pollutants from feed, water, soil, and ambient air into the lactational pathway (15-18).

Owing to their bioaccumulative nature and toxicological relevance, even low-level contamination of dairy matrices can warrant rigorous investigation. The present study offered a comprehensive assessment of heavy metal residues in bovine milk from Shahrekord, Iran. The distribution of trace metal contamination observed in this study highlighted the multifactorial nature of exposure pathways in dairy production systems. The pronounced seasonal divergence in Hg occurrence suggests that environmental dynamics such as increased volatilization, atmospheric deposition, and changes in forage composition during warmer months may substantially influence bioaccumulation in lactating cattle. The higher susceptibility of pasteurized milk to Hg presence also points to the complexity of industrial milk collection networks, where blending practices and broader geographic sourcing can mask localized contamination patterns and introduce heterogeneity that is not evident in farm-level raw milk sampling. Moreover, the negligible levels of Pb and As across all samples reflected a relatively low regional burden of these metals, consistent with areas lacking intensive mining or smelting activities; however, the isolated detection of Cd indicated that point sources linked to agricultural inputs or soil characteristics cannot be overlooked. Collectively, these findings underscore the importance of surveillance frameworks that incorporate seasonal variability, supply-chain structure, and environmental monitoring, as failure to account for such factors may result in a systematic underestimation of toxic metal exposure through dairy products (19).

The relatively elevated Hg concentrations in pasteurized milk may also be attributable to specific feed components including fishmeal and marine-derived inputs known to facilitate Hg biomagnification within the food chain (2, 19). These observations align with those of Bonyadian *et al.* (2022), who reported mean Hg levels of 13.14 and 16.5 µg/L in raw milk during warm and cold seasons in Shahrekord, Iran respectively (20). Collectively, these findings underscore the need for reinforced regulatory measures concerning feed composition, water quality assurance, and systematic monitoring of industrial emissions within the region. Cd was detected in only one sample. Potential contributors include the widespread use of Cd-bearing phosphate fertilizers, industrial emissions, or leaching from dairy processing equipment (21-23).

Given Cd's high toxicity, long biological half-life, and documented propensity for renal and hepatic accumulation, enhanced surveillance particularly in agricultural–industrial interface zones is imperative. Preventive actions should include systematic evaluation of processing equipment and phased replacement of materials susceptible to metal leaching. Neither Pb nor arsenic As was detected in our samples; however, their absence does not eliminate the need for continued monitoring. Considering their environmental persistence, cumulative toxicity, and extensive distribution in regions affected by traffic emissions, mining activities, and agrochemical inputs, sustained surveillance remains essential, especially in high-risk agro-industrial environments (24-27).

Although both national and international regulatory bodies have established measures to mitigate this issue, comprehensive milk-specific maximum limits for most heavy metals remain scarce, and existing regulations primarily target powdered milk and infant formula rather than general dairy products (28). Regulation of the European Union (EU) 2023/915 sets a maximum permitted concentration of Pb in raw and heated milk, as well as in milk used for dairy product manufacturing at 20 µg/L. This threshold aligns with the Iranian national standard and the Codex Alimentarius guidelines (20, 29, 30). In contrast, Cd lacks specific limits for milk itself; permissible concentrations are defined only for infant formula and foods for young children (5–20 µg/L) (31). Similarly, As thresholds are established solely for children's food products, with maximum levels of 20 µg/L in powdered formulations and 10 µg/L in liquid forms as specified by Regulations (EU) 2023/915 and 2023/465 (32). Despite the absence of As in dairy products, these regulations recognize milk and dairy products as potential contributors to dietary As exposure, highlighting the need for continuous monitoring (32).

Hg is regulated in milk only under Commission Regulation (EU) 2018/73, which specifies a maximum residue limit of 10 µg/L for total Hg compound. Notably, this restriction is framed under pesticide residue legislation rather than environmental contaminant standards (33). Similarly, the Iranian National Standard and the Codex Alimentarius do not provide explicit limits for Cd, Hg, or As in milk and dairy products, reflecting a global regulatory gap. The US Food and Drug Administration (FDA) likewise lacks specific standards for these metals in milk, relying instead on general toxicological assessments and manufacturing controls. Nevertheless, recent FDA guidance sets a Pb threshold of 10 µg/L for most processed infant foods and 20 µg/L for select categories, including some dairy products (34).

Regional studies in Iran highlighted both temporal and spatial variability of heavy metal residues in milk. Bonyadian *et al.* reported Pb levels of 60.72 µg/L in raw milk and 13.57 µg/L in pasteurized milk, and Cd concentrations of 2.87 and 1.03 µg/L, respectively, in Shahrekord, Iran (35). Another work by Bonyadian *et al.* determined the mean Hg levels of 13.14 and 16.5 µg/L in raw milk during the warm and cold seasons, respectively (20). Ansari *et al.* reported that in Shahrekord, the mean concentrations of Pb, Cd, As, and Hg were 7 and 14 µg/L, 11 and 5 µg/L, 8 and 11 µg/L, and 2 and 2 µg/L in spring and summer, respectively (36). Other investigations in Zanjan (37), East Azerbaijan (38), Gorgan (39), and Tabriz (40) similarly demonstrated wide variability in Pb, Cd, and As concentrations, reflecting differences in regional agricultural practices, feed and water sources, proximity to industrial facilities, livestock management systems, and seasonal influences (38). Collectively, these findings underscore the importance of considering environmental, seasonal, and supply-chain factors when assessing heavy metal contamination in milk. The diversity of reported levels and regulatory gaps highlights the need for nationally and internationally coordinated risk assessment frameworks and the establishment of comprehensive, milk-specific monitoring programs to ensure consumer safety.

Conclusion

This study identified Hg as the predominant heavy-metal contaminant in bovine milk from Shahrekord, Iran with heightened occurrence in pasteurized samples and during the warm season, reflecting both environmental and supply-chain influences. The sporadic detection of Cd, though limited, underscores the presence of localized point-source contamination that warrants targeted investigation and preventive action. While Pb and As were not detected, their potential for persistence and cumulative toxicity necessitates continued monitoring. Collectively, these findings highlighted the urgent need for milk-specific regulatory limits for critical heavy metals, seasonally informed monitoring strategies encompassing farm and industrial scales, stricter control of feed and processing inputs, and focused assessments at identified contamination hotspots. Implementing such measures would strengthen food safety frameworks, reduce consumer exposure, and provide a model for proactive heavy-metal risk management in dairy systems.

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Authors' Contribution

MA: Supervision, Formal analysis, Investigation, Visualization, Writing – original draft, Writing – review & editing. MR: Investigation, Formal analysis, Writing – review & editing. HM: Conceptualization, Formal analysis, Writing – review & editing.

Conflict of Interest

The authors declare that they have no conflict of interest.

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