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Seasonal Occurrence and Quantification of Pb, Cd, and Hg in Commercial Pasteurized Milk Brands Distributed in Tehran

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Abstract

Milk is a major dietary component, particularly for vulnerable population groups, which makes monitoring its contamination by heavy metals essential for public health protection. This study investigated the concentrations of lead (Pb), cadmium (Cd), and mercury (Hg) in 40 samples from 10 commercial pasteurized/sterilized milk brands available in the Iranian market, collected across the four seasons in 2023. Heavy metals were quantified using validated standard methods, with a certified reference material (CRM) and reagent blanks analyzed to verify analytical accuracy and precision.

Of the 40 samples, Pb was undetectable in 3 (7.5%), below the maximum permissible limit (MPL) of 0.02 mg/kg in 36 (90%), and exceeded the MPL in only one sample (2.5%). Seasonal trends revealed higher average Pb levels in spring and autumn relative to summer and winter. Cd, concentrations were undetectable in 26 samples (65%) and measurable but below the MPL in 14 (35%). Hg was undetectable in 18 samples (45%) and quantifiable, albeit at low levels, in 22 (55%). Overall, Pb was the predominant contaminant, while Cd and Hg occurred less frequently and at negligible levels.

Keywords pasteurized milk; heavy metals; matrix digestion; atomic absorption spectrometry

1. Introduction

Milk is a fundamental component of human nutrition, especially for vulnerable age groups such as infants and children. However, due to environmental pollution and industrial activities, milk can become contaminated with heavy metals, which pose significant health risks due to their toxicity and bioaccumulative nature [1, 2]. Among these contaminants, lead (Pb), cadmium (Cd), and mercury (Hg) are of particular concern owing to their adverse effects on multiple organ systems including the nervous system, kidneys, and liver [3].

Heavy metals enter the milk supply chain through var-

ious pathways, including contamination of animal feed and drinking water, environmental deposition from industrial effluents, and during milk processing and storage [4, 5]. Regulatory agencies worldwide have established maximum permissible limits (MPLs) for these metals in milk to protect public health. For instance, the Codex Alimentarius Commission [6], the European Commission [7] and Iranian National Standardization Organization (INSO) [8] have set the maximum allowable lead concentration in milk at 0.02 mg/kg. Similarly, cadmium limits vary across countries, with FAO/WHO [9] recommending 0.01 mg/kg and other standards ranging from 0.0026



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mg/kg (IDF) to 0.02 mg/kg in Turkey and Malaysia [10]. In India and Egypt, permissible levels for lead and cadmium differ slightly, reflecting regional risk assessments [5, 11].

In Iran, multiple studies conducted between 2008 and 2024 have reported lead concentrations in milk samples ranging from below detection limits up to 0.369 mg/kg, with an average around 0.014 mg/kg [12-17]. Notably, higher lead levels have been observed in industrial regions such as Khuzestan and Hamedan, occasionally exceeding national standards [16, 18]. Cadmium levels in Iranian milk have similarly been documented within a range of 0.0001 to 1.0 mg/kg, with mean values approximately 0.003 mg/kg [13, 14, 16-18]. These findings underscore the impact of rapid industrialization and inadequate environmental controls in certain regions.

Mercury contamination, although less extensively studied, has been detected in milk samples from Iran and other countries at generally low concentrations within safety limits, except for isolated high values reported in Pakistan [19-22]. Given the toxicological implications of mercury exposure, further monitoring is warranted.

Effective management and reduction of heavy metal contamination in milk require multifaceted approaches, including stringent regulation of industrial discharges, monitoring of animal feed and water quality, and application of advanced purification technologies for wastewater treatment [23, 24]. Additionally, ensuring hygienic practices during milk processing and storage is critical to minimize contamination risks. Despite these efforts, persistent contamination remains a concern, particularly in developing countries. Therefore, continuous surveillance of heavy metal levels in milk and dairy products is essential to safeguard public health. This study aims to provide a comprehensive assessment of heavy metal concentrations in milk distributed in Tehran, contributing to the understanding of contamination patterns.

2. Experimental

2.1. Chemicals and Reagents

All chemicals used were of analytical grade or higher purity. Concentrated nitric acid (HNO_3 , 65% w/w), hydrochloric acid (HCl , 37% w/w), and hydrogen peroxide (H_2O_2 , 30% w/w) were obtained from Merck (Germany). A 6 mol.L⁻¹ HCl solution was prepared by diluting 500 mL of concentrated HCl to 1 L with ultrapure deionized water.

A freshly prepared 0.3% (w/v) sodium borohydride (NaBH_4) solution stabilized with 0.5 g NaOH in 100 mL deionized water was used as the reducing agent for cold vapor/hydride-generation measurements of mercury.

Certified 1000 mg·L⁻¹ stock standard solutions of Pb, Cd, and Hg (ChemLab, Belgium) were used to prepare

calibration standards. Working standards were prepared daily by serial dilution in deionized water. All containers used for sample and standard preparation were pre-cleaned with 10% HNO_3 and thoroughly rinsed with deionized water.

The certified reference material ERM®-BD151 (skimmed milk powder, European Reference Materials, 2013/2017) was used for validation, providing certified concentrations of 0.207 ± 0.014 mg/kg for Pb, 0.106 ± 0.013 mg/kg for Cd, and 0.52 ± 0.04 mg/kg for Hg, among other elements.

2.2. Instruments and Apparatus

Metal analyses were performed using two atomic absorption spectrometers: a Varian 220FS equipped with a graphite furnace atomizer and SPECTRAA control software for the determination of Pb and Cd, and a Varian SpectrAA 10 Plus coupled with a VGA-77 continuous-flow hydride-generation system for Hg measurement under cold vapor conditions. Closed-vessel acid digestion was carried out using a microwave digestion system, while an electric hot plate and a temperature-controlled laboratory oven were employed for auxiliary heating steps. All sample digestions and dilutions were performed in acid-washed, high-density polyethylene (HDPE) vessels and volumetric flasks to minimize contamination.

2.3. Sampling

Milk samples were collected from ten different commercial brands produced in Tehran Province during the four seasons of 2023 (spring, summer, autumn, and winter). In each season, a total of ten samples were obtained, representing various fat contents and production dates. For consistency, all samples were collected directly from retail outlets and immediately transported to the laboratory under refrigerated conditions. Each sample was assigned a unique identifier, and its production date, fat content, and batch information were recorded. The sampling strategy aimed to provide a representative overview of seasonal variations in lead, cadmium, and mercury concentrations in pasteurized milk. All samples were stored at 4 °C until further preparation and analysis.

2.4. Closed-Vessel Acid Digestion Procedure for the Determination of Lead and Cadmium

Milk contains substantial organic components that interfere with atomic absorption measurements; thus, complete matrix destruction is essential. Dry ashing according to Iranian National Standard INSO. 9266 [25] was initially evaluated but was found insufficient to eliminate matrix effects, resulting in poor accuracy and repeatability for

CRM and spiked samples. Consequently, closed-vessel microwave digestion following BS EN 13805:2014 [26] was employed.

Approximately 5.00 g of each homogenized milk sample was accurately weighed (± 0.0001 g) into polyethylene digestion vessels. Then, 5 mL concentrated HNO₃ and 1 mL H₂O₂ (30%) were added. The vessels were tightly sealed and placed in an oven at 200 °C for 2 h.

After cooling, the digests were diluted with deionized water, filtered when necessary, and quantitatively transferred into 10 mL volumetric flasks and made up to volume. The resulting solutions were analyzed using graphite furnace atomic absorption spectrometry (GF-AAS) for Pb and Cd determination. The same digestion protocol was applied to the certified reference material (CRM), except that 0.30 g of CRM was weighed according to its certified concentration.

2.5. Microwave-Assisted Digestion Followed by Cold Vapor AAS for Mercury Determination

Approximately 3.00 g of each milk sample was weighed into microwave digestion vessels. To each vessel, 2 mL concentrated HNO₃ and 1 mL H₂O₂ (30%) were added. Samples were digested using microwave irradiation at 1500 W, at a controlled temperature of 180 °C for 30 minutes.

After cooling to room temperature, the digests were quantitatively transferred to volumetric flasks and diluted to a final volume of 50 mL with deionized water. For method verification, an additional digestion series was performed using 1.5 g sample mass with a final dilution volume of 25 mL. Mercury concentrations were measured using atomic absorption spectrometry equipped with a hydride-generation unit (VGA-77) under cold vapor conditions.

2.6. Quality Assurance and Quality Control (QA/QC)

To assess method accuracy and precision, certified reference material (CRM) was processed alongside test samples. Acceptable recovery ranges followed the Codex Alimentarius guideline (70–120%). For mercury, recovery was also evaluated using spiked samples.

Instrumental limits of detection (LOD) and quantification (LOQ) were established on the basis of the standard deviation obtained from at least five consecutive measurements of a reagent blank and the slope of the calibration curve, using the following equations:

$$LOD = \frac{3 \times SD_b}{m} \quad \text{Eq (1)}$$

$$LOQ = \frac{10 \times SD_b}{m} \quad \text{Eq (2)}$$

where SD_b and m respectively represent the standard

deviation of the blank measurements and the slope of the calibration curve. Although minor day-to-day fluctuations in absorbance responses were observed, routine performance verification ensured that calibration stability remained within the laboratory's predefined confidence limits. Consequently, the calculated LOD and LOQ values were deemed reliable for the analytical conditions employed in this study. The methods' LOQs were calculated by spiking a blank sample at estimated LOQs and evaluating the recovery and precision of the results. Concentration values falling below the LOQ were reported as ">LOQ," whereas those below the LOD were designated as not detected (ND). A reagent blank (prepared using all digestion and analytical reagents in the absence of sample matrix) was processed with each batch of samples. When the analytical signal of a sample was lower than that of the corresponding blank, the result was reported as ND.

3. Results and Discussion

Results of CRM recovery, spiking tests, LOD/LOQ values, and repeatability assessments are presented in Table 1. The calculated instrumental and method limits of quantification (LOQs) were experimentally verified by spiking the samples at the theoretically determined concentrations and evaluating the recovery and repeatability of the obtained results.

Table 1. Analytical figure of merits of the extraction and determination method of Pb, Cd and Hg in Milk

		Heavy metal		
		Pb	Cd	Hg
Figures of merit				
Calibration	linear dynamic range (LDR) (μg. L ⁻¹)	6 – 40	0.5 – 5	1 – 10
	R ²	0.9995	0.9953	0.9963
	LOD (μg. L ⁻¹)	0.91	0.07	0.29
	LOQ (μg. L ⁻¹)	3.0	0.25	1.00
Method LOQ _A (mg. kg ⁻¹)		0.006	0.001	0.02
Average Recovery		87.6%	98.2%	107.2%
CRM sample	Assigned value (mg. kg ⁻¹)	0.207	0.106	0.52
	Determined value (mg. kg ⁻¹)	0.181	0.104	0.55
Repeatability (RSD%) (n=5)		7.93%	4.64%	5.19%

3.1 Real sample analysis results

The concentrations of heavy metals (lead, cadmium, and mercury) in pasteurized milk samples collected from Tehran Province in 2023 were determined using the described methods, with results summarized in Tables 2. Recovery corrections were applied based on CRM measurements. For lead, the mean recovery was 87.6% from four replicate CRM analyses, while cadmium required no net adjustment due to approximately 100% recovery. Mercury results were corrected using a mean recovery factor of 107%. In all cases, no recovery correction was applied

Table 2. Seasonal Heavy Metal Concentrations (Pb, Cd, Hg) in Commercial Milk Samples (Tehran, 2023)

NO.	Brand Code	Fat Content (%)	Recovery-Adjusted Pb (mg/kg)	Results vs. MPL Pb (0.02 mg/kg)	Cd (mg/kg)	Results vs. MPL Cd (0.0026 mg/kg)	Recovery-Adjusted Hg (mg/kg)
Spring							
1	B1	Semi-skimmed milk (1.5-2.5%)	<LOQ	Pass	ND	Pass	ND
2	B2	Whole milk ($\geq 3\%$)	0.013	Pass	0.0013	Pass	0.022
3	B3	Super milk ($\sim 2.5\%$)	0.015	Pass	ND	Pass	ND
4	B4	Whole milk	0.023	Pass	ND	Pass	<LOQ
5	B5	Low-fat milk (<1.5%)	0.006	Pass	ND	Pass	<LOQ
6	B6	Low-fat milk	ND	Pass	ND	Pass	<LOQ
7	B7	Whole milk	<LOQ	Pass	ND	Pass	<LOQ
8	B8	Low-fat milk	0.035	Fail	<LOQ	Pass	<LOQ
9	B9	Whole milk	<LOQ	Pass	ND	Pass	<LOQ
10	B10	Low-fat milk	0.024	Pass	<LOQ	Pass	<LOQ
Summer							
1	B1	Semi-skimmed milk	0.008	Pass	ND	Pass	<LOQ
2	B2	Whole milk	<LOQ	Pass	ND	Pass	ND
3	B3	Super milk	<LOQ	Pass	ND	Pass	<LOQ
4	B4	Whole milk	0.009	Pass	<LOQ	Pass	ND
5	B5	Low-fat milk	0.015	Pass	ND	Pass	ND
6	B6	Low-fat milk	<LOQ	Pass	ND	Pass	0.022
7	B7	Whole milk	0.013	Pass	<LOQ	Pass	0.015
8	B8	Low-fat milk	0.011	Pass	<LOQ	Pass	ND
9	B9	Whole milk	ND	Pass	ND	Pass	ND
10	B10	Low-fat milk	0.014	Pass	<LOQ	Pass	ND
Autumn							
1	B1	Semi-skimmed milk	0.010	Pass	ND	Pass	<LOQ
2	B2	Whole milk	<LOQ	Pass	ND	Pass	ND
3	B3	Super milk	<LOQ	Pass	ND	Pass	<LOQ
4	B4	Whole milk	<LOQ	Pass	ND	Pass	<LOQ
5	B5	Low-fat milk	0.012	Pass	<LOQ	Pass	ND
6	B6	Low-fat milk	0.012	Pass	ND	Pass	ND
7	B7	Whole milk	0.019	Pass	<LOQ	Pass	ND
8	B8	Low-fat milk	0.020	Pass	<LOQ	Pass	ND
9	B9	Whole milk	0.017	Pass	ND	Pass	ND
10	B10	Low-fat milk	0.022	Pass	<LOQ	Pass	ND
Winter							
1	B1	Semi-skimmed milk	<LOQ	Pass	<LOQ	Pass	ND
2	B2	Whole milk	0.008	Pass	ND	Pass	ND
3	B3	Super milk	0.018	Pass	ND	Pass	ND
4	B4	Whole milk	<LOQ	Pass	ND	Pass	0.024
5	B5	Low-fat milk	0.008	Pass	ND	Pass	0.016
6	B6	Low-fat milk	0.011	Pass	<LOQ	Pass	<LOQ
7	B7	Whole milk	ND	Pass	ND	Pass	<LOQ
8	B8	Low-fat milk	0.009	Pass	ND	Pass	<LOQ
9	B9	Whole milk	<LOQ	Pass	ND	Pass	0.024
10	B10	Low-fat milk	0.009	Pass	<LOQ	Pass	<LOQ

to samples with instrumental readouts below the LOQ to prevent bias. For samples below the LOQ in seasonal averaging (e.g., for lead), values were assigned as zero to avoid overestimation.

Overall, the milk samples exhibited low levels of heavy metal contamination, with most concentrations complying with regulatory limits. For lead, the majority of samples were within permissible thresholds, except for one sample from the B8 brand in the spring that exceeded the limit of 0.020 mg/kg. Seasonal mean concentrations varied slightly, with the lowest observed in winter (0.006 mg/kg) and higher values in spring and autumn (both 0.011 mg/kg); However, pairwise statistical comparisons between seasons using Student's t-test showed

no statistically significant differences ($p > 0.05$), indicating the absence of a systematic seasonal effect. No clear correlation was found between milk fat content and lead levels, and while B8 and B10 brands showed marginally higher means (Figure 1-b), these remained below limits except for the noted outlier. Approximately 37.5% of samples had lead concentrations below the limit of detection (LOD) or LOQ, 20% were ≤ 0.010 mg/kg, and 10% exceeded the threshold, though only one was confidently non-compliant after accounting for method repeatability and analytical uncertainty. Inter-annual consistency was lacking, suggesting sporadic rather than persistent contamination events.

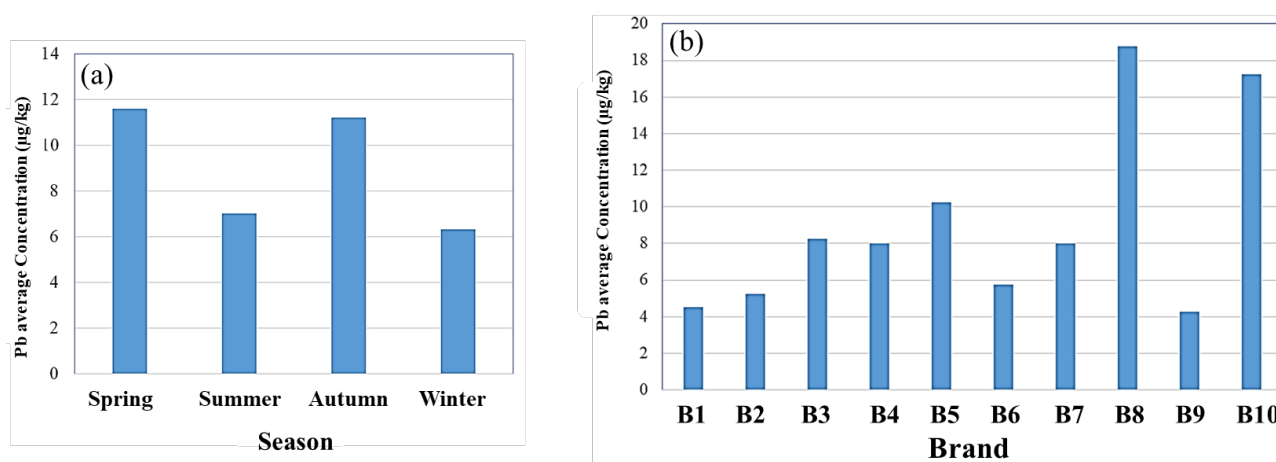


Fig 1. (a) Seasonal variations in the average Pb concentration in commercial milk samples, and (b) comparison of average Pb levels among the ten studied brands. For clarity of presentation, Pb concentrations are expressed in µg/kg. Values below the LOQ were excluded from the calculations.

Although seasonal differences were not statistically significant, minor fluctuations in mean metal concentrations may reflect natural variability associated with environmental and production-related factors. Seasonal changes in cattle feed composition, particularly the relative contribution of fresh forage versus stored feed, can influence trace metal intake and subsequent transfer into milk. Additionally, variations in environmental exposure, including soil–plant transfer, water sources used for livestock, and atmospheric deposition, may contribute to subtle seasonal variability without producing a consistent or reproducible seasonal trend [27, 28]. Differences in milk processing and supply-chain dynamics across seasons, such as batch mixing, storage duration, and sourcing from multiple farms, may further dilute or obscure potential seasonal signals.

Cadmium levels were uniformly below the maximum allowable limit across all samples, with most falling below

the LOQ in digested solutions, indicating negligible environmental exposure. These findings are consistent with prior studies on cadmium in Iranian milk. Notably, three of the four B10 brand samples contained detectable but insignificant cadmium residues, posing no health concerns.

Unexpectedly, mercury was detected above the LOQ in several samples, though a measurable absorbance in the reagent blank was subtracted from all readings for accuracy. In most cases, levels were below the instrumental LOD but above the qualitative detection threshold, signifying trace presence. Six samples exceeded the LOQ, but these values were marginally above the threshold and analytically negligible. Overall mercury contamination remained minimal, aligning with the low bioaccumulation potential in dairy products under typical conditions, and all levels were far below regulatory limits. The sporadic detections may arise from environmental or processing factors.

In summary, the analyzed milk samples from Tehran Province demonstrated compliance with heavy metal standards, with isolated instances of elevated lead likely attributable to episodic contamination. These results underscore the generally low risk of heavy metal exposure through pasteurized milk in the region, though ongoing monitoring is recommended to address potential variability.

4. Conclusion

This study provides a comprehensive assessment of lead (Pb), cadmium (Cd), and mercury (Hg) levels in ten commercial pasteurized and sterilized milk brands distributed in Tehran across four seasons in 2023. The findings indicate that the choice of sample digestion method significantly affects Pb quantification, with spiked sample recoveries proving unreliable, while CRM ensured accurate and reproducible measurements. This limitation was not observed for Cd or Hg analyses. In mercury determination, blank sample contributions were notable, emphasizing the necessity of subtracting blank absorbance to obtain accurate results.

Lead was identified as the primary metallic contaminant in milk, though the vast majority of samples remained below the regulatory limit (0.02 mg/kg). Cadmium levels were consistently low and within permissible limits in all samples. Mercury concentrations were generally below the quantification limit but mostly above the detection limit, suggesting a potential early indication of concern regarding Hg contamination in milk. No consistent relationships were observed between heavy metal concentrations and milk brand, fat content, or sampling season.

Overall, these results underscore the importance of rigorous analytical protocols, including appropriate digestion methods and quality control measures, for reliable heavy metal monitoring in milk. Continuous surveillance is recommended to safeguard public health, particularly with regard to emerging mercury contamination.

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Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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