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Original Article

Assessment of arsenic, cadmium, and lead in biological and environmental matrices in dairy production systems in Isfahan, Iran

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Abstract

Background: Contamination with heavy metals such as arsenic (As), cadmium (Cd), and lead (Pb) in dairy production systems poses significant public health risks due to their bioaccumulative nature. **Aims:** This study investigated the levels of As, Cd, and Pb in multiple matrices within dairy farms in Isfahan Province, Iran, and assessed associated health risks, particularly among children. **Methods:** A total of 180 samples (water, milk, feed, feed supplements, manure, and tail hair) were collected from 30 industrial dairy farms. Samples were stratified by herd size, milk yield, and proximity to industrial zones. Metal concentrations were analyzed using atomic absorption spectroscopy. Hazard quotients (HQs) for Pb exposure through milk consumption were estimated. **Results:** Pb levels in raw milk exceeded national standards (0.02 mg/L) in over 50% of farms, particularly in small, and those located near industrial areas. Cd and As levels in milk were generally below detection limits, though As, Cd, and Pb exceeded regulatory limits in tail hair on a significant number of farms. Strong and significant correlations were observed between Pb levels in milk and those in supplements (r_s=0.68), manure (r_s=0.81), and tail hair (r_s=0.59), suggesting systemic exposure. HQs for Pb intake via milk remained below 1 in both adults (0.027) and children (0.173), though children faced higher relative risks. **Conclusion:** Contamination in small farms near industrial zones underscores the need for more stringent feed quality regulations and more frequent biomonitoring using non-invasive markers like tail hair, which has proven effective as a biomarker for chronic metal exposure.

Key words: Biomonitoring, Dairy production, Hazard quotient, Heavy metals, Iran

Introduction

Heavy metals such as arsenic (As), cadmium (Cd), and lead (Pb) are introduced into the environment through natural processes, such as volcanic activity and rock weathering, and also anthropogenic activities including industrial operations, mining, and agriculture. These toxic elements possess high bioaccumulative potential and can pose serious health risks to both animals and humans through the consumption of contaminated food products such as milk (Briffa *et al*., 2020). Livestock raised near industrial zones or human settlements can act as effective biomonitors of environmental pollution, thus comprehensive monitoring in such regions is essential to ensure the safety of water and food supplies (Ravanipour *et al*., 2021; Kazemi Moghaddam *et al*., 2022). Biomonitoring refers to the systematic measurement of environmental pollutants or their biological effects in living organisms, with the aim of assessing long-term exposure (Perillo *et al*., 2021). In this study, the term biological matrices refers to biological samples such as raw milk, tail hair, and feces, which are utilized in biomonitoring to assess internal exposure to heavy metals in dairy cattle (Esteban and Castaño, 2009). In this context, biological matrices such as hair, milk, blood, or feces serve as valuable tools for detecting internal contamination levels in animals and are particularly useful in chronic environmental studies.

In Iran, several studies have reported concentrations of heavy metals in the drinking water and feed of livestock which sometimes exceeding the permissible limits established by the Institute of Standards and Industrial Research of Iran (ISIRI) (Shahbazi *et al*., 2016; ParsiMehr *et al*., 2020; Ravanipour *et al*., 2021; Kazemi Moghaddam *et al*., 2022). For instance, the ISIRI thresholds for heavy metals in drinking water are 0.01 mg/L for As and Pb, and 0.003 mg/L for Cd (ParsiMehr *et al*., 2020). Dairy Production Systems encompass a broader concept than merely “dairy farms”. These systems include not only the physical location of animal housing but also a wide range of environmental, managerial, nutritional, and sanitary factors influencing milk production. From water sources and feed composition to proximity to industrial sites, farm management quality, and herd health status, these factors collectively shape the production environment (Lemma *et al*., 2018). Within such a complex framework, simultaneous monitoring of environmental components (such as water, feed, supplements, and manure) and biological matrices (like milk and tail hair) can provide a comprehensive picture of contamination levels and their potential sources. This approach is crucial for accurately assessing risks to both animal and human health.

Isfahan province is one of the primary dairy production hubs in Iran, hosting numerous large-scale industrial farms situated near steel and petrochemical industries. This geographic proximity may pose increased risk of contamination to the milk production chain. Previous studies in this region have confirmed the presence of Pb and Cd contamination in soil, forage, and water, highlighting the intricate relationship between environmental conditions and the transfer of pollutants into animal products (Kamkar *et al*., 2010; Parsi Mehr *et al*., 2020; Perillo *et al*., 2021). Despite growing awareness, most national studies have focused on a single matrix, typically milk, without incorporating broader environmental samples such as water, feed, or manure, or biological indicators like tail hair (Raeeszadeh *et al*., 2022; Nassiri *et al*., 2024; Sadeghian *et al*., 2024). Additionally, limitations such as small sample sizes or insufficient consideration of proximity to industrial zones have restricted the generalizability of results. Among various biological matrices, tail hair in cattle represents a novel and non-invasive biomarker for chronic exposure to heavy metals. This stable tissue reflects long-term accumulation patterns of elements and is particularly valuable in environmental toxicology studies (Roggeman *et al*., 2013; Katz, 2019). Although validated internationally, this method remains underutilized to assess chronic exposure in Iranian industrial dairy farms.

This study was designed to address the aforementioned gaps by employing a multi-matrix biomonitoring approach across 30 industrial dairy production systems in Isfahan province. Farms were classified based on herd size, milk yield, and distance from industrial areas to evaluate how environmental and managerial factors affect contamination levels. Sampling was performed on six matrices: water, raw milk, feed, supplements, manure, and tail hair. Additionally, the hazard quotient (HQ) for Pb intake via milk consumption, particularly in children, was calculated to assess potential public health risks.

The main objectives of this study were as follows: To determine the concentrations of As, Cd, and Pb in six key matrices: water, feed, supplements, raw milk, manure, and cattle tail hair in industrial dairy production systems in Isfahan province. To analyze the relationship between contamination levels and farm characteristics, including herd size, milk yield, and proximity to industrial zones. To evaluate the effectiveness of cattle tail hair as a non-invasive biomarker for chronic heavy metal exposure and finally to estimate the health risk associated with Pb intake through milk consumption, especially in children.

Materials and Methods

Study area and farm classification

This cross-sectional study was conducted on 30 industrial dairy farms located in Isfahan province, Iran. Farms were randomly selected from the official registry to ensure a representative distribution in terms of herd size, milk production levels, and proximity to industrial zones. Ethical approval for the study was obtained from the Animal Ethics Committee of Shiraz University (IACUC No.: 4687/63). To facilitate analysis of environmental and operational factors influencing heavy metal contamination, farms were classified into subgroups based on three criteria: herd size (large: ≥600 cows; small: <600 cows), milk yield (high: ≥39 kg/day; low: <39 kg/day), and proximity to industrial zones (“Near” farms are defined as those located at a distance of ≤4 km from industrial areas, major roads, or urban centers, while “far” farms are situated at a distance of >4 km from above mentioned areas).

Sample collection and handling

Between September 2020 and May 2021, six types of samples were collected from each farm, yeilding in a total of 180 samples. The matrices included were drinking water (1 L, pooled from troughs and sources), raw milk (500 ml, pooled from three milking sessions), feed (1 kg, mixed ration), dietary supplements (500 g, pooled from available additives), manure (1 kg, from barn floors), and tail hair (50 g, from three randomly selected cows per barn). All samples were labeled and transported under cooled conditions to the analytical laboratory, where they were prepared and processed for heavy metal analysis.

Sample preparation and analytical procedure

Sample digestion was performed following standardized protocols. Water and raw milk samples were digested using a nitric acid-hydrogen peroxide mixture (HNO_3:H_2O_2 = 4:1, v/v). Briefly, 5 ml of each sample was mixed with 8 ml of concentrated nitric acid (HNO_3, 65-70%) and 2 ml of 30% (v/v) hydrogen peroxide (H_2O_2). The mixture was heated at 120°C on a hot plate until a clear and colorless solution was obtained. After cooling to room temperature, the digest was filtered through Whatman No. 42 filter paper and diluted to a final volume of 25 ml with ultrapure deionized water prior to analysis (AOAC, 2019). Dietary supplements were digested using a nitric acid-perchloric acid mixture (HNO_3:$HClO_4$ = 3:1, v/v). Approximately 0.5 g of homogenized sample was mixed with 6 ml concentrated HNO_3 and 2 ml $HClO_4$ and heated gradually to 150180°C until a clear solution was obtained. After cooling, the digest was diluted with deionized water to a fixed volume prior to analysis (AOAC, 2019). Tail hair samples were thoroughly washed, air-dried, and digested using concentrated HNO_3. The concentrations of As, Cd, and Pb were determined using graphite furnace atomic absorption spectrometry (AAS) with Zeeman background correction (Varian SpectrAA 220Z, Varian Inc., USA), in accordance with the methodology described by Perillo *et al.* (2021). Instrument calibration was conducted using certified metal standards, with calibration curves demonstrating coefficients of determination (R^2) exceeding 0.995. Each analytical batch included certified reference materials and procedural blanks to ensure data quality, and all samples were analyzed in triplicate. The detection threshold of the device was 0.001 mg/L for water and milk, 0.01 mg/kg for feed, manure, and tail hair, and for supplements, 0.001 mg/kg for As and 0.01 mg/kg for Cd and Pb. Total recovery rates ranged from 85% to 110%, consistent with established quality control criteria (Haswell, 1991; Peters *et al.*, 2003). The use of Zeeman background correction further enhanced measurement accuracy by minimizing spectral interferences.

Health risk assessment of Pb exposure via milk consumption

The non-carcinogenic health risk associated with Pb exposure through milk consumption was assessed using the HQ method, following the approach described by Sobhanardakani *et al.* (2016) and Dashtizadeh *et al.* (2019). The HQ was calculated using the equation:

$$HQ = \frac{EF \times ED \times FIR \times C}{RFD \times Bw \times AT} \times 10^{-3}$$

Where,

EF: Is the exposure frequency (365 days/year)

ED: Is the exposure duration (30 years for adults and 6 years for children)

FIR: Is the daily milk ingestion rate (175 ml/day for adults and 200 ml/day for children)

C: Is the mean Pb concentration in raw milk obtained from this study (0.0379 mg/L)

RfD: Is the oral reference dose for Pb (0.0035 mg/kg/day)

BW: Is the average body weight (70 kg for adults and 15 kg for children)

AT: Is the averaging time (equal to ED × 365 days), as established by Barnes *et al.* (1988)

An HQ value below 1 suggests that the estimated level of Pb exposure is unlikely to pose significant health risks.

Statistical analysis

Statistical analyses were conducted using IBM SPSS Statistics version 27.0 (Armonk, NY, USA). The distribution of heavy metal concentrations was assessed using the Shapiro-Wilk test, which confirmed non-normality. Therefore, non-parametric statistical tests were employed throughout. The Kruskal-Wallis test was used to compare metal concentrations across farm categories (based on herd size, milk yield, and proximity to industrial areas), followed by Dunn's test for pairwise comparisons where appropriate. The Mann-Whitney U-test was applied for two-group comparisons. Associations among heavy metal concentrations across matrices were evaluated using Spearman's rank correlation coefficients. Chi-square (χ^2) tests were used to analyze relationships between categorical variables, such as proximity to industrial zones and exceedance of ISIRI standards (Table 1). All tests were conducted at a significance level of 0.05.

Results

A total of 180 samples were collected from 30 industrial dairy farms in Isfahan province, comprising six matrices: drinking water, raw milk, feed, dietary supplements, manure, and tail hair. Farms were classified by herd size, milk yield, and proximity to industrial zones. Concentrations of As, Cd, and Pb were evaluated against the ISIRI maximum permissible limits: such as 0.01 mg/L for As in water, 0.003 mg/L for Cd in water, and 0.02 mg/L for Pb in raw milk (Table 1). Heavy metal levels exceeded ISIRI standards in several matrices. Pb in raw milk violated the allowable limit in 16 of 30 farms (53.3%). Elevated As concentrations were detected in drinking water at one small farm near industrial zones. Cd levels exceeded the threshold in feed from one farm and in tail hair across 29 of 30 farms, indicating chronic exposure. Summary of exceedance data by farm category are presented in Table 2, and concentration comparisons across farm characteristics are shown in Tables 3 and 4.

Arsenic (As)

Arsenic was undetectable in all raw milk samples and all drinking water samples except one, where a small farm near industrial zones recorded a value of 0.020 mg/L, exceeding the ISIRI limit (0.01 mg/L). Supplements from a single low-yielding small farm near industrial zones also showed elevated As (0.015 mg/kg). Arsenic concentrations in manure were significantly higher in high-yielding farms (mean±SD: 0.440 ± 0.396 mg/kg, median: 0.412 mg/kg) compared to low-yielding farms (mean±SD: 0.142 ± 0.410 mg/kg, median: 0 mg/kg; P=0.006). Tail hair samples from nine farms (four large and five small) exceeded the reference threshold of 0.05 mg/kg. Although no significant difference in As levels were found based on herd size or milk yield (P>0.05), a moderate correlation was observed between As levels in tail hair and manure (r_s=0.41, P=0.025).

Cadmium (Cd)

Cadmium was below the detection limit (0.001 mg/L) in all water and raw milk samples. However, feed from one small farm located near industrial zones contained 0.58 mg/kg of Cd that was bellow the ISIRI limit (1

Table 1: Regulatory limits for arsenic (As), cadmium (Cd), and lead (Pb) in cattle-related products and environmental matrices based on Iranian, European, and American standards

Standard values/metals	Water (mg/L)			Raw milk (mg/L)			Feed (mg/kg)			Supplements (mg/kg)			Manure (mg/kg)			Hair (mg/kg)		
	As	Cd	Pb	As	Cd	Pb	As	Cd	Pb	As	Cd	Pb	As	Cd	Pb	As	Cd	Pb
Iranian (ISIRI)	0.01	0.003	0.01	-	0.02	0.02	2	1	3	10	10	5	20	20	25	0.05	0.02	0.5
European (EFSA)	0.01	0.003	0.01	0.5	0.01	0.02	2	0.5	5	10	10	10	30	10	100	0.015	0.02	0.5
American (FDA)	0.01	0.003	0.01	0.5	0.05	0.02	2.68	1.2	10	8.54	4.34	57	45	20	100	0.02	0.02	0.2

Table 2: Number of farms exceeding regulatory limits for arsenic (As), cadmium (Cd), and lead (Pb) by farm characteristics in Isfahan province

Category	Subgroup	n	Water (As)	Supplements (As)	Supplements (Pb)	Raw milk (Pb)	Hair (As)	Hair (Cd)	Hair (Pb)
Herd size	>600 head	15	0	0	0	6	4	15	0
	≤600 head	15	1	1	2	10	5	14	3
Distance *	Near	19	1	1	1	10	6	18	2
	Far	11	0	0	1	6	3	11	1
Milk production	≥39 kg/day	15	1	1	2	8	5	15	2
	<39 kg/day	15	0	0	0	8	4	14	1

* Distance: "Near" farms are defined as those located at a distance of ≤4 km from industrial areas, major roads, or urban centers, while "far" farms are situated at a distance of >4 km

Table 3: Comparison of arsenic (As), cadmium (Cd), and lead (Pb) concentrations in water (mg/L), feed (mg/kg), and supplements (mg/kg) across dairy farms in Isfahan province, stratified by farm characteristics

Category	Subgroup	n	Water (As)	Feed (Cd)	Feed (Pb)	Supplements (As)	Supplements (Cd)	Supplements (Pb)
Herd size	>600 head	15	nd***	0.17±0.15 (0.20)	0.28±0.23 (1.884)	0.61±0.75 (0)	0.34±0.36 (0.12)	1.82±1.04 (0.44)
	≤600 head	15	0.02	0.18±0.16 (0.15)	0.72±0.17 (0.73)	1.53±3.21 (0.47)	0.45±0.53 (0.19)	2.77±2.75 (1.96)
	P-value			0.016	0.001	NS	NS	NS
Distance *	Near	19	0.02	0.21±0.16 (0.18)	0.56±0.31 (0.61)	1.48±2.88 (0.49)	0.53± 0.52 (0.26)	2.37±2.39 (1.74)
	Far	11	nd	0.11±0.12 (0.07)	0.39±0.28 (0.45)	0.36±0.25 (0.31)	0.17±0.03 (0.16)	2.17±1.55 (2.27)
	P-value			0.067	0.076	0.015	0.012	NS
Milk production	≥39 kg/day	15	nd	0.22±0.13 (0.18)	0.53±0.34 (0.51)	0.69±0.73 (0.48)	0.45±0.48 (0.22)	2.61±2.58 (1.99)
	<39 kg/day	15	0.02	0.13±0.16 (0.07)	0.47±0.27 0.52)	1.45±3.24 (0.31)	0.34±0.43 (0.16)	1.98±1.5 (1.54)
	P-value			0.016	NS**	NS	NS	NS

Values are presented as mean ± standard deviation (median). Statistical comparisons were performed between subgroups using non-parametric tests. * Distance: "Near" farms are defined as those located at a distance of ≤4 km from industrial areas, major roads, or urban centers, while "far" farms are situated at a distance of >4 km, ** NS: Indicates non-significant differences (P>0.05), and *** nd: Non detected. Bolded P-values indicate statistically significant differences (P<0.05)

Table 4: Comparison of arsenic (As), cadmium (Cd), and lead (Pb) concentrations in raw milk (mg/L), manure (mg/L), and tail hair (mg/kg) across dairy farms in Isfahan province, stratified by farm characteristics

Category	Subgroup	n	Raw milk (Cd)	Raw milk (Pb)	Manure (As)	Manure (Cd)	Manure (Pb)	Hair (As)	Hair (Cd)	Hair (Pb)
Herd size	>600 head	15	0.02±.002 (0.002)	0.02±.01 (0.02)	0.21±0.34 (0)	0.015±0.03 (0)	1.75±1.31 (1.38)	0.038±0.07 (0)	0.08±0.05 (0.062)	nd***
	≤600 head	15	0.001±.002 (0)	0.05±.06 (0.03)	0.37±0.49 (0)	0.014±0.04 (0)	2.35±2.28 (1.61)	0.048±0.07 (0)	0.09±0.09 (0.06)	0.25±0.49 (0)
	P-value		NS	NS	NS	NS	NS	NS	NS	0.035
Distance *	Near	19	0.001±0.002 (0)	0.04±0.05 (0.03)	0.41±0.49 (0.287)	0.021±0.05 (0)	2.07±2.07 (1.32)	0.042±0.06 (0)	0.07±0.03 (0.06)	0.14±0.39 (0)
	Far	11	0.002±0.002 (0)	0.04±0.03 (0.03)	0.09±0.15 (0)	0.003±0.01 (0)	2.01±1.50 (1.39)	0.045±0.08 (0)	0.1±0.10 (0.062)	0.10±0.34 (0)
	P-value		NS	NS	0.06	NS	NS	NS	NS	NS

Milk production	≥39 kg/day	15	0.002±0.002 (0.002)	0.04±0.05 (0.03)	0.44±0.40 (0.41)	0.013±0.03 (0)	2.57±2.23 (1.95)	0.056±0.07 (0)	0.08±0.08 (0.06)	0.13±0.41 (0)
	<39 kg/day	15	0.001±0.002 (0)	0.04±0.04 (0.02)	0.14±0.40 (0)	0.016±0.04 (0)	1.53±1.24 (1.20)	0.029±0.06 (0)	0.08±0.05 (0.07)	0.12±0.32 (0)
	P-value		NS**	NS	0.006	NS	NS	NS	NS	NS

Values are presented as mean ± standard deviation (median). Statistical comparisons were performed between subgroups using non-parametric tests. * Distance: "Near" farms are defined as those located at a distance of ≤4 km from industrial areas, major roads, or urban centers, while "far" farms are situated at a distance of >4 km, ** NS: Indicates non-significant differences (P>0.05), and *** nd: Non detected. Bolded P-values indicate statistically significant differences (P<0.05)

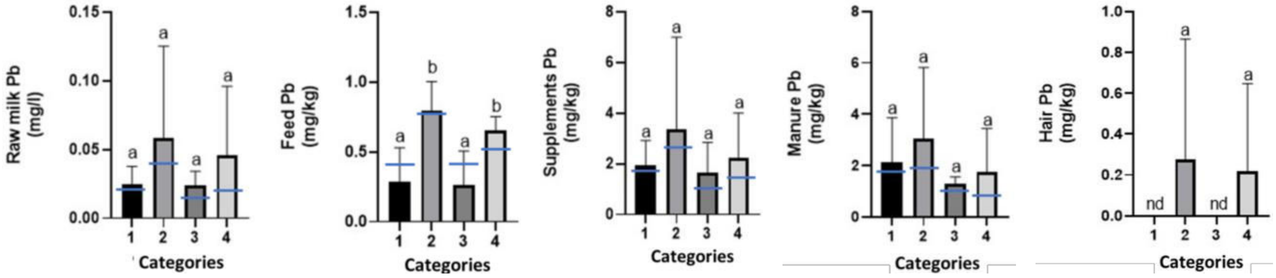

Fig. 1: Lead (Pb) concentrations in raw milk, feed, supplements, manure, and tail hair across four farm categories: (1) high-yield, large herd, (2) high-yield, small herd, (3) low-yield, large herd, and (4) low-yield, small herd. The data illustrate a pattern in which higher Pb inputs from feed and supplements are associated with elevated Pb levels in biological outputs (milk, manure, and hair). Bars represent mean values, error bars indicate standard deviations, and horizontal lines within each bar denote medians. Statistically significant differences between groups are indicated by different lowercase letters (P<0.05), and non-detectable values are labeled as "nd". Sample size: n=30 farms

Table 5: Pb contamination in raw milk: a hazard quotient (HQ) analysis for children and adults across individual dairy farms in Isfahan province

HQ	Origins of raw milk (farms in Isfahan, Iran)														
Farm number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Adult	0.02	0.034	0.026	0.024	0.028	0.019	0.038	0.014	0.148	0.008	0.008	0.014	0.088	0.023	0.026
Child	0.128	0.216	0.165	0.151	0.178	0.118	0.244	0.086	0.944	0.051	0.054	0.087	0.559	0.145	0.167
Number	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Adult	0.005	0.02	0.011	0.013	0.03	0.023	0.008	0.013	0.092	0.015	0.017	0.005	0.015	0.008	0.019
Child	0.034	0.129	0.071	0.084	0.189	0.149	0.053	0.083	0.589	0.094	0.109	0.034	0.094	0.051	0.123

mg/L). Tail hair samples from 29 of 30 farms (96.7%) contained Cd concentrations that surpassed the reference threshold of 0.02 mg/kg, indicating widespread chronic exposure. Cd concentrations in feed were significantly higher in high-yielding farms (median: 0.18 mg/kg) than low-yielding farms (median: 0.073 mg/kg; P= 0.016). Supplements in near farms also showed elevated Cd levels compared to far farms (median: 0.27 *vs* 0.16 mg/kg; P=0.012). No significant difference in Cd concentrations were observed across farm categories based on herd size or milk yield (P>0.05), likely due to undetectable levels in water and milk. A strong positive correlation was found between Cd levels in feed and supplements (rs=0.62, P<0.001).

Lead (Pb)

Lead levels in drinking water were below the detection limit in all farms. In contrast, raw milk from 16 of 30 farms (53.3%) exceeded the ISIRI limit of 0.02 mg/L, with a mean Pb concentration of 0.038 ± 0.043 mg/L. Elevated Pb levels in raw milk were more common in small (10 of 15 farms, 66.7%) compared to large farms (6 of 15 farms, 40%). No statistically significant association was found between Pb exceedance and proximity to industrial zones (P>0.05). Supplements from two small high-yielding farms exceeded the Pb threshold (5 mg/kg). Pb levels in tail hair were above the reference threshold in samples from three small farms. Although manure Pb concentrations remained below ISIRI limits across all farms, strong correlations were observed between Pb concentrations in raw milk and those in supplements (r_s=0.68, P<0.001), manure (r_s=0.81, P<0.001), and tail hair (r_s=0.59, P<0.001). A moderate correlation was also found between Pb in tail hair and manure (r_s=0.47, P=0.009), highlighting systemic exposure patterns. As illustrated in Fig. 1, farms with lower milk yield and smaller herd size exhibited a consistent pattern of elevated Pb levels across multiple matrices.

Health risk assessment (HQ analysis)

HQs were calculated for both adults and children based on the mean Pb concentration in raw milk (0.038 mg/L). All HQ values remained below 1, indicating no immediate non-carcinogenic risk. However, HQ values were consistently higher in children (mean: 0.173) than in adults (mean: 0.027), reflecting their greater vulnerability due to higher intake-to-body-weight ratios. Individual farm HQ values are presented in Table 5.

Discussion

This study provides comprehensive evidence of heavy metal contamination, specifically As, Cd, and Pb, in industrial dairy farms across Isfahan province, Iran. By analyzing six matrices across 30 farms, the study identified key contamination patterns associated with farm characteristics such as herd size, milk yield, and proximity to industrial zones. The integration of biological (hair, milk) and environmental (water, feed, manure, supplements) matrices strengthens the interpretation of systemic exposure pathways.

Lead (Pb) exposure and risk

Lead contamination was the most prominent concern in this study. Over half of the farms (53.3%) had Pb levels in raw milk exceeding the ISIRI safety limit (0.02 mg/L), consistent with previous studies in Iran reporting similarly high Pb concentrations in dairy products (Shahbazi *et al*., 2016; Noaman *et al*., 2021; Sharifi *et al*., 2022). Smaller farms and those near industrial zones exhibited higher Pb concentrations in milk, suggesting potential links to feed quality, low-cost additives, or poor regulatory oversight, especially regarding the use of unregulated toxin binders such as bentonite (Kamkar *et al*., 2010). Strong correlations between Pb in milk and Pb in manure (r_s=0.81), supplements (r_s=0.68), and tail hair (r_s=0.59) reinforce the hypothesis of dietary and environmental exposure as primary contributors. These findings support previous work in other regions (Draghi *et al*., 2024) and highlight the value of tail hair as a biomarker of long-term Pb exposure (Roggeman *et al*., 2013). While HQs for Pb in milk were below the safety threshold of 1 for both adults and children, the higher HQs which were observed in children (up to 0.944) underscore their increased susceptibility. This finding is consistent with prior health risk assessments emphasizing pediatric vulnerability to Pb-related neurotoxicity (Qu *et al*., 2018; Abedi *et al*., 2020).

Cadmium (Cd) and chronic exposure

Although Cd was undetectable in milk and water, elevated levels were detected in feed from one small farm near industrial zones, and tail hair samples showed elevated Cd in 96.7% of all farms. This widespread detection suggests chronic, low-level exposure likely linked to contaminated feed ingredients or the use of phosphate fertilizers (Kubier *et al*., 2019). Higher Cd levels in feed from high-yielding farms (median: 0.18 *vs* 0.07 mg/kg; P=0.016) may reflect the use of more nutrient-dense or imported rations, which could inadvertently contain elevated metal residues (Li *et al*., 2005, 2014). Supplements from farms near industrial zones also showed significantly higher Cd concentrations (median: 0.27 *vs* 0.16 mg/kg; P=0.012), implicating environmental emissions as another potential source (Alloway, 2013; Zhou *et al*., 2019). The strong correlation between Cd in feed and supplements (r_s=0.62) supports a dietary exposure route. Despite low direct dietary risk from milk (due to undetectable levels), the high prevalence of Cd in hair samples demonstrates the utility of tail hair as a non-invasive, long-term biomonitor (Roggeman *et al*., 2013; Perillo *et al*., 2021).

Arsenic (As) and localized contamination

Arsenic contamination was rare, being detected above regulatory limits in only one farm water sample and one supplement sample. Nevertheless, tail hair from nine farms exceeded the reference threshold, and manure from high-yielding farms had significantly higher As levels (P=0.006), possibly due to increased feed and water intake (Datta *et al*., 2012). Moderate correlation between As in tail hair and manure (r_s=0.41) suggests parallel routes of accumulation and excretion. These findings are consistent with earlier studies linking chronic As exposure in cattle to contaminated water and soil sources (Kazi *et al*., 2016; Katz, 2019). Though less pervasive than Pb or Cd, As contamination still poses localized risks and warrants attention in hotspot zones.

Environmental and operational factors

Farms located near industrial zones (≤4 km) consistently exhibited higher As and Cd levels in supplements (P<0.05), indicating environmental contamination from nearby steel and petrochemical facilities (Kamkar *et al*., 2010; Zhou *et al*., 2019). However, Pb levels in milk were not significantly associated with proximity, suggesting that proximity to industrial zones alone does not explain the observed Pb levels. This underscores the need for stricter feed and supplement quality control measures across all locations.

Implications for public health and policy making

While current HQ values suggest limited acute risk, particularly for adults, the high Pb concentrations detected in raw milk and the widespread presence of Cd in tail hair samples point toward a chronic exposure scenario with potential long-term health consequences, especially for children. The health risk is further intensified by structural differences in milk distribution systems. In large-scale dairy processing centers, pooled milk from multiple farms may dilute contamination from a single source, potentially reducing the exposure level in final products. However, in traditional dairy shops, widely present across Iran, raw milk and dairy products such as yogurt, cheese, butter, and doogh are often sourced from a single farm. In such cases, if milk from that farm contains high levels of heavy metals, the absence of dilution increases the likelihood of significant exposure for consumers. This is particularly concerning in regions near industrial zones, where contamination risks are higher and regulatory oversight may be weaker. Therefore, even single-case instances of contamination can pose serious health threats in traditional distribution settings. The findings of this study underscore the urgent need for regulatory monitoring of feed and supplement ingredients, the incorporation of tail hair-based biomonitoring into herd health surveillance programs, the development of spatial risk maps to identify high-risk zones, and targeted educational outreach to smallholders about contamination sources and safe feeding practices. Taken together, these actions are essential for protecting public health and ensuring the safety of dairy products, particularly in areas where single-source milk is directly consumed or sold.

Limitations

This study has several limitations. Its cross-sectional design restricts causal inference between farm characteristics and contamination levels. The sample size, while statistically sufficient for within-region comparisons, may not capture national variability. Additionally, seasonal variations in metal concentrations were not assessed, and soil analyses were not performed, both of which could provide valuable contextual data in future research.

This study presents strong evidence of localized heavy metal contamination in dairy production systems in Isfahan province, Iran, with Pb posing the most immediate concern. Although As and Cd concentrations in raw milk and drinking water were generally below national standards, Pb levels in raw milk exceeded the ISIRI limit in more than half of the surveyed farms, particularly in small farms. Additionally, elevated concentrations of As, Cd, and Pb were detected in tail hair on several farms, indicating chronic exposure risks. The consistent detection of Cd in tail hair across nearly all farms and strong correlations between Pb levels in milk, supplements, manure, and hair confirm the systemic nature of contamination and underscore the role of dietary and environmental sources. Although HQ values for Pb intake through milk were below 1 in both adults and children, the higher values observed in children reflect their greater vulnerability and highlight the need for continued vigilance. Tail hair was validated as a reliable, non-invasive biomarker for chronic exposure, enhancing the utility of biomonitoring approaches in livestock management. To improve food safety and reduce exposure, particularly in vulnerable populations, this study recommends the implementation of stricter quality control measures on animal feed and supplements, expanded use of biomarker-based monitoring tools, development of spatial risk maps for contamination hotspots, and targeted educational programs for farmers. While the cross-sectional nature of this research limits causal inference, and the findings are region-specific, the results provide a compelling basis for future longitudinal studies and policy making measures aimed at mitigating heavy metal exposure in dairy systems.

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Conflict of interest

The authors declare that there is no conflict of interest.

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