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Determination of lead and cadmium levels in the milk of some dairy-producing animals

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تحديد مستويات الرصاص والكادميوم في حليب بعض الحيوانات المنتجة للألبان

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Abstract:
This study evaluated the concentrations of toxic heavy metals, specifically lead (Pb) and cadmium (Cd), in raw milk samples from cows, goats, and camels collected from various locations in the Western Mountain region and its surroundings in Libya. A total of 11 milk samples (comprising 7 distinct sample groups representing different animal types and locations, analyzed in triplicate) were collected during January and February 2024 from Al-Hira, Al-Asabiʿa, Al-Rabta, Gharyan, Mizdah, Al-Zintan, and Al-Swani. Heavy metal levels were determined using atomic absorption spectrophotometry (AAS) following wet acid digestion. The results showed that Pb concentrations ranged from 0.001 to 0.070 ppm, which were entirely within the permissible limit (0.20 ppm) across all samples. Conversely, Cd concentrations ranged from 0.001 to 0.090 ppm, with 12.1% (4 out of 11) of the samples exceeding the international permissible limit of 0.05 ppm. Notably, all cow milk samples from Al-Swani exhibited significantly high Cd levels, averaging 0.0767 ± 0.0153 ppm, alongside one goat milk sample from Al-Asabiʿa. Statistical analysis using the Kruskal-Wallis test confirmed highly significant spatial differences for Cd concentrations among the studied areas (H=22.344, P=0.013). The findings highlight the critical need for continuous monitoring of heavy metals, particularly cadmium, and emphasize further environmental investigations into feed, water, and soil in the affected zones.

Keywords: Raw milk, Lead, Cadmium, Atomic absorption spectrophotometry, Environmental pollution, Libya.

الملخص:
هدفت هذه الدراسة إلى تقييم تراكيز العناصر الثقيلة السامة، متمثلة في الرصاص (Pb)والكادميوم((Cd . في عينات من الحليب الخام للأبقار والماعز والإبل، والتي جُمعت من مواقع مختلفة بمنطقة الجبل الغربي ومحيطها في ليبيا. شملت الدراسة تحليل 11عينة حليب (موزعة على 7 مجموعات جغرافية وحيوانية مختلفة، وبواقع 3 مكررات لكل مجموعة) جُمعت خلال شهري يناير وفبراير 2024 من مناطق: الهيرة، الأصابعة، الرابطة، غريان، مزدة، الزنتان، والسواني. تم تقدير تراكيز العناصر باستخدام تقنية مطيافية الامتصاص الذري (AAS) بعد إجراء الهضم الحمضي الرطب. وأظهرت النتائج أن تراكيز الرصاص تراوحت بين 0.001 و0.070 جزء في المليون (ppm)، وكانت جميعها ضمن الحدود

الدولية المسموح بها (0.20 ppm) في المقابل، تراوحت تراكيز الكادميوم بين 0.001 و0.090 جزء في المليون، حيث تجاوزت 12.1% من العينات (4 عينات من أصل 11) الحد المسموح به عالمياً (0.05 ppm) وسجلت عينات حليب الأبقار في منطقة السواني أعلى متوسط تركيز للكادميوم بلغ 0.0767 ± 0.0153 جزء في المليون متجاوزة الحد الآمن، تليها عينة من حليب الماعز في الأصابعة. وأكد التحليل الإحصائي باستخدام اختبار كروكسال - واليس وجود فروق ذات دلالة إحصائية واضحة لتراكيز الكادميوم بين المناطق المدروسة. توصي الدراسة بضرورة المراقبة المستمرة للعناصر الثقيلة في الحليب، وإجراء بحوث بيئية موسعة لفحص الأعلاف والمياه والتربة في المناطق المتأثرة.

الكلمات المفتاحية : الحليب الخام، الرصاص، الكادميوم، مطيافية الامتصاص الذري، التلوث البيئي، ليبيا.

Introduction:

Milk is widely recognized as a fundamental dietary component for humans, particularly for infants and children, due to its comprehensive nutritional profile [1]. As a natural secretion of the mammary glands of dairy livestock, it provides essential macro- and micronutrients necessary for growth and physiological development [2]. However, the nutritional quality of milk can be severely compromised by environmental contaminants [3]. Among these, toxic heavy metals, specifically lead (Pb) and cadmium (Cd), represent a critical global concern due to their non-biodegradable nature, long biological half-lives, and high potential for bioaccumulation within the food chain [4].

The pathways of heavy metal contamination in milk are multi-faceted, primarily involving the ingestion of contaminated feed and drinking water by dairy animals [5]. Environmental pollution driven by rapid urbanization, industrial emissions, traffic exhaust, and the unsustainable reuse of untreated wastewater for agricultural irrigation leads to the accumulation of Pb and Cd in soil and forage plants [6]. Consequently, these metals are absorbed by livestock, translocated into systemic circulation, and ultimately excreted into the milk [7].

From a public health perspective, chronic exposure to even low concentrations of these non-essential elements poses severe toxicological risks. Lead accumulation is known to induce neurotoxicity, hematopoietic disorders, and irreversible hepatic and renal damage [8]. Similarly, cadmium exposure is heavily linked to severe nephrotoxicity (kidney failure), bone demineralization (osteoporosis), cardiovascular disorders, and is classified as a potent human carcinogen [4, 9]. Given that milk is consumed directly without processing that can remove heavy metals, monitoring its chemical safety is paramount to ensure consumer protection [1].

In Libya, and particularly within the Western Mountain region and its adjacent agricultural plains, livestock farming relies heavily on local environmental resources [10]. Variations in geographic locations, proximity to urban or industrial activities, and differences in husbandry practices may significantly influence the levels of heavy metals in dairy products [7,10]. Despite these risks, comprehensive and updated regional data regarding heavy metal surveillance in local milk remain limited. Therefore, this study was designed to determine and evaluate the concentrations of lead (Pb) and cadmium (Cd) in raw milk samples from cows, goats, and camels across different locations in the Western Mountain region, comparing the obtained levels with international safety standards to assess potential dietary risks.

Materials and Methods:

1. **Reagents and Equipment**: The experimental work of this study was conducted in the Chemistry Laboratory, Faculty of Science – Al-Asabiʿa, from January 21, 2024, to February 13, 2024. All chemical reagents used were of high analytical grade, including ultra-pure Nitric acid (HNO_3, 65%) and Hydrogen peroxide (H_2O_2, 30%). Deionized / Distilled water was utilized for all dilution and cleaning procedures. All glassware was soaked in 10% HNO_3 for 24 hours and rinsed thoroughly with distilled water before use to prevent cross-contamination.
2. **Sample Collection**: A total of 11 distinct sample groups of raw milk were collected from various locations across the Western Mountain region and its surroundings (Al-Hira, Al-Asabiʿa, Al-Rabta, Gharyan, Mizdah, Al-Zintan, and Al-Swani). The livestock sampled included cows, goats, and camels. Each sample group was analyzed in triplicate (n =3), resulting in a total of 11 individual analyses. Samples were collected in sterile plastic containers, kept in an ice box during transit, and immediately stored at −20◦C until chemical digestion. The details of the collected samples are systematically organized in Table 1.

Table (1): Detailed Matrix of Collected Milk Samples

Sample Group	Sample Code	Livestock Type	Collection Location	Collection Date	Replicates (n)
1	A	Cow	Al-Hira	07/01/2024	3
2	S	Cow	Al-Asabiʿa (Qashut)	07/01/2024	3
3	D	Cow	Gharyan (Al -Bawadi)	11/02/2024	3
4	C	Cow	Al-Swani	05/01/2024	3
5	N	Goat	Al-Rabta	11/02/2024	3
6	B	Goat	Al-Rabta	11/02/2024	3
7	F	Goat	Al-Asabiʿa	05/01/2024	3
8	G	Goat	Al-Zintan	13/02/2024	3
9	I	Camel	Mizdah	14/02/2024	3
10	E	Camel	Al-Zintan	14/02/2024	3
11	H	Camel	Al-Asabiʿa	13/02/2024	3

3. **Sample Digestion and Preparation**: Sample preparation was carried out using the wet acid digestion method. Briefly, 5 mL of raw milk was transferred into a clean glass beaker, and 5 mL of concentrated.

$HNO_3$was added. The mixture was heated on an electric hot plate at a controlled temperature of approximately 120–150 °C (inside a fume hood) until the evolution of brown acid fumes ceased and a clear, yellowish solution was obtained. After cooling the beaker to room temperature, 1 mL of $H_2O_2$was added dropwise to enhance the organic matter oxidation and clear any matrix residuals. The mixture was slightly reheated, allowed to cool, filtered using What man No. 42 filter paper, and quantitatively transferred into a volumetric flask, making up the final volume to 25 mL with distilled water. Reagent blank solutions containing only acids without milk were prepared simultaneously following the identical protocol.

4. **Analytical Determination and Instrumental Conditions:** The digested milk solutions and reagent blanks were transferred to the Petroleum Research Center (PRC) in Tripoli, Libya, for final quantitative determination. The analysis of lead (Pb) and cadmium (Cd) was performed using a Flame Atomic Absorption Spectrophotometer (F-AAS) optimized under standard automated operating conditions sustained with an air-acetylene flame: Lead (Pb): Monitored at its primary resonance wavelength of 217.0 nm (slit width: 0.7 nm). Cadmium (Cd): Quantified at its distinctive absorption wavelength of 228.8 nm (slit width: 0.7 nm). Quality control, calibration configurations, and matrix matching were independently managed at the center utilizing high-purity certified standard reference materials. To ensure the analytical reliability of the generated dataset, the method validation metrics were strictly evaluated. The multi-point calibration curves displayed highly reliable linearity over the working concentration ranges, achieving a correlation coefficient ($R^2 > 0.998$) for both lead (Pb) and cadmium (Cd) prior to sample analysis. The Limit of Detection (LOD) and Limit of Quantitation (LOQ) were calculated based on the standard deviation of the blank responses (n = 11) using the formulas LOD = 3σ/S and LOQ = 10σ/S, where σ is the standard deviation of the blank and S is the slope of the calibration curve. The calculated LOD and LOQ values were 0.005 ppm and 0.015 ppm for Pb, and 0.002 ppm and 0.006 ppm for Cd, respectively. Analytical values obtained below the calculated LOD were verified and reported accordingly

Results and Discussion:

1. **Lead (Pb) Concentrations in Milk**:

The concentration of lead (Pb) across all analyzed milk samples ranged from 0.001 to 0.070 ppm (Table 2). When benchmarking these findings against the international maximum permissible limit (MPL) of 0.20 ppm established by the Codex Alimentarius Commission (FAO/WHO), all analyzed samples fell safely below the legal thresholds, representing 100% compliance.

The highest Pb accumulations were observed in goat milk from Al-Asaba'a (sample F_1) and Al-Zintan (sample G_3), alongside camel milk from Al-Asaba'a (sample H_3), with each reaching a peak of 0.070 ppm. Conversely, the lowest concentrations (0.001 ppm) were scattered among various cow and goat milk matrices. Statistically, these low baseline values indicate that atmospheric deposition of lead from leaded gasoline combustion has significantly decreased in these regions, which aligns with recent regional surveys in North Africa.

The minor fluctuations recorded among geographic zones can be attributed to localized baseline differences in veterinary drug exposures, proximity to secondary roads, and minor variations in livestock

forage sources. Although Pb levels do not currently present an acute toxicological threat, its cumulative behavior inside human biological systems warrants a continuous dietary monitoring program, especially since milk serves as a core nutrient for vulnerable groups like infants and children.

2. Cadmium (Cd) Concentrations in Milk:

Cadmium (Cd) levels in the tested milk matrix varied extensively, spanning from 0.001 to 0.090 ppm. In stark contrast to lead, Cd levels raised substantial food safety concerns, with 12.1% (4 out of 11) of the total individual samples breaching the international standard limits of 0.05 ppm.

The most prominent contamination hotspot was explicitly identified in Al-Swani, where all cow milk replicates (samples C_1, C_2, and C_3) exceeded the safe thresholds, recording 0.080, 0.060, and 0.090 ppm, respectively. Additionally, one goat milk sample from Al-Asabi'a (sample F_3) exceeded the limit with a value of 0.060 ppm.

From an environmental standpoint, the severe elevation of cadmium in Al-Swani's milk can be scientifically justified by the heavy anthropogenic and agricultural activities characterizing this perimeter. Al-Swani acts as a strategic agricultural basin that suffers from intensive long-term utilization of chemical phosphate fertilizers, which are historically documented to contain substantial cadmium impurities that shift easily into the soil-plant-animal pathway. Furthermore, the potential utilization of untreated wastewater or compromised shallow groundwater for irrigating local fodder stands as a primary vector for Cd biomagnification in lactating cows. These findings heavily correlate with recent local literature addressing heavy metal spikes in the biological fluids of livestock in western Libya.

3. Comparative Synthesis of Metal Contamination:

A comparative view of the two toxic metals clearly indicates that cadmium poses a significantly higher environmental and dietary risk than lead within the studied areas. While peak lead values (0.070 ppm) remained far below their respective MPL (0.20 ppm), peak cadmium values (0.090 ppm) nearly doubled the acceptable limits (0.05 ppm).

This clear disparity can be associated with the high mobility of cadmium ions in agricultural ecosystems. Crops absorb cadmium from contaminated soils far more readily than lead, facilitating its rapid translocation into livestock feed and subsequent transfer into the mammalian mammary glands. The significant variation in Cd profiles across the studied locations emphasizes that heavy metal profiles in milk are heavily dictated by regional industrialization and farming management rather than the physiological differences of the animal species alone.

Table (2): Quantitative Matrix of Pb and Cd Concentrations in Collected Milk Samples:

Milk Type	Sampling Area	Sample Code	Lead (Pb), ppm	Cadmium (Cd), ppm	Status vs. Standards
Cow Milk	Al-Heera	A1	0.060	0.001	Safe
		A2	0.002	0.001	Safe
		A3	0.020	0.003	Safe
	Al-Asaba'a	S1	0.030	0.001	Safe
		S2	0.060	0.004	Safe
		S3	0.040	0.003	Safe
	Gharyan	D1	0.050	0.001	Safe
		D2	0.010	0.003	Safe
		D3	0.030	0.005	Safe
	Al-Swani	C1	0.002	0.080	Exceeds Limit (Cd)
		C2	0.004	0.060	Exceeds Limit (Cd)
		C3	0.001	0.090	Exceeds Limit (Cd)
Goat Milk	Al-Rabta (Group N)	N1	0.040	0.010	Safe
		N2	0.060	0.040	Safe
		N3	0.020	0.030	Safe
	Al-Asaba'a	F1	0.070	0.010	Safe
		F2	0.040	0.050	Safe
		F3	0.030	0.060	Exceeds Limit (Cd)
	Al-Rabta (Group B)	B1	0.002	0.001	Safe
		B2	0.001	0.003	Safe
		B3	0.006	0.007	Safe
	Al-Zintan	G1	0.050	0.001	Safe
		G2	0.030	0.003	Safe
		G3	0.070	0.004	Safe

Camel Milk	Mizdah	I1 I2 I3	0.050 0.020 0.010	0.001 0.003 0.001	Safe Safe Safe
	Al-Zintan	E1 E2 E3	0.010 0.040 0.030	0.001 0.001 0.003	Safe Safe Safe
	Al-Asaba'a	H1 H2 H3	0.040 0.050 0.070	0.001 0.003 0.002	Safe Safe

Note: Codex Alimentarius Maximum Permissible Limits (MPL) are 0.20 ppm for Pb and 0.05 ppm for Cd.

4. Statistical Analysis and Distribution Variations:

The statistical evaluation of the generated dataset highlighted significant spatial and matrix-driven variations in the concentrations of both investigated trace elements. The mean concentrations of lead (Pb) peaked at 0.0533±0.0153ppm in camel milk from Al-Asaba'a, dropping to a minimum of 0.0023 ± 0.0015ppm in cow milk from Al-Swani. Conversely, the mean cadmium (Cd) concentrations exhibited a completely inverted profile, maximizing at a highly critical level of 0.0767±0.0153ppm in cow milk from Al-Swani, while falling to baseline levels of 0.0017±0.0012ppm with in several cow and camel milk groups (Table 3).

To test the significance of these geographic and biological variations, a parametric One-way Analysis of Variance (ANOVA) was initially applied. The ANOVA test revealed statistically significant differences across the studied groups for both Pb (F=2.698, P=0.025) and Cd (F=16.206, P<0.001). However, because parametric assumptions can be compromised by small sample sizes (n=3per group), a robust non-parametric Kruskal–Wallis test was cross-examined to validate these findings.

Interestingly, the Kruskal–Wallis test confirmed a highly significant statistical difference for cadmium levels (H=22.344, P=0.013), whereas the significance for lead was marginally weakened, shifting just outside the conventional alpha threshold (H=18.243, P=0.051). This marginal discrepancy between the two tests regarding Pb indicates that its environmental distribution is heavily influenced by localized micro-environments and individual animal exposure variances rather than a systemic regional contamination pattern. The exceptionally high F-value (16.206) and highly significant P-value (<0.001is less than 0.001<0.001) obtained for cadmium mathematically confirm that the pollution profiling in Al-Swani is not a random variance. This statistical divergence strongly verifies the presence of an active point source or fixed environmental vector, such as agrochemical runoff or contaminated irrigation schemes, uniquely impacting the Al-Swani agricultural plain.

Table (3): Mean Concentration (±Standard Deviation) of Pb and Cd Across Examined Milk Matrices:

Milk Type and Sampling Area	Pb Mean ±SD (ppm)	Cd Mean ± SD (ppm)	Compliance Status
Cow milk – Al-Heera	0.0273 ± 0.0297	0.0017 ± 0.0012	Safe
Cow milk – Al-Asaba'a	0.0433 ± 0.0153	0.0027 ± 0.0015	Safe
Cow milk – Gharyan	0.0300 ± 0.0200	0.0030 ± 0.0020	Safe
Cow milk – Al-Swani	0.0023 ± 0.0015	0.0767 ± 0.0153	Exceeds Cd limit
Goat milk – Al-Rabta (N)	0.0400 ±0.0200	0.0267 ±0.0153	Safe
Goat milk – Al-Asaba'a	0.0467 ±0.0208	0.0400 ±0.0265	Safe
Goat milk – Al-Rabta (B)	0.0030 ± 0.0027	0.0037 ±0.0031	Safe
Goat milk – Al-Zintan	0.0500 ±0.0200	0.0027±0.0015	Safe
Camel milk – Mizdah	0.0267±0.0208	0.0017±0.0012	Safe
Camel milk – Zintan	0.0267± 0.0153	0.0017±0.0012	Safe
Camel milk – Al-Asaba'a	0.0533±0.0135	0.0020±0.0010	Safe

Note: Data represents the calculated mean ± standard deviation based on triplicate analyses (n=3).

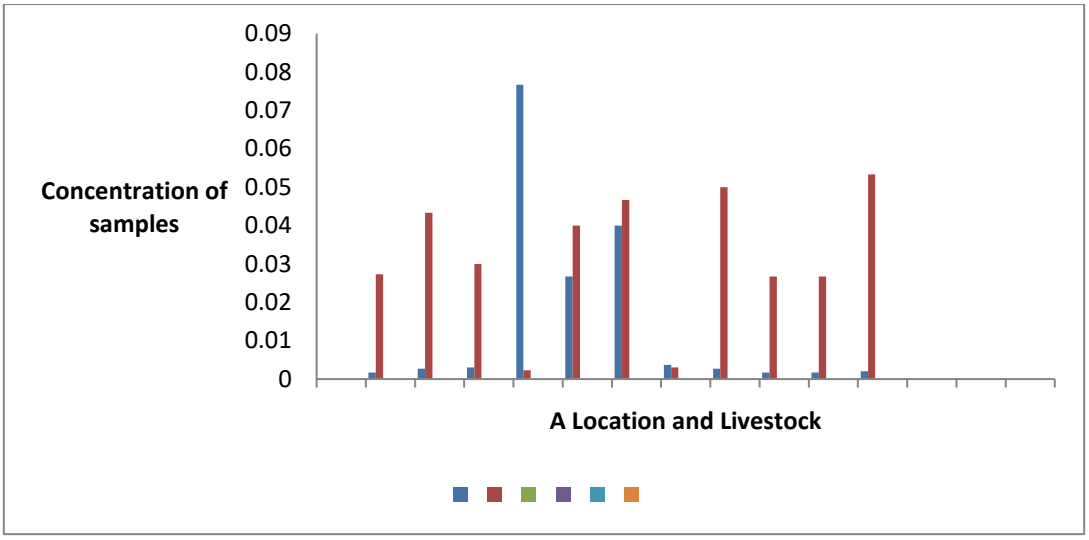


Figure 1. Graphical profiling of Pb and Cd distribution vs Codex guidelines)

Conclusion:

The present study provided a comprehensive evaluation of toxic heavy metal residues, specifically lead (Pb) and cadmium (Cd), in raw milk samples from cows, goats, and camels across major livestock areas in the Western Mountain region of Libya and its surrounding agricultural plains. The generated analytical dataset confirms distinct profiles for the two investigated elements. In terms of lead, all analyzed milk matrices demonstrated full compliance with international safety thresholds, as the maximum detected Pb level (0.070 ppm) remained confidently below the Codex Alimentarius permissible limit of 0.20 ppm, indicating no immediate toxicological risk to consumers regarding lead exposure.

In stark contrast, cadmium levels presented substantial environmental and food safety concerns, with 12.1% of the overall analyzed samples breaching the international standard ceiling of 0.05 ppm. The statistical and spatial profiling using the Kruskal–Wallis test verified highly significant distribution variances for cadmium (H=22.344, P=0.013), identifying Al-Swani as a major contamination hotspot. In Al-Swani, 100% of the cow milk replicates exceeded the safe thresholds, exhibiting a critical average concentration of 0.0767±0.0153ppm.

Additionally, borderline Cd values observed in goat milk from Al-Asaba'a call for immediate vigilance. Collectively, these findings mathematically demonstrate that while systemic heavy metal pollution is absent across most of the Western Mountain territory, localized anthropogenic factors in agricultural areas like Al-Swani, such as the heavy usage of chemical phosphate fertilizers or impaired groundwater for irrigation, are actively shifting toxic cadmium into the soil-plant-animal food chain.

Recommendations:

To mitigate the dietary risks associated with heavy metal bioaccumulation in dairy products and ensure public health protection, the following coordinated actions are strongly recommended at both the producer and regulatory levels:

- **First: For Dairy Producers and Farmers:**
- **Enforce Feed and Water Safety:** Transition livestock to safe, clean, and verified forage sources, strictly prohibiting the grazing of dairy animals near heavy traffic roads, industrial processing sites, or open municipal waste zones.
- **Regular Monitoring:** Livestock owners in high-risk zones, particularly Al-Swani and parts of Al-Asaba'a, should proactively subject their farm water supplies and local fodder crops to seasonal testing for element monitoring.
- **Optimize Husbandry Hygiene:** Maintain strict compliance with standard dairy management practices, ensuring routine sanitary maintenance of milk storage containers and prioritizing targeted veterinary care to enhance animal metabolic resilience against toxic exposures.
- **Second: For Regulatory Authorities and Government Agencies:**
- **Establish Continuous Surveillance Systems:** Implement national routine monitoring programs for chemical and environmental contaminants (pesticides, heavy metals, and mycotoxins) across all commercial milk collection nodes in western Libya.
- **Equip Regulatory Laboratories:** Provide municipal food control centers and laboratory units with technical calibration tools, ensuring local access to continuous monitoring resources.

- **Enforce Wastewater Treatment Legislation:** Accelerate the construction and rehabilitation of municipal and industrial wastewater processing stations to eliminate toxic heavy metal runoff before it reaches shallow agricultural aquifers and local soil systems.
- **Regulate Fertilizer Quality:** Impose strict standard limitations on imported agricultural chemical fertilizers, specifically ensuring that phosphate fertilizers undergo tight screening to minimize cadmium impurities transferred to agricultural lands.

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