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Heavy metals in raw cow, goat and sheep milk: Associated dietary exposure and human health risk assessment in eastern Algeria

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ABSTRACT

In recent years, the presence of heavy metals in milk has emerged as an important public health issue, owing to their potential detrimental effects on milk quality. The present study aimed to assess the occurrence and levels of heavy metals in raw cow, goat, and sheep milk obtained from eastern Algeria. Sixty milk samples were analysed for Cd, Cr, Cu, Ni and Fe using atomic absorption spectrometry. Results demonstrated that Fe (5.49 ± 21.93) was the dominant metal in cow milk, followed by Cu (0.86 ± 0.02) and Cr (0.56 ± 0.05). In goat milk, Fe (9.77 ± 0.69) and Cu (2.79 ± 0.04) were highest, while in sheep milk, Cu (1.99 ± 0.04) exceeded Fe (1.78 ± 0.16) and Cr (0.79 ± 0.07). Several investigated trace elements, including copper, chromium and iron, exceeded the established maximum residue limits (MRLs). In contrast, Ni was mostly below the detection limit, but Cd was present in goat and sheep milk with low concentrations. PCA explained 57.6% of the total variance. Meanwhile, the non-carcinogenic risk assessment showed that the estimated daily intakes of all investigated metals were below their respective oral reference doses (RfDs). THQ values remained < 1, whereas TTHQ exceeded the safety threshold for goat and sheep milk, particularly in children.

Keywords: heavy metals, milk, health risk, non-carcinogenic, Algeria.

INTRODUCTION

Milk ranks among the most consumed foods worldwide and constitutes an essential component for human nutrition owing to its high nutritional value. It provides high levels of vitamins, proteins, lipids, carbohydrates, essential minerals, and numerous bioactive compounds that are indispensable for development and the maintenance of normal physiological and biochemical functions (Boukria et al., 2020). Driven by population growth, evolving dietary patterns, and increased awareness of the nutritional benefits of milk and dairy products, global consumption has steadily increased over recent decades (Claeys et al., 2013).

Despite its nutritional importance, milk can also represent an important dietary pathway for human exposure to environmental contaminants, particularly heavy metals (Bousbia et al., 2019; Boussadia et al., 2025). Both natural geological processes and anthropogenic activities contribute to the release of these contaminants into the environment, including mining, industrial emissions, intensive agriculture, urbanisation, wastewater irrigation, and petroleum-related pollution (Parker et al., 2022). Heavy metals may accumulate in soil, water, and forage crops before being transferred to livestock through feed and drinking water, ultimately entering the human food chain through dietary intake of contaminated foods (Karimi et al., 2023; Pompilio et al., 2021).

Numerous factors influence the concentrations of trace elements in milk, including animal species and breed, physiological status, lactation stage, feeding practices, environmental conditions, geographical location, and farm management systems (Dobrzanski et al., 2005).

The proper functioning of enzymatic systems, immune responses, oxygen transport, antioxidant mechanisms, and cellular metabolism relies on essential micronutrients, such as iron (Fe), copper (Cu), and zinc (Zn) (Capcarova et al., 2024; Jaafarzadeh et al., 2023; Su et al., 2021). Several non- essential metals, including cadmium (Cd) and nickel (Ni), have no known essential biological functions and may exert toxic effects even at relatively low concentrations (Wu et al., 2022).

Chronic dietary exposure to heavy metals has become a growing global concern because of their persistence, capacity for bioaccumulation, and adverse effects on human health. Long-term exposure may induce oxidative stress, disrupt endocrine and metabolic functions, impair renal and hepatic function, affect neurological development, and contribute to cardiovascular disorders and cancer (Wu et al., 2022). Among these contaminants, cadmium is considered a particularly hazardous environmental pollutant because of its prolonged biological retention and tendency to accumulate in vital organs (Kar et al., 2025).

Consequently, regular monitoring of heavy metal contamination in milk is indispensable for ensuring food safety and protecting consumers from potential health hazards. In Algeria, livestock farming represents an important component of the agricultural sector, with cattle, goats, and sheep contributing substantially to national milk production (Bousbia et al., 2019). The demand for goat and sheep milk has risen steadily recently, owing to their nutritional value, digestibility, and suitability as alternatives for individuals suffering from cow's milk allergy (Khan et al., 2024; Tesse et al., 2009). Moreover, goats and sheep are well adapted to harsh climatic conditions and extensive production systems, making them particularly important for milk production in arid and semi-arid regions (Silanikove, 2000). However, reliance on natural grazing may also increase animal exposure to environmental contaminants, potentially enhancing the passage of heavy metals from the environment into milk.

Although heavy metal contamination in cow's milk has been widely investigated, comparative information on heavy metal levels in cow, goat, and sheep milk produced under extensive and semi-extensive farming systems in northeastern Algeria remains scarce.

To address these concerns, the present study aimed to (i) determine the concentrations of Cd, Cr, Cu, Fe, and Ni in raw cow, goat, and sheep milk collected from extensive and semi-extensive farms in Guelma Province, northeastern Algeria; (ii) compare contamination patterns among the three animal species; (iii) investigate the relationships among heavy metals using principal component analysis (PCA); and (iv) assess dietary exposure together with the potential non-carcinogenic health risks associated with the consumption of raw milk in both adults and children. The results support food safety surveillance, public health protection and serve as valuable baseline data for future environmental monitoring programs in Algeria.

MATERIALS AND METHODS

Ethics statement

Milk samples were collected from farms after obtaining informed consent from the participating farmers. Before sample collection, participants were informed about the objectives of the study and provided consent for milk sampling and for the use of the generated data and analytical results for scientific research and publication.

Study site

The study was carried out in Guelma Province, northeastern Algeria (36°28′ N, 7°26′ E), at an altitude of approximately 290 m above sea level. The province is located within an important agricultural region and is surrounded by the Maouna, Debagh, and Houara mountain ranges. Its fertile soils, together with water resources supplied by the Seybouse River and a major dam supporting an extensive irrigation network, contribute to the region's importance for agricultural and livestock production.

Milk sampling

For the determination of heavy metal concentrations, sixty raw milk samples were obtained from cows (30 samples), goats (15 samples) and sheep (15 samples) during the spring season (March to June). Sampling was conducted on extensive livestock farms located in the Guelma Province of northeastern Algeria, namely Aïn

Larbi, Lekhzara, and El Fedjoudj (Figure 1). For each sample, approximately 200–400 mL of raw milk was collected under hygienic conditions into sterile glass bottles. To preserve their physicochemical properties and minimise degradation before heavy metal determination, all samples were stored at -20 °C immediately upon arrival at the laboratory. After collection, milk samples were transported under a continuous cold chain in insulated coolers containing ice packs.

Sample digestion and metal analysis

A mixture of 5 ml of 68% nitric acid (HNO_3) and 2 ml of 30% hydrogen peroxide (H_2O_2) was added to a 5 ml aliquot of each milk sample in a digestion vessel. The digested samples were subsequently cooled to room temperature and diluted with distilled water to a final volume of 50 ml containing 1% (v/v) nitric acid.

Before this step, digestion was performed using an electric hot plate at 120 to 220 °C until the solution was gradually concentrated and a clear digest was obtained. The obtained digests were then subjected to filtration through a 0.45-µm membrane filter (Merck, Lyon, France) before heavy metal analysis. Blank samples, consisting of the digestion reagents without milk, were prepared and processed following the same digestion protocol as the milk samples. Cd, Cr, Cu, Fe, and Ni concentrations in milk samples were determined employing a flame atomic absorption spectrophotometer (FAAS) using an AAnalyst 400 instrument (PerkinElmer, USA). Quantitative analysis was performed using external calibration with standard solutions for each analyte. The calibration curves for Cd, Cr, Fe, Ni, and Cu were constructed using standard points. The linearity of each calibration curve was evaluated by determining the coefficient of determination (R^2) for each analysed metal. All analyses were undertaken in three independent replicates, and the results are presented as the mean values.

Analytical quality control

The analytical method was confirmed by evaluating its linearity, precision, accuracy, limit of detection (LOD), and limit of quantification (LOQ). Calibration curves for Cd, Cr, Fe, Ni, and Cu were constructed using five calibration standards, with coefficients of determination (R^2) greater than 0.99 for all analytes. The LOD and LOQ were derived from reagent blank measurements, using factors of three and ten times the standard deviation of the blanks, respectively. The obtained LOD (LOQ) values for Cd, Cr, Fe, Ni, and Cu were 3×10^{-3} (1×10^{-2}), 1×10^{-2} (3.3×10^{-2}), 2×10^{-2} (6.7×10^{-2}), 1.5×10^{-2} (5×10^{-2}), and 5×10^{-2} (1.7×10^{-2}) mg/kg, respectively. Method accuracy was assessed through recovery experiments using the standard addition technique at three concentration levels, yielding recoveries ranging from 90 to 106%. Precision was assessed by triplicate analysis of each milk sample ($n = 60$); all analysed metals exhibited relative standard deviation (RSD) values below 10%. Measurements exceeding this limit were discarded, and the corresponding samples were re-digested and reanalysed.

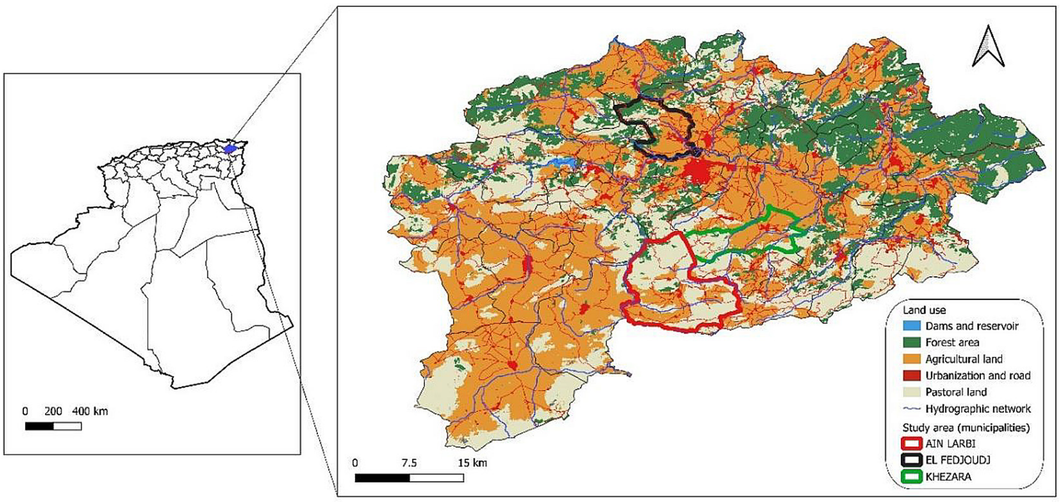

Figure 1. Geographic location of the investigated municipalities, mapped using open-source QGIS software

Human health risks assessment

Estimated daily intake

EDI values were calculated separately for children and adults using their respective average body weights of 20 kg and 60 kg (Adesida, 2025), to identify the population group most vulnerable to dietary exposure. The EDI (mg/kg BW/day) was determined as shown in the equation below:

$$EDI = \frac{MS \times C}{BW} \tag{1}$$

where: *MS* represents the average daily raw milk consumption (0.20 kg/day) for adults and (0.10 kg/day) (Serraino et al., 2019) for children, as commonly applied in dietary exposure assessments, *C* denotes the metal concentration in milk (mg/kg), and *BW* denotes the average body weight (kg).

Target hazard quotient

Non- carcinogenic risks resulting from long-term dietary exposure to toxic elements were assessed based on the target hazard quotient (THQ), a widely accepted approach developed by the US EPA. The THQ was calculated using the following equation:

$$THQ = \frac{EDI}{RfDo} \tag{2}$$

where: *EDI* is the estimated daily intake of the metal (mg/kg BW/day), and *RfDo* is the oral reference dose (mg/kg BW/day).

The reference doses adopted in this study were 0.001, 0.04, 0.02, 0.70, and 0.003 mg/kg/day for Cd, Cu, Ni, Fe, and Cr, respectively (Akele et al., 2017). *THQ* values above 1 are considered indicative of potential non-carcinogenic health risks, whereas a *THQ* value less than 1 suggests that exposure is not expected to pose significant health risks (Copat et al., 2012).

Total target hazard quotient

To evaluate the cumulative non-carcinogenic health risk associated with exposure to multiple heavy metals, the total target hazard quotient was calculated. The TTHQ was obtained by summing the individual THQ values of all analysed metals using the equation below:

$$\begin{aligned} TTHQ = THQ\ toxic\ element\ 1 + \\ + THQ\ toxic\ element\ 2 \dots + \\ + THQ\ toxic\ element\ n \end{aligned} \tag{3}$$

A *TTHQ* value > 1 indicates a potential cumulative health risk, whereas a value < 1 suggests that adverse non-carcinogenic effects are unlikely.

Data processing

All statistical analyses were performed using R software (version 4.3.1). The Gaussian distribution of heavy metal concentrations in raw cow, goat and sheep milk samples was first assessed using the Shapiro-Wilk test. Comparison between milk types and sampling periods was then conducted using the non-parametric Kruskal-Wallis test. Additionally, standardised data were subjected to a principal component analysis (PCA) to explore the variability and examine the relationships among heavy metal concentrations in the different milk matrices. This multivariate approach allowed the identification of trends and potential contamination sources and was performed using FactoMinoR software (Husson et al., 2020). EDI, THQ, and TTHQ were also calculated. Statistical significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

Heavy metal levels across raw cow, goat and sheep milk

The concentrations of five heavy metals (Cu, Cr, Fe, Ni, and Cd) were quantified in raw milk samples using atomic absorption spectrometry. Descriptive statistics for the concentrations of these metals in raw cow, goat, and sheep milk samples collected from extensive farms in Guelma Province, northeastern Algeria, are presented in Table 1.

Copper

Copper concentrations were between 0.35 and 6.00 mg/kg, 0.45 to 12.50 mg/kg, and 0.20 to 12.50 mg/kg in raw cow, goat, and sheep milk, respectively (Table 1). The corresponding mean concentrations were 0.86 ± 0.02 mg/kg for cow milk, 2.79 ± 0.04 mg/kg for goat milk, and 1.99 ± 0.04 mg/kg for sheep milk. Goat milk exhibited the highest mean copper concentration, followed by sheep milk, whereas cow milk had the lowest concentration.

The overall Kruskal-Wallis test was significant, while pairwise comparisons showed a significant difference between cow and sheep milk ($P = 0.02$), whereas comparisons involving goat milk were not statistically significant ($P > 0.05$) (Table 1, Figure 2). The compliance of milk samples with the established maximum residue limits (MRLs) varied considerably among the investigated metals. Copper exhibited the highest rate of non-compliance, with 100% of the analysed samples exceeding the MRL of 0.01 mg/kg established by the International Dairy Federation (IDF, 1979).

Figure 2 presents box plots of Cu levels in raw cow, goat, and sheep milk. Considerable variability was observed across the three milk types, with the lowest median Cu concentration in cow milk and the highest in sheep milk, while goat milk displayed intermediate Cu concentrations. Overall, Cu concentrations differed significantly among the three milk types ($P < 0.05$). Notably, Cu concentrations in all analysed milk samples surpassed the maximum permissible limit recommended by FAO/WHO (2012).

These findings are in agreement with previous studies conducted in Algeria (Boudebbouz et al., 2022; Lebsir et al., 2023), which also reported elevated Cu levels in raw milk. The observed levels may reflect environmental exposure and warrant attention regarding potential consumer health risks. The relatively higher concentrations observed in goat and sheep milk may be attributed to species-specific physiological characteristics (Parker et al., 2022), differences in feeding practices (Karimi et al., 2023), or greater environmental exposure to trace elements (Mahdinezhad Hargalan et al., 2025). Overall, the Cu concentrations recorded in the current study fell within the range previously reported in the literature (Bilandžić et al., 2021; Boudebbouz et al., 2024), suggesting that the observed values are comparable to those reported for raw milk under similar environmental conditions.

Chromium

As shown in Table 1. Cr concentrations varied among the three milk types. The mean concentrations of Cr were 0.56 ± 0.05 mg/kg in raw cow milk, 0.70 ± 0.07 mg/kg in raw goat milk, and 0.79 ± 0.07 mg/kg in raw sheep milk. The maximum concentrations reached 2.15 mg/kg in cow milk, 1.65 mg/kg in goat milk, and 1.90 mg/kg in sheep milk. Sheep milk exhibited the maximum mean Cr concentration, with goat milk ranking second, whereas cow milk showed the lowest concentration (0.79 ± 0.07 > 0.70 ± 0.07 > 0.56 ± 0.05 mg/kg) (Figure 3; Table 1). Chromium also showed a high frequency of exceedance of the (MRL) of 0.20 mg/kg, with 66.7%, 66.7%, and 86.7% of cow, goat, and sheep milk samples,

Table 1. Descriptive statistics and percentages of samples exceeding the MRLs for Cu, Cr, Fe, Ni, and Cd (mg/kg) in raw cow, goat, and sheep milk collected from Guelma Province, northeastern Algeria

Sample	Parameter	Heavy metal (mg/kg)				
		Cu	Cr	Fe	Ni	Cd
Cow milk	Mean ±SD Min Max	0.86 ± 0.02 0.35 6	0.56 ± 0.05 0 2.15	5.49 ± 21.93 0 120.75	BDL	BDL
Goat milk	Mean ±SD Min Max	2.79 ± 0.04 0.45 12.5	0.70 ± 0.07 0 1.65	9.77 ± 0.69 0 64.8	0.05 ± 0.09 0 0.25	0.03 ± 0.003 0 0.45
Sheep milk	Mean ±SD Min Max	1.99 ± 0.04 0.2 12.5	0.79 ± 0.07 0 1.9	1.78 ± 0.16 0 9.8	0.013 ± 0.05 0 0.2	0.006 ± 0.02 0 0.1
p value		0.02	0.21	0.28	0.36	0.20
MRLs		0.01	0.2	0.37	1	0.0026
% Samples exceeding MRLs		100 % [a, b, c]	66.7[a, b] 86.7[c]	46.7[a], 66.7[b, c]	0 [a, b, c]	ND[a] 6.7[b] 6.7[c]

Note: SD – Standard deviation, BDL – below detection limit, ND: not determinable, [a]: Represents percentage of cow milk samples exceeding MRLs, [b]: Represents percentage of goat milk samples exceeding MRL$_S$, [c]: Represents percentage of sheep milk samples exceeding MRLs.

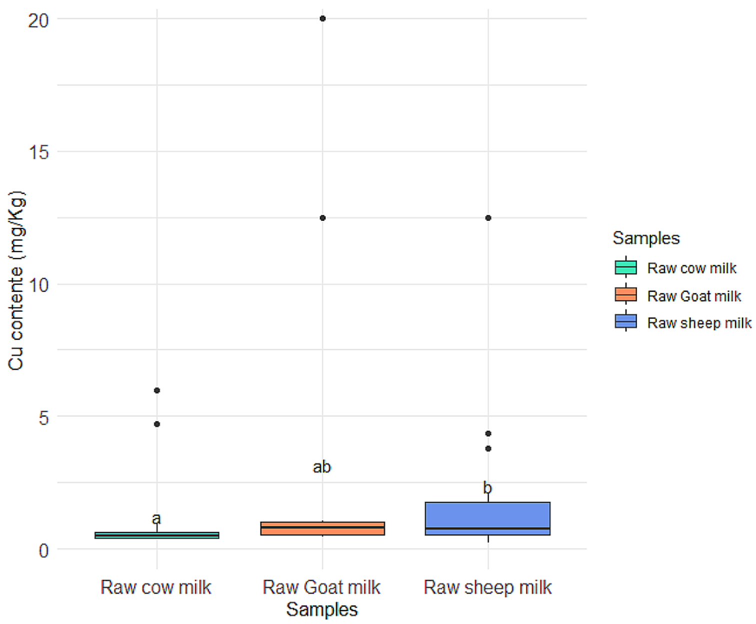

Figure 2. Box plot analysis representing the copper levels (mg/kg) in raw milk; cow, goat and sheep samples. Lines inside the box represent the median. Whiskers represent the spread of the data (minimum and maximum). Dots outside the whiskers are outliers

respectively (Table 1). Despite the higher mean Cr concentrations observed in sheep and goat milk, the three milk types did not differ significantly ($p = 0.21$) (Table 1).

As an essential trace element, chromium is typically found in milk at low concentrations, although its levels may vary depending on animal species, environmental conditions (Tobi et al., 2023), feeding practices, and contamination sources (Lebsir et al., 2023). The relatively higher Cr concentrations observed in sheep and goat milk may be associated with grazing behavior, as small ruminants are more likely to ingest soil particles and environmental contaminants while grazing (Rahimi et al., 2023).

Similar findings have been reported by Tahir et al. (2023). Although most samples complied with typical background levels, some maximum Cr concentrations surpassed the recommended guideline values established by the FAO/WHO.

Iron

Fe concentrations in raw cow, goat, and sheep milk are shown in Table 1. Goat milk exhibited the highest mean Fe concentration (9.77 ± 0.69 mg/kg), followed by cow milk (5.49 ± 21.93 mg/kg), whereas sheep milk showed the lowest mean concentration (1.78 ± 0.16 mg/kg) (Figure 4, Table 1). Iron concentrations varied considerably among samples, particularly in cow milk (0–120.75 mg/kg) and goat milk (0–64.80 mg/kg), while sheep milk exhibited a narrower range (0–9.80 mg/kg). The exceedance of the reference value (0.37 mg/kg) was observed in 46.7%, 66.7%, and 66.7% of cow, goat, and sheep milk samples, respectively (Table 1). Despite the apparent differences in mean concentrations, statistical analysis indicated no significant differences among the three milk types ($p = 0.28$).

According to (FAO/WHO, 2012), iron concentrations in milk are typically low, generally between 0.3 and 1.0 mg/kg across the different animal species (Battisti et al., 2024). In the present study, Fe levels frequently exceeded these reference ranges, suggesting that the elevated levels may be associated with environmental contamination, feeding practices, or other external sources rather than normal physiological variation (Khan et al., 2024).

Nickel and cadmium

The concentrations of nickel and cadmium were generally low in the analysed raw milk samples, with a considerable proportion of measurements below the detection limit (BDL) in cow milk, indicating limited contamination (Table 1). Detectable concentrations were mainly observed in goat and sheep milk, with mean

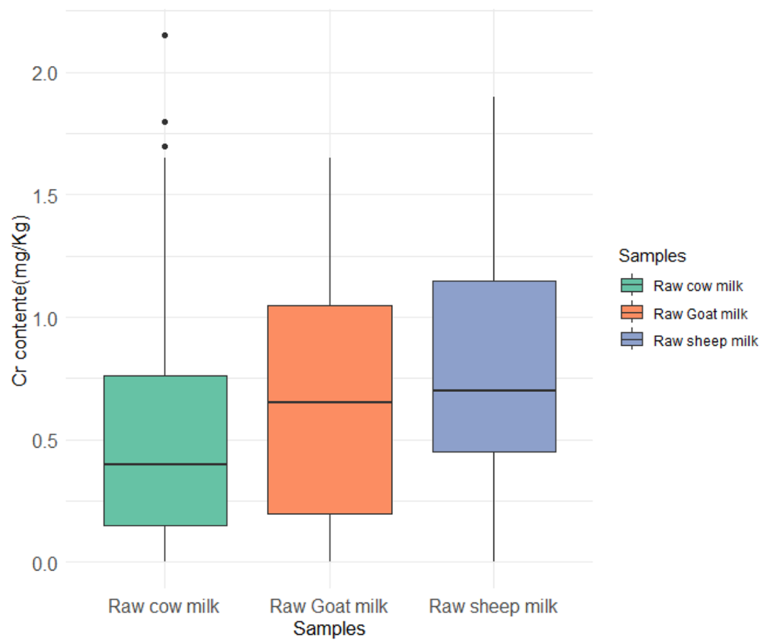

Figure 3. Box plot analysis representing the chromium levels (mg/kg) in raw milk; cow, goat and sheep samples. Lines inside the box represent the median. Whiskers represent the spread of the data (minimum and maximum). Dots outside the whiskers are outliers.

Ni and Cd concentrations of 0.05 ± 0.09 mg/kg and 0.03 ± 0.003 mg/kg, respectively, while 0.013 ± 0.05 mg/kg and 0.006 ± 0.02 mg/kg, respectively. Maximum concentrations reached 0.25 mg/kg for Ni and 0.45 mg/kg for Cd in goat milk and 0.2 mg/kg for Ni and 0.1 mg/kg for Cd in sheep milk. No statistically significant differences in Ni and Cd concentrations were observed among the three milk types ($P > 0.05$). However, goat milk showed greater variability and higher Cd concentrations than sheep milk, as illustrated in Figure 5.

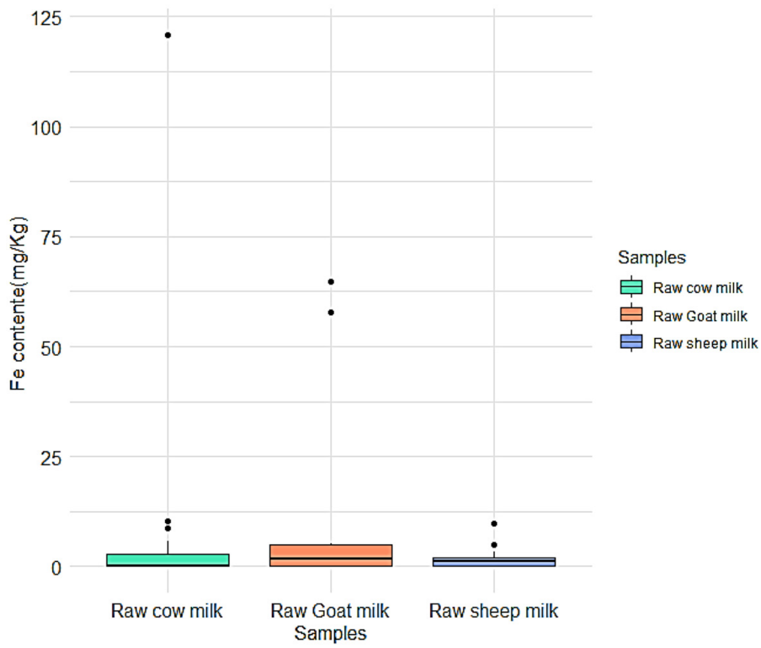

Figure 4. Box plot analysis representing Iron levels (mg/kg) in raw milk; cow, goat and sheep samples. Lines inside the box represent the median. Whiskers represent the spread of the data (minimum and maximum). Dots outside the whiskers are outliers

Nickel was detected in four of the 15 goat milk samples and in one of the 15 sheep milk samples. While the remaining samples were below the detection limit. The detected Ni concentrations were between 0 and 0.25 mg/kg in goat milk and reached 0.20 mg/kg in the single positive sheep milk sample. All detectable concentrations were below the MRLs of 1mg/kg; consequently, none of the analysed samples exceeded the Ni MRL (0%) (Table 1).

A similar pattern was observed for cadmium (Cd), which was below the detection limit in cow milk. The mean Cd concentration in goat and sheep milk exceeded the MRL of 0.0026 mg/kg, although only a small proportion of samples (6.7%) exceeded this limit. The detection of Ni and Cd mainly in goat and sheep milk may be associated with differences in dietary exposure and environmental conditions, as discussed before, as well as species- related differences in the uptake and transfer of metals into milk (Dugald Maclachlan, 1998)

The findings of the current research contrast with those reported by Boudebbouz et al. (2022), who found that 5.68% of the analysed milk exceeded the MRL for Ni, while 100% exceeded the corresponding MRL for Cd. Overall, the frequency of exceedance followed the order Cu > Cr > Fe > Cd > Ni, identifying copper and chromium as the primary contaminants of concern.

Compared with previously published studies (Table 2), heavy metal levels measured in the current research generally fall within the ranges reported for raw milk from different regions worldwide, including Algeria, Romania, Turkey, Sudan, Slovakia, China, Ethiopia, Egypt, Iran, India, and Libya. However, notable variations were observed among studies, particularly for Cu, Cr, and Fe, reflecting differences in environmental conditions, agricultural management, animal feeding regimes, grazing behavior, soil characteristics, industrial emissions, and species-specific physiological factors. These results indicate that trace metal accumulation in milk is affected by a complex interaction of environmental and biological determinants. Although the concentrations reported in the current research are broadly comparable with those described in the international literature, the relatively elevated levels of Cu and Cr were detected in some samples and warrant continued monitoring to evaluate potential environmental contamination and ensure food safety.

PCA for the assessment of trace metal variability in milk

The first two principal component analyses (dim 1 = 36.8% and dim 2 = 20.8%) collectively account for approximately 57.6% of the total variance, as shown in Figure 6, indicating a satisfactory representation of the data set variability. Dim 1 is mainly connected with cadmium (r = 0.63), iron (r = 0.80) and copper (r = 0.86), which are positively correlated and aligned along the positive axis. This suggests that these metals share similar sources or accumulation behaviours in milk samples. In contrast, chromium (r = 0.73) and nickel (r = 0.70) are strongly correlated with dim 2 and are positioned in the upper part of the plot (Figure

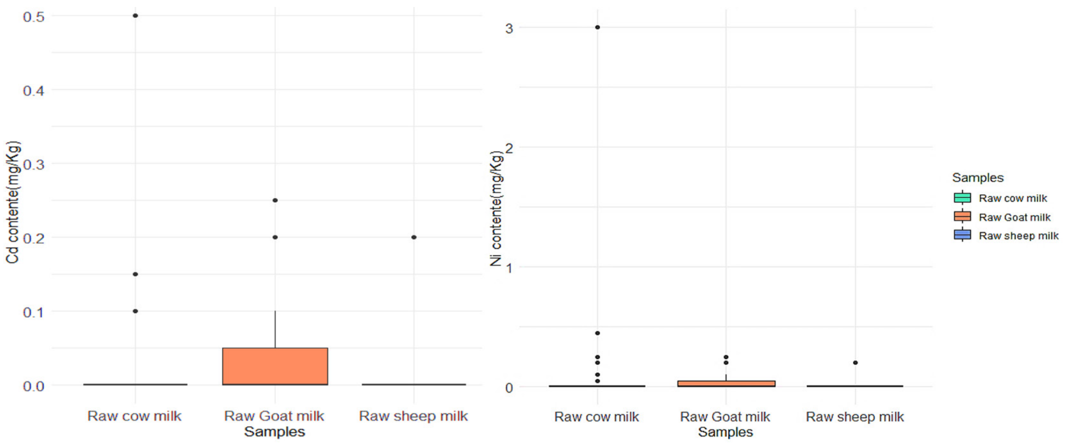

Figure 5. Box plot analysis representing cadmium (left) and Nickel (right) levels (mg/kg) in raw milk; cow, goat and sheep samples. Lines inside the box represent the median. Whiskers represent the spread of the data (minimum and maximum). Dots outside the whiskers are outliers

Table 2. Levels of Cr, Cu, Cd, Ni and Fe heavy metals in different milk types as documented in international literature (mg/kg)

Country/region (Observation)	Species	Heavy metal levels (mg/kg),					References
		Cr	Cu	Cd	Ni	Fe	
Algeria/ Guelma (Extensive farms)	Cow/ goat/ sheep	0.56 ± 0.05 0.70 ± 0.07 0.79 ± 0.07	0.86 ± 0.02 2.79 ± 0.04 1.99 ± 0.04	BDL 0.03 ± 0.003 0.006 ± 0.02	BDL 0.05 ± 0.01 0.013 ± 0.05	5.49 ± 21.93 9.77 ± 0.69 1.78 ± 0.16	The current study
Algeria/Bejaïa	Goat	-	0.05 ± 0.001	0.018± 0.001	-	-	(Lebsir et al., 2023)
Algeria/Guelma	Cow	0.18 ± 0.20	0.14 ± 0.08	0.03 ± 0.01	0.39 ± 0.68	0.76 ± 1.25	(Boudebbouz et al., 2022)
Turkey (Close to highways)	Cow	0.13 ± 0.006	0.62 ± 0.01	0.039 ± 0.002	-	4.2 ± 0.16	(Bigucu et al., 2016)
Slovak (Agricultural sector)	Cow	-	2.12± 0.61	0.27 ± 0.006	0.84 ± 0.13	4.2 ± 0.16	(Capcarova et al., 2019)
Algeria/Bejaïa	Goat	-	0.05 ± 0.0024	0.015 ± 0.0021	-	-	(Balli et al., 2023)
Romania/Rodnei (Mountains)	Cow	-	0.047	0.001	-	-	(Cadar et al., 2015)
Ethiopia (Close to highways)	Cow	-	0.09 ± 0.12	BDL	BDL	-	(Hussen et al., 2014)
Ethiopia	Cow	0.64 ± 0.029	1.12 ± 0.06	0.29 ± 0.01	-	-	(Akele et al., 2017)
Egypt (Industrial air pollution)	Cow	0.35 ± 0.10	1.2 ± 0.4	0.02 ± 0.002	-	5.1 ± 1.3	(El Sayed et al., 2011)
Iran	Goat	-	-	-	0.04	-	(Arianejad et al., 2015)
India (High- altitude)	Cow	-	0.30± 0.01	0.0089 ± 0.0002	-	4.91 ± 0.37	(Giri et al., 2021)
Libya	Cow	-	-	0.0009	-	-	(Elatrash & Atoweir, 2014)
China	Cow	-	0.03	0.00007	0.0057	0.35	(Zhou et al., 2017)
Slovak (Contaminated areas)	Sheep	-	0.14 ±0.06	1.66 ±1.86	-	-	(Stanovi et al., 2016)
Romania	Sheep	4.58 ± 1.65	808.72 ± 134.94	0.21 ± 1.60	1.44 ± 0.05	-	(Fechete et al., 2024)

Note: SD standard deviation, BDL below detection limit, - relevant data were unavailable.

6), indicating a different origin or geochemical behaviour. Principal component analysis is applied to simplify complex datasets, converting correlated variables into a reduced set of independent components that preserve most of the original information.

PCA results revealed a significant positive correlation between iron and cadmium, as indicated by the small angle between their vectors, while copper also shows a similar trend but with slightly lower contribution. Conversely, chromium and nickel are closely grouped, indicating a similar pattern of variability, possibly linked to environmental or feed-related factors (Elafify et al., 2023). The grouping of samples reveals spatial distribution between cow, goat and sheep milk, providing that the overall metal profiles are not completely distinct among species. However, goat milk samples tend to spread more widely along dim 1, suggesting higher variability in copper, iron and cadmium concentrations. In contrast, cow and sheep milk samples appear more clustered around the origin, indicating relatively homogeneous metal concentrations. These findings align with previous research (Donia et al., 2024; Sumner, 2020), highlighting that heavy metal distribution in milk is affected more by environmental conditions than by animal species alone.

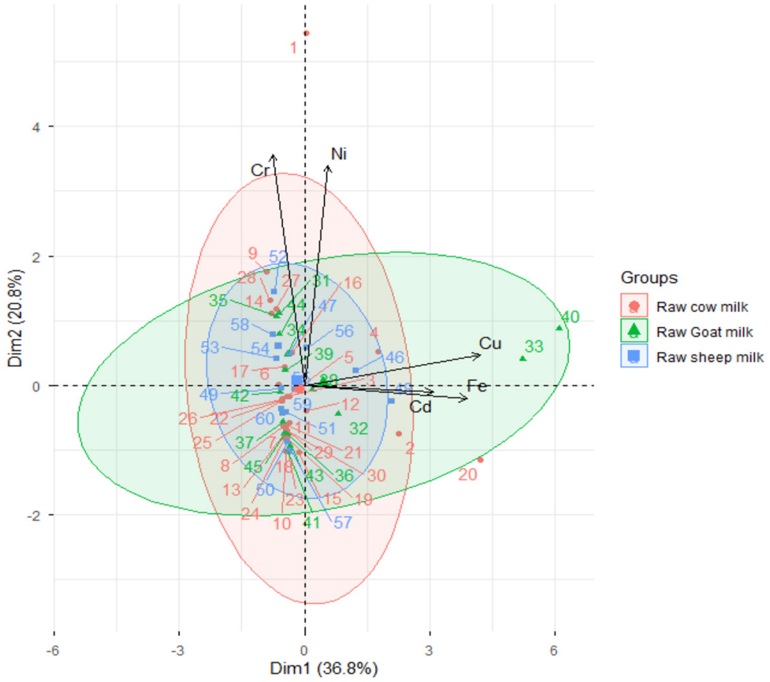

Figure 6. Principal component analysis (PCA) biplot illustrates the distribution of raw cow, goat and sheep milk samples according to their heavy metal composition as well as the relationships between the analysed elements (Cr, Ni, Cu, Fe, and Cd)

Human health risks assessment

Estimated daily intake

EDI values of iron, copper, chromium, cadmium and nickel via the consumption of raw cow, goat and sheep milk are illustrated in Table 3. Overall, for all detected metals, the EDI values were higher in children than in adults. Among the three milk types, raw goat milk showed the highest EDI values for most of the analysed metals in both population groups. In adults, the highest EDI values were observed for Fe (0.0326 mg/kg BW/day), followed by Cu (0.0093 mg/kg BW/day), Cr (0.0026 mg/kg BW/day), Cd (0.0001 mg/kg BW/day), and Ni (0.00017 mg/kg BW/day). Likewise, children consuming raw goat milk had the greatest dietary exposure, with EDI values of 0.0489, 0.0140, 0.0035, 0.00015, and 0.00025 mg/kg BW/day for Fe, Cu, Cr, Cd and Ni, respectively (Table 3).

Compared with respective oral reference doses (RfDs) established by (US EPA, 2024), the calculated EDI values for all investigated metals remained below the recommended thresholds. These results indicate that the intake of these metals via the consumption of raw milk is unlikely to pose significant non- carcinogenic health risks to either adults or children. Although children experienced relatively higher exposure than adults did, the estimated intakes remained well within the recommended safety limits. Similar findings have been documented by Boudebbouz et al. (2022), who attribute the higher exposure among children to their lower body weight rather than to their metal concentrations in milk.

Target hazard quotient (THQ) and total target hazard quotient (TTHQ)

Target hazard quotient and total target hazard quotient values of the studied heavy metals for both adults and children are presented in Table 4.

The estimated individual THQ values for the investigated metals (Cd, Cr, Cu, Fe and Ni) remain within acceptable regulatory limits for adults. With hazard indices below one, the data confirm that these milk types do not pose an active non-carcinogenic threat to consumers. Among the metals, chromium showed relatively higher THQ values compared to others, particularly in sheep milk (0.87) and goat milk (0.77), although still within the acceptable limit (Table 4). Conversely, the cumulative risk assessment demonstrated that goat milk (1.16) and sheep milk (1.05) exhibited TTHQ values slightly above the acceptable limit of one, suggesting that the combined exposure to multiple heavy metals could increase potential health risks. In contrast, cow milk (0.72)

Table 3. EDI values in raw cow, goat, and sheep milk samples for both adult and child individuals.

Category	Sample type	EDI (mg/kg BW/day)				
		Fe	Cu	Cr	Cd	Ni
Adult	RCM	0.0183	0.002867	0.001867	-	-
	RGM	0.032567	0.0093	0.002333	0.0001	0.000167
	RSM	0.005933	0.006633	0.002633	-	-
Child	RCM	0.02745	0.0043	0.0028	-	-
	RGM	0.04885	0.01395	0.0035	0.00015	0.00025
	RSM	0.0089	0.00995	0.00395	-	-

Note: RCM; Raw cow milk, RGM; Raw goat milk, RSM; Raw sheep milk.

Table 4. THQ and TTHQ values of raw cow, goat, and sheep milk samples for non-carcinogenic health risks for both adults and children

Individuals	Sample type	Fe	Cu	Cr	Cd	Ni
Adult	RCM	0.02	0.07	0.62	-	-
	RGM	0.04	0.23	0.77	0.1	0.008
	RSM	0.008	0,16	0.87	-	-
Child	RCM	0.17	0.48	4.2	-	-
	RGM	0.31	1.56	5.25	0.67	0.05
	RSM	0.05	1.11	5.92	-	-
TTHQ (Adult)	RCM			0.72		
	RGM			1.16		
	RSM			1.05		
TTHQ (Child)	RCM			4.86		
	RGM			7.86		
	RSM			7.10		

Note: RCM; Raw cow milk, RGM; Raw goat milk, RSM; Raw sheep milk.

remained within the safe range, indicating lower overall risk for adults.

For children, the situation was notably different. Several THQ values exceeded the safety threshold, particularly for chromium, which reached 4.2 in cow milk, 5.25 in goat milk and 5.92 in sheep milk. Copper also showed elevated THQ values, especially in goat milk (1.56) and sheep milk (1.11). These findings indicate a higher susceptibility of children to metal exposure due to their lower physiological mass and higher sensitivity to contaminants (Silanikove, 2000). The TTHQ values for children were significantly above the safe limit for all milk types, with values of 4.86 (RCM), 7.86 (RGM) and 7.10 (RSM), respectively.

Consequently, the consumption of these milk samples presents a potential non-carcinogenic health risk to children; these outcomes align with previously published findings where the most elevated THQ values were documented in Iran by Homayonibezi et al. (2021).

Homayonibezi et al. (2021) documented markedly elevated THQ values in Iran, where THQ values for Cr reached 19.6 for adults and 91.2 for children in Asuluyeh, while corresponding values of 16.9 and 79.0 were reported in Kaki. Likewise in Pakistan, Kabir et al. (2017) reported that THQ values of Cd were 2.64 for adults and 12.3 for children. Comparable findings have also been described in Algeria by Lebsir et al. (2023), who observed elevated THQ values of Cd in El Milia (0.554 adults; 2.58 children).

CONCLUSIONS

The current research provides valuable data regarding trace element levels in milk and their potential health implications. Several investigated metals, including copper, chromium and iron across the three species (cow, goat and sheep) surpassed the maximum residue limits set by

IDF, highlighting potential concerns regarding milk quality and consumer exposure. Nickel and cadmium were below the detection limit in cow milk but detected at low concentrations in goat and sheep milk. The first two principal component analyses (dim 1 = 36.8% and dim 2 = 20.8%) revealed distinct relationships among the investigated metals, collectively accounting for approximately 57.6% of the total variance, indicating a satisfactory representation of the data set variability. Although the estimated daily intakes of all investigated metals were below their respective oral reference doses (RfDs) established by the US EPA. In particular, all individual THQ values for the analysed metals (Cd, Cr, Cu, Fe and Ni) were within the safety limit for adults, while TTHQ values for goat milk and sheep milk slightly exceeded the safe limit. In contrast, the situation was notably different for children; several THQ values exceeded the safety threshold, and the TTHQ values were higher instead of those observed in adults, reflecting their lower body weight and potentially greater sensitivity to contaminants.

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