

Evaluation of Mineral and Trace Metals in Buffalo, Cow and Goat Milk with Special Emphasis on Health Risk Assessment

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Abstract

This study aims to evaluate minerals and trace metals in milk, emphasizing health risk assessment. The milk samples were collected from buffaloes, cows and goats. The sodium, potassium, calcium, and magnesium contents of the milk samples ranged from 30.55–58.36 mg/100 g, 95.7–138.04 mg/100 g, 102.34–142.14 mg/100 g, and 10.34–30.35 mg/100 g, respectively. The lead (Pb), selenium, and arsenic levels were below the limit of quantification (BLQ), indicating the minimal presence of these metals. Iron (Fe) and copper (Cu) were reported in the milk samples, with Fe concentrations exceeding Cu concentrations. Noncarcinogenic risk-related parameters such as estimated daily intake (EDI) and hazard index (HI) were also analyzed. In conclusion, a region's population is not at risk of noncarcinogenic health effects from heavy metal consumption through milk intake, as the HI is < 1.

Key words: Estimated daily intake, Hazard index, Heavy metals, Noncarcinogenic risk

Milk is often considered an ideal food due to its balanced nutritional profile. It offers quick energy and is easily digested and absorbed [1-2]. It is a rich source of proteins, vitamins, calcium, amino acids, fatty acids, and bioactive compounds that are essential for human growth and health, promoting strong bones and teeth [3]. Milk provides vital elements such as calcium (Ca), potassium (K), magnesium (Mg), sodium (Na), phosphorus (P), and chloride (Cl), in addition to trace elements like copper (Cu), iron (Fe), and chromium (Cr). The concentration of minerals and trace elements in raw milk, however, can fluctuate based on internal factors like lactation stage, breed, and the health of the cow, along with external influences such as seasonal changes, diet, and environmental conditions [4-7]. While many of these minerals and trace elements are vital for maintaining health, the presence of certain heavy metals—even in trace amounts—can pose significant health risks. Contamination may occur due to environmental pollution, industrial waste, agricultural runoff, or contaminated feed and water sources. Chronic exposure to toxic metals such as lead (Pb), cadmium (Cd), arsenic (As), and mercury (Hg) can have deleterious effects on human health, particularly in vulnerable populations such as infants and children who consume milk regularly.

Along with rapid industrialization and urban growth, the contamination of milk with metals has become a major concern, particularly in developing nations, because of the potential long-term health consequences. Toxic metals can enter animal systems through polluted land, water, air, and industrial activities [8]. High levels of hazardous elements in the environment, including the air, soil, water, and food chain, have raised significant global concerns [9]. Metal pollution sources include mining operations, urban runoff, sewage sludge,

synthetic fertilizers, fungicides, and pesticides [10-12]. While certain trace metals like iron (Fe), zinc (Zn), and copper (Cu) are essential in small amounts for human health due to their nutritional and medicinal properties [13-14]. Their excessive levels can be harmful and lead to various health complications [15-16]. Metals such as arsenic (As), cadmium (Cd), chromium (Cr), mercury (Hg), and lead (Pb) have no recognized biological role in human physiology and can be toxic even in minute quantities [17]. Long-term exposure to these toxic metals, even in small doses, can pose serious health risks, especially for infants and children [18-19]. These risks include a variety of health issues, such as organ dysfunction, respiratory issues, cognitive impairment, central nervous system disruptions, liver, prostate, and kidney problems, hypertension, ulcers, cancer, asthma, osteoporosis, hormonal imbalances, gastrointestinal problems, immune system weakening, and infertility [20-24]. The capacity of heavy metals to accumulate in organisms due to their long half-lives, high absorption rates, and low excretion rates amplifies their dangers as they move up the food chain [25]. Numerous studies have detected toxic heavy metals in milk [26-27]. To avoid toxicity, nonessential metals must be maintained below established safety limits, as their excessive levels can lead to serious health issues, even death [28]. Therefore, it is essential to monitor and regulate metal concentrations in milk to prevent harmful effects and ensure consumer safety.

India is the largest producer of milk globally, contributing to 24% of worldwide milk production. Milk production plays a crucial role in the economy and provides significant employment opportunities, particularly in Haryana. The state of Haryana is vital to India's dairy industry, with an annual milk production of 98.09 tonnes. This study aims to

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assess the levels of minerals and trace elements in buffalo, cow, and goat milk from various regions of Haryana, providing a scientific basis for enhancing milk quality and evaluating the related health risks.

MATERIALS AND METHODS

Study area and sample collection

Milk samples were collected from four cities, namely, Gurugram, Rohtak, Hisar, and Panipat of Haryana, in two different seasons (summer and winter). Cities have been chosen for potential milk production. Gurugram is situated southwest of Delhi and has a mean elevation of 217 m above sea level, with its location specified at 28° 7' 21" N and 77° 1' 44" E. Rohtak is an industrial city with an area of 1745 square km and an altitude of 220 m (720 ft). It is located at 3 0° 1' N and 75°

17' E. Hisar is located at 29.09°N and 75.43°E and has an average elevation of 215 above sea level, and Panipat is situated at 29.39°N, 76.97°E. Buffaloes and cattle were fed seasonal fodder, such as cotton balls, oil cakes (khal), straws (tuna), sugarcane tops, millets (bajra), fenugreek, and goats, which were used for grazing. Fresh samples of buffalo, cow, and goat milk were collected from four locations in two seasons—spring and winter. A total of one hundred twenty milk samples (sixty from each season) were collected from healthy lactating animals. Buffaloes, cows, and goats were randomly selected from each of the four cities. Several precautions were taken during milk sampling. The samples were collected in sterile 50 ml Falcon tubes and kept in an ice box. In the laboratory, milk samples were stored at -80°C until further examination. The average milk production was significantly high in the winter season.

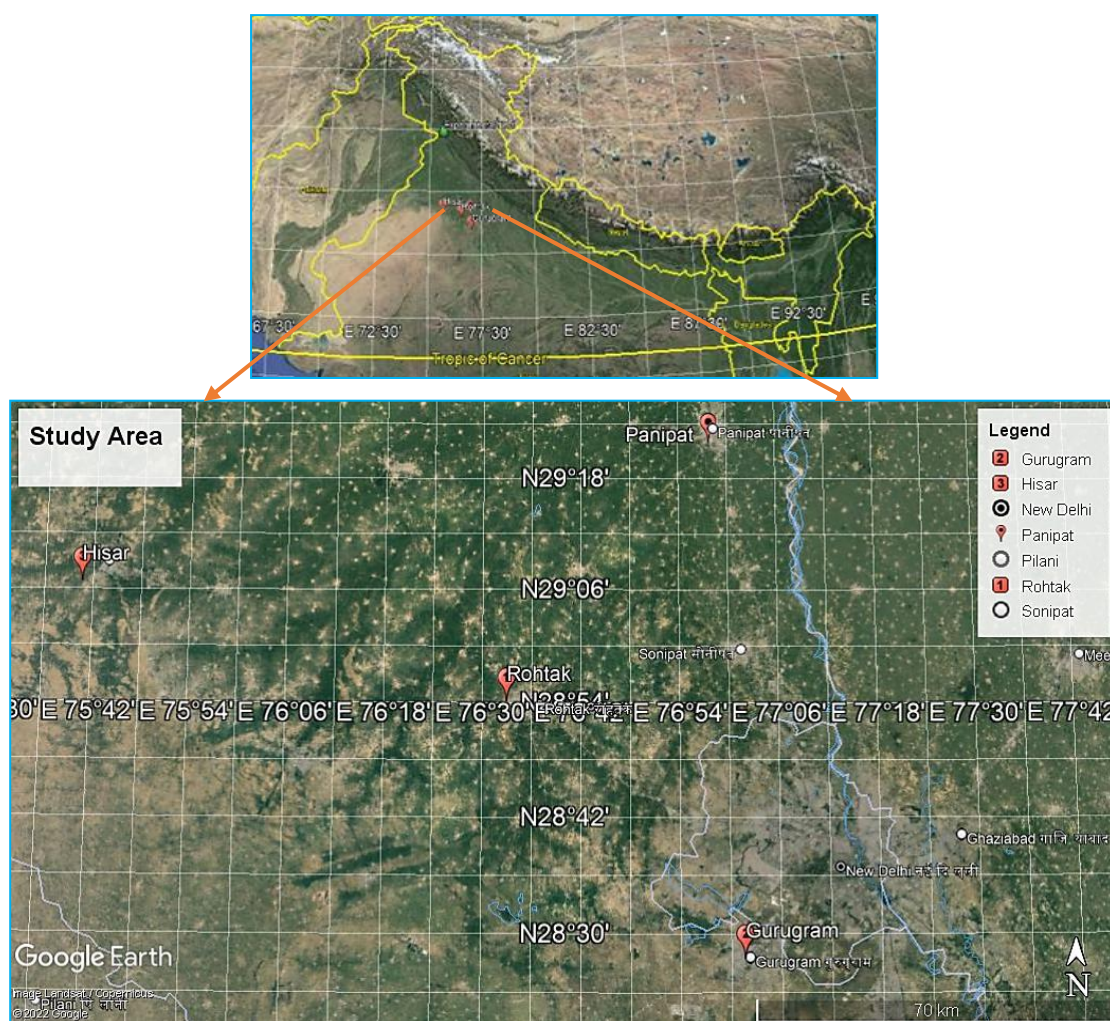


Fig 1 Sampling locations of milk samples

Sample processing

The process involves adding 10 ml of nitric acid to a 0.5 ml milk sample. The tube was incubated for 30 minutes at room temperature and then heated for 25 minutes at 70°C. After cooling, 2 ml of perchloric acid (HClO₄) was added, followed by heating for 25 minutes at 135°C. The solution was then treated with 2 ml of HClO₄ and 2 ml of HNO₃ and heated for another 25 minutes at 135°C. Finally, the solution was mixed with 2 ml HNO₃ and 5 ml HCl, cooled and transferred to a 50 ml Falcon tube. The volume was adjusted to 50 ml with distilled water and was examined via atomic absorption spectrometer (AAS) [29].

Health risk assessment

Human health risk assessment involves the measurement of parameters, including the average intake of metal (DIM) and health risk index (HRI), via different formulas. The average daily intake of metal was calculated with Eq. (1) [30-32].

Estimated daily intake of metal (EDI)

$$EDI = \frac{C_{\text{metal}} \times D_{\text{food intake}}}{BW} \dots\dots\dots \text{Eq. (1)}$$

where C_{metal} is the estimated daily intake of metal in milk (mg/kg), $D_{\text{food intake}}$ is the average daily intake of milk, i.e., 90 ml [33], and $B_{\text{body weight}}$ is the average body weight of an Indian adult, which is 60 kg, and that of children is 15 kg for the study.

Health risk index (HRI)

The health index proposed by the U.S. Environmental Protection Agency defines the health risk index as a maximum daily intake limit that does not result in any hazardous health effects [34]. It is the ratio of the average daily intake of a metal ($\text{mg kg}^{-1}\text{d}^{-1}$) to the oral reference dose of that metal (Eq. 2). The oral RfD for copper (Cu) is 0.04 mg/kg/day, and that for iron (Fe) is 0.7209 mg/kg/day.

$$\text{HRI} = \frac{\text{EDI}}{\text{RfD}} \dots\dots\dots \text{Eq. (2)}$$

If the value of the health risk index (HRI) is greater than 1, there is a possibility of health risk in the exposed population [35-36].

The total HRIs were calculated by adding the hazard quotient (HQ) of each metal, as shown in Eq. 3 [37].

$$\text{THRI} = \text{HRI (metal1)} + \text{HRI (metal 2)} \dots\dots\dots \text{Eq. (3)}$$

Statistical analysis

Analysis of variance (ANOVA) via SPSS was performed to evaluate the significant variations among the four locations, followed by Tukey's post hoc test to detect the significance of differences among the treatments at a 95% confidence interval. In addition, a t test was performed to observe seasonal variation. All the data are presented as the means \pm standard errors.

RESULTS AND DISCUSSION

Variations in the mineral concentrations of buffalo, cow, and goat milk collected from various districts of Haryana during the winter and summer seasons

Sodium

Sodium is the primary ion in extracellular fluid and regulates nerve and muscle function, plasma volume, and acid-base balance. The sodium content in the buffalo milk samples varied across different districts, ranging from 30.55 to 58.36 mg/100 g. This finding is comparable to that of a previous study that reported a sodium content of 42.39 mg/100 g in buffalo milk [22]. In contrast, the sodium content in cow milk samples ranged from 48.80 to 35.48 mg/100 g. Similarly, the sodium content in the goat milk samples also varied across different districts. The highest concentration was found in Hisar (50.75 ± 1.22 mg/100 g), while the lowest was in Panipat (35.11 ± 1.05 mg/100 g). This range is comparable to that reported in previous studies, which reported a sodium content of 54.06 mg/100 g in goat milk³⁶. Moreover, other studies have reported sodium contents in goat milk of 52.89 mg/100 g, 0.037 g/100 g, 118 mg L⁻¹ and 150 mg L⁻¹ [38-40].

Potassium

Potassium is a vital intracellular ion. It is crucial for nerve impulse transmission, muscle function, and blood pressure regulation. The potassium content in milk samples from buffaloes, cows, and goats ranged from 95.75–133.20, 99.10–125.27, and 104.22–138.04 mg/100 g, respectively. Previous studies reported potassium concentrations of 118.05 mg/100 g and 144.88 mg/100 g in buffalo and cow milk, respectively [22]. Previous studies have reported a wide range of potassium contents in goat milk: 174.85 mg/100 g, 0.203 g/100 g, 355.1 mg L⁻¹, and 1,690 mg L⁻¹ [38-40].

Calcium

Calcium is an essential mineral crucial for developing tissues, bones, and teeth. Adequate intake is needed to maintain

bone health and reduce the risk of osteoporosis. The calcium content in milk samples from buffaloes, cows, and goats ranged from 105.58–157.70, 106.75–136.65, and 102.34–142.14 mg/100 g, respectively. Previous studies reported higher calcium contents in buffalo milk, at 178.59 mg/100 ml and 204 mg/100 g, respectively; and in cow milk, at 120.24 mg/100 and 134.87 mg/100 g, respectively [41]. Additionally, other studies have reported calcium concentrations in goat milk of 1,620 mg/L and 0.114 g/100 g [38-39].

Magnesium

The magnesium content in milk samples from buffaloes, cows, and goats ranged from 10.34-30.35, 12.00-26.65, and 12.00-28.45 mg/100 g, respectively. These values are comparable to those of previous studies, which reported magnesium contents of 8.29 mg/100 ml and 23.53 mg/100 g in buffalo milk and 12.65 mg/100 and 10.87 mg/100 g in cow milk [22], [41]. Previous studies reported magnesium contents in goat milk of 19.94 mg/100 ml, 10.81 mg/100 g, and 0.014 g/100 g [22], [38], [41].

Trace elements

The concentrations of trace metals in milk samples, as presented in (Table 1), revealed that lead (Pb), selenium, and arsenic levels were below the limit of quantification (BLQ), indicating the minimal presence of these metals. Iron (Fe) and copper (Cu) were reported in the milk samples, with Fe concentrations exceeding those of Cu. However, other studies have reported varying levels of Pb, ranging from 0.04 to 4.404 ppm [42-45]. Arsenic has also been detected in cow milk, with a mean value of 0.01 mg L⁻¹ in industrialized areas of Dhaka, Bangladesh [46]. Additionally, 0.029 mg/kg and 0.11 ± 0.08 mg/kg selenium has been detected in goat milk [38], [47].

Iron

Iron is essential for various bodily functions, including red blood cell production, infection prevention, and immune system function. It is a key component of hemoglobin and facilitates oxygen transport. In this study, the iron content in buffalo milk ranged from 0.04–0.14 mg/100 g (0.10 ± 0.01 in winter and 0.07 ± 0.00 in summer), that in cow milk ranged from 0.01–0.09 mg/100 g (0.06 ± 0.00 in winter and 0.05 ± 0.01 in summer), and that in goat milk ranged from 0.03–0.09 mg/100 g (0.07 ± 0.01 in winter and 0.06 ± 0.01 in summer). These findings align with those of previous studies, which reported iron levels between 0.078 and 0.15 mg/100 g [48-49]. Notably, these concentrations are relatively high, ranging from 0.36-9.07 mg L⁻¹ [39-40], [47], [50].

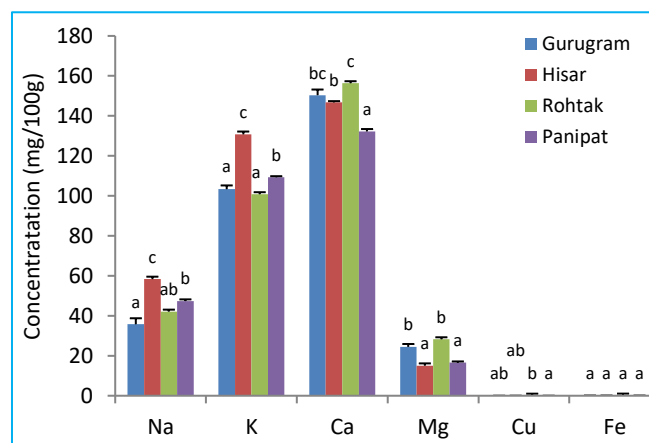


Fig 2 Graph representing mineral and trace element contents in buffalo milk collected during the summer and winter seasons

Copper

Copper plays a vital role in various bodily functions, including assisting in iron absorption and supporting enzyme function in glucose metabolism and hemoglobin synthesis. It is crucial for the proper functioning of multiple enzymes, including energy production (cytochrome C oxidase), antioxidant protection (superoxide dismutase), pigment formation (tyrosinase), collagen and elastin formation (lysyl oxidase), and blood clotting (clotting factor V). However, milk and dairy products are not significant sources of copper. The copper content in buffalo, cow, and goat milk ranged from 0.01–0.04 mg/100 gm, 0.01–0.05 mg/100 gm, and 0.01–0.02 mg/100 gm, respectively. Previous studies reported similar values, i.e., 0.36 mg L⁻¹ and 0.12 mg L⁻¹, respectively [39],

[47]. On the other hand, some studies reported higher cu values in the range of 0.0016-0.5 mg/100gm [51-53]. Similarly, some researchers reported copper levels in milk samples of 0.33 mg/kg and 0.14 µg/kg, respectively by [54].

Copper (Cu) and iron (Fe) are essential nutrients that play crucial roles in dairy product quality and nutritional value. However, excessive levels of these metals can harm dairy technology, as they can catalyze lipid oxidation, leading to quality defects and spoilage. Specifically, concentrations of Fe and Cu exceeding 1.5 mg/kg in milk can limit the shelf-life of cream and butter, making long-term storage challenging. Copper (Cu) has a set MRL of 1.5 mg/kg in milk and dairy products, as specified by the Codex Alimentarius Commission [55].

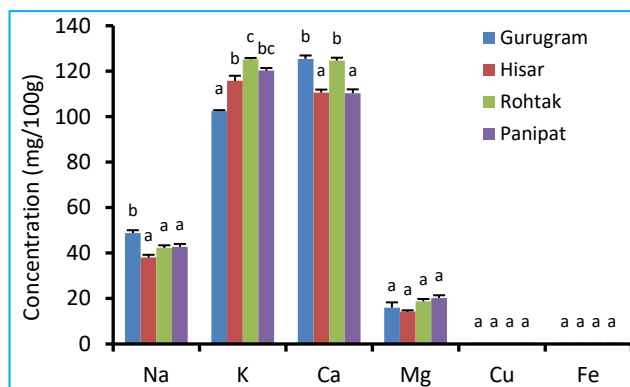


Fig 3 Graph showing the mineral and trace element contents in cow milk collected during the summer and winter seasons

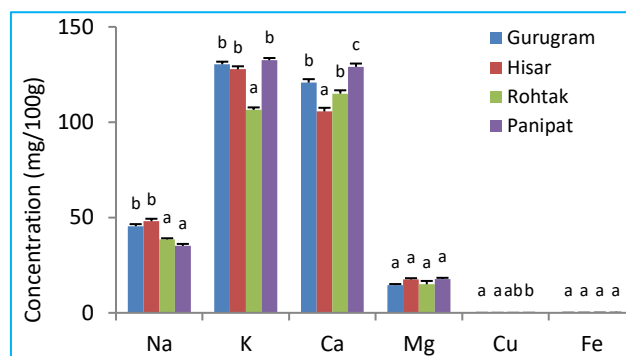
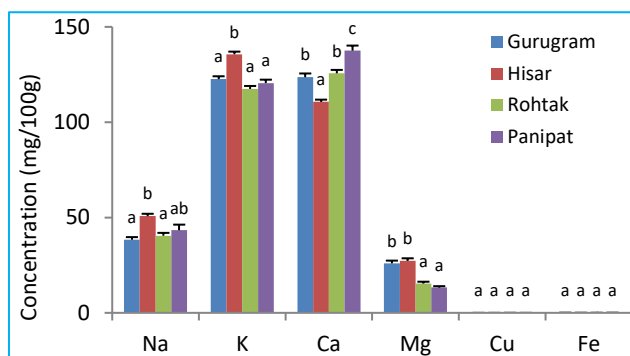
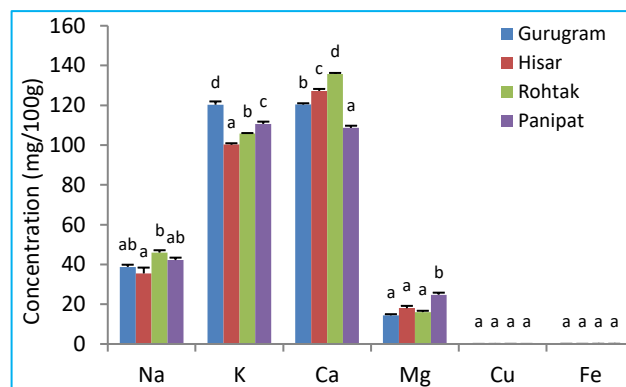


Fig 4 Graph representing mineral and trace elements in goat milk collected during the summer and winter seasons

Seasonal variation in mineral and trace element levels

The mineral compositions of buffalo, cow, and goat milk exhibit seasonal variations between winter and summer. These seasonal variations may be attributed to factors such as changes in cattle feed, climate, and regional differences. In buffalo milk, the sodium and potassium contents are significantly greater in

winter. Conversely, the calcium and magnesium levels were higher in the summer than in the other months, as shown in (Fig 5). Cow milk and goat milk presented relatively high concentrations of calcium and magnesium in summer, as shown in (Fig 6-7). Notably, copper and iron levels remained relatively consistent across both seasons [56-57].

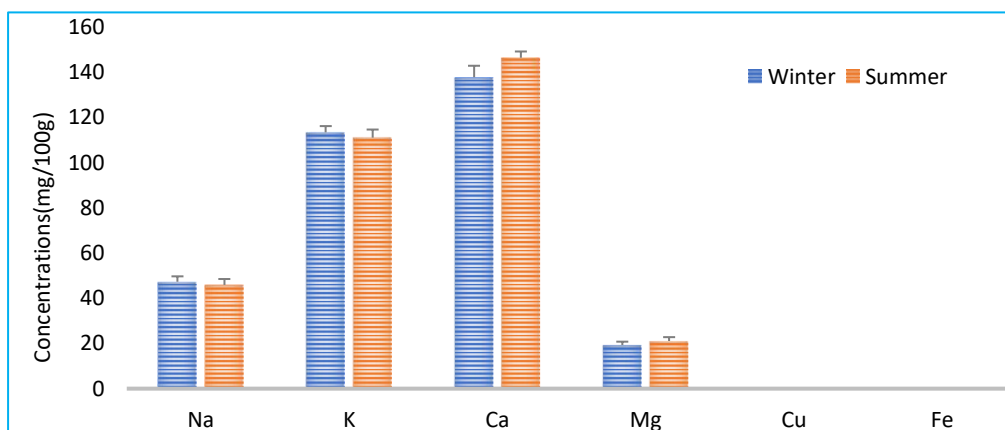


Fig 5 Mineral and trace element levels in buffalo milk during the winter and summer seasons

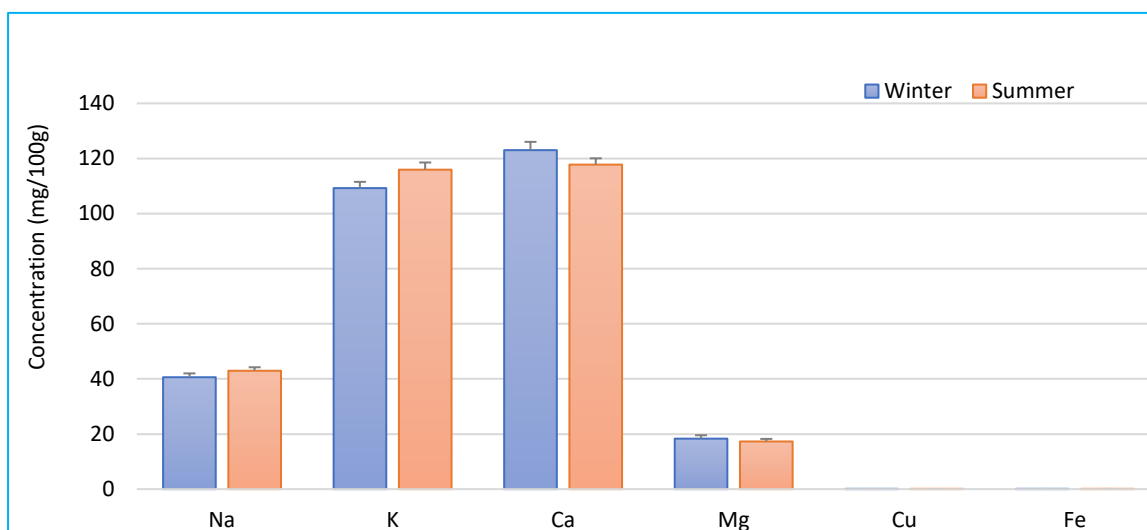


Fig 6 Mineral and trace element levels in cow milk during the winter and summer seasons

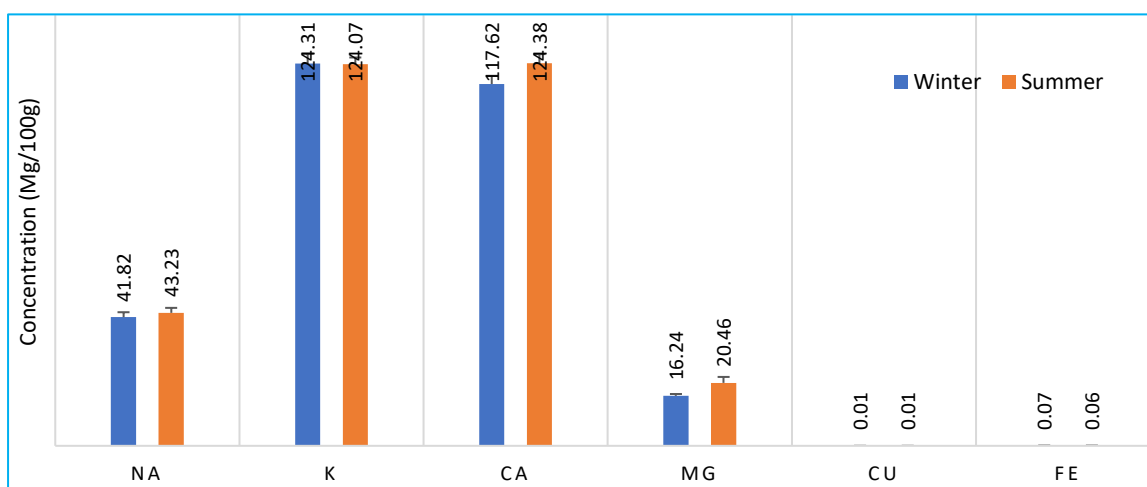


Fig 7 Mineral and trace element levels in goat milk during the winter and summer seasons

Estimated daily intake

The copper levels in the milk samples analyzed in this study are within safe limits, well below the toxicity threshold of 0.4 mg/L [58]. Furthermore, the daily copper intake from these milk samples is significantly lower than the recommended daily intake of 2–3 mg/day [58], ranging from 0.00013–0.0039 mg/day for adults and 0.00044–0.00179 mg/day for children.

The daily iron intake ranged from 0.0001–0.0015 for adults and 0.0004–0.0060 for adults, as shown in (Table 1). These findings suggest that milk and milk products are not significant copper or iron sources. The levels of copper and iron in the analyzed milk samples are well within safe limits, contributing minimally to daily intake and posing no significant health risk for both adults and children.

Table 1 Estimated daily intake (EDI) of buffalo, cow, and goat milk in the summer season

	Species	Gurugram	Hisar	Panipat	Rohtak
Copper (adult)	Buffalo	0.00015	0.00030	0.00015	0.00045
Copper (child)		0.00060	0.00120	0.00060	0.00180
Iron (adult)		0.00120	0.00120	0.00105	0.00090
Iron (child)		0.00480	0.00480	0.00420	0.00360
Copper (adult)	Cow	0.00030	0.00030	0.00045	0.00015
Copper (child)		0.00120	0.00120	0.00180	0.00060
Iron (adult)		0.00060	0.00090	0.00090	0.00060
Iron (child)		0.00240	0.00360	0.00360	0.00240
Copper (adult)	Goat	0.00015	0.00015	0.00011	0.00030
Copper (child)		0.00060	0.00060	0.00042	0.00120
Iron (adult)		0.00120	0.00075	0.00120	0.00060
Iron (child)		0.00480	0.00300	0.00480	0.00240

Hazard quotient and health risk index

This study evaluated the non-carcinogenic risk of heavy metal consumption through milk intake in a region's population.

The hazard index (HI) and total hazard index (THI) were used to assess risk. The results revealed that all HI values for both seasons were below the threshold (< 1), indicating that there

was no significant health risk. The THI, which considers the cumulative risk of multiple metals, was also below the threshold (< 1) for summer, winter, and overall. Therefore, the region's population is not at risk of non-carcinogenic health effects from

heavy metal consumption through milk intake, as the HI is < 1. These findings suggest that the milk consumed in the region is safe from heavy metal contamination. These findings are consistent with those of previous studies [59-62].

Table 2 Estimated daily intake (EDI) of buffalo, cow, and goat milk in the winter season

Attributes	Species	Gurugram	Hisar	Panipat	Rohtak
Copper (adult)	Buffalo	0.00030	0.00015	0.00060	0.00045
Copper (child)		0.00120	0.00060	0.00240	0.00180
Iron (adult)		0.00135	0.00180	0.00150	0.00135
Iron (child)		0.00540	0.00720	0.00600	0.00540
Copper (adult)	Cow	0.00030	0.00045	0.00030	0.00075
Copper (child)		0.00120	0.00180	0.00120	0.00300
Iron (adult)		0.00090	0.00075	0.00105	0.00105
Iron (child)		0.00360	0.00300	0.00420	0.00420
Copper (adult)	Goat	0.00009	0.00011	0.00030	0.00015
Copper (child)		0.00036	0.00042	0.00120	0.00060
Iron (adult)		0.00075	0.00090	0.00105	0.00135
Iron (child)		0.00300	0.00360	0.00420	0.00540

Table 3 Health risk index (HRI) of buffalo, cow, and goat milk in the summer season

Attributes	Species	Gurugram	Hisar	Panipat	Rohtak
Copper (adult)	Buffalo	0.004	0.008	0.004	0.011
Copper (child)		0.015	0.030	0.015	0.045
Iron (adult)		0.002	0.002	0.001	0.001
Iron (child)		0.007	0.007	0.006	0.005
Copper (adult)	Cow	0.008	0.008	0.011	0.004
Copper (child)		0.030	0.030	0.045	0.015
Iron (adult)		0.001	0.001	0.001	0.001
Iron (child)		0.005	0.004	0.006	0.006
Copper (adult)	Goat	0.000	0.004	0.003	0.008
Copper (child)		0.001	0.015	0.011	0.030
Iron (adult)		0.030	0.001	0.002	0.001
Iron (child)		0.120	0.004	0.007	0.003

Table 4 Health risk index (HRI) of buffalo, cow and goat milk in the winter season

Attributes	Species	Gurugram	Hisar	Panipat	Rohtak
Copper (adult)	Buffalo	0.008	0.004	0.015	0.011
Copper (child)		0.030	0.015	0.060	0.045
Iron (adult)		0.002	0.002	0.002	0.002
Iron (child)		0.007	0.010	0.008	0.007
Copper (adult)	Cow	0.008	0.011	0.008	0.019
Copper (child)		0.030	0.045	0.030	0.075
Iron (adult)		0.001	0.001	0.001	0.001
Iron (child)		0.005	0.004	0.006	0.006
Copper (adult)	Goat	0.002	0.003	0.008	0.004
Copper (child)		0.009	0.011	0.030	0.015
Iron (adult)		0.001	0.001	0.001	0.002
Iron (child)		0.004	0.005	0.006	0.007
THRI		0.107	0.112	0.175	0.195

CONCLUSION

In conclusion, our investigation into milk quality in Haryana has revealed significant insights with implications for both health and sustainability. The absence of increased heavy metal content in the analyzed milk samples indicates that the present agricultural practices might effectively mitigate the risk of contamination and address concerns about the rise in industrialization. Moreover, the present study also highlights the need for caution and proactive strategies. By prioritizing environmentally friendly practices and regular monitoring, we can safeguard public health and promote a thriving dairy

industry that benefits both people and the planet. This research serves as a test of the power of responsible agricultural practices and the pursuit of human well-being, paving the way for a healthier and more sustainable future in the dairy sector by prioritizing people's well-being and environmental supervision.

Contributors

Mrs. Neeru Yadav has completed her Master's degree in Bioscience from Banasthali University, Jaipur, Rajasthan. She is currently pursuing her Ph. D. work in Baba Mastnath University, Rohtak, Haryana. Her research area is environmental toxicology. She collected all the samples,

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