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Microbiological safety, adulteration, and heavy metal–associated health risks in raw cow and buffalo milk from Punjab, Pakistan

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Abstract: Although milk is an essential part of human nutrition, its safety is still a serious public health problem in poorer nations where raw milk intake is widespread. This study compares the microbiological quality, physicochemical composition, adulteration, heavy metal contamination, and related health hazards of raw cow and buffalo milk from Central and Southern Punjab, Pakistan. Eighty samples were analyzed utilizing common techniques, such as USEPA risk models and ICP-AES. Microbiological safety criteria were not met by either kind of milk, with buffalo milk exhibiting far greater levels of contamination. Additionally, buffalo milk had a better nutritional makeup with greater levels of fat, protein, and total solids. Nevertheless, extensive adulteration was found, including the addition of water (12.5–14.8%) and the presence of neutralizers (8–10%) and detergents (5–7%). Permissible limits were surpassed by heavy metals, especially lead (0.025–0.038 mg/L) and arsenic (0.010–0.014 mg/L). Total Target Hazard Quotient (7.2–12.5), Hazard Index (8.5–15.0), and lifetime Cancer Risk (1.2×10^{-3}–2.5×10^{-3}) were all found to be above safe criteria in the health risk assessment. These results point to substantial health risks, both carcinogenic and non-carcinogenic, particularly for groups with heavy consumption. Despite the exceptional nutritional qualities of buffalo milk, environmental pollution, adulteration, and inadequate cleanliness impair its safety, underscoring the need for more stringent regulation and oversight.

Keywords: Raw cow milk; Raw buffalo milk; Microbiological quality; Heavy metals; Health risk assessment

1. Introduction

Milk is defined as the lacteal secretion obtained from the complete milking of one or more healthy dairy animals, excluding colostrum and secretions produced within 15 days before and 5 days after calving. According to the Pasteurized Milk Ordinance of the United States Public Health Service, milk must contain a minimum of 8.25% solids-not-fat and not less than 3.25% fat (Hussain et al., 2010). Historically, milk consumption dates back to approximately 4000 BC, and milk from various species—including cow, buffalo, goat, ewe, and sheep—has long been utilized as a primary nutrient source and for manufacturing dairy products such as yogurt, cheese, butter, and cream due to its high biological and nutritional value (Wassay et al., 2025).

Milk is a complex biological fluid composed of approximately 87% water, 3.5% fat, 3.3% protein, 4.9% lactose, and 0.7% minerals. Milk fat mainly consists of triacylglycerides (≈98%), along with minor fractions of free fatty acids, diacylglycerides, and cholesterol, and serves as a carrier for fat-soluble vitamins A, D, and E. Owing to the presence of more than 400 fatty acids—approximately 70% of which are saturated—milk fat exhibits

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considerable compositional complexity. Proteins in milk are primarily divided into casein (≈80%) and whey proteins (≈20%), both of which contribute significantly to the functional and nutritional properties of dairy products (Gustavsson et al., 2014).

Cow milk remains the most widely consumed and commercially utilized milk worldwide, with Holstein-Friesian cows being the dominant breed for dairy production (Riaz et al., 2026). Growing consumer awareness and evolving dietary trends have shifted the concept of milk quality beyond basic composition toward enhanced nutritional value, improved fatty acid profiles, and better technological properties, particularly for cheese manufacture and functional dairy products (Smit et al., 2000). Milk is also a vital source of essential minerals, which, despite their low concentrations, play crucial roles in physiological and metabolic processes (Khalid et al., 2025). These minerals exist in milk as free ions, salts, or in association with proteins, lipids, and carbohydrates, and their concentrations vary according to breed, feeding regime, and farm management practices (Stocco et al., 2019). Lactose, the principal carbohydrate in milk, contributes significantly to milk's energy value and total solids, with its concentration influenced by feed quality and geographical factors (Cashman, 2006).

Pakistan is among the world's leading milk-producing countries, ranking fourth globally after India, China, and the United States. The national livestock population comprises approximately 38.8 million buffaloes and 46.1 million cattle, with buffalo milk accounting for nearly 68% of total milk production due to the superior adaptability of buffaloes to local climatic conditions. Punjab province alone contributes nearly 49% of the country's total milk output (Yasmin et al., 2012). Buffalo milk represents more than 12% of global milk production and dominates dairy output in tropical and subtropical regions because of buffaloes' disease resistance and ability to utilize low-quality fodder efficiently (Deb et al., 2016). Pakistan and India together account for nearly 90% of global buffalo milk production (Medhammar et al., 2012).

Despite its economic and nutritional importance, raw milk in Pakistan faces severe safety challenges. It is estimated that approximately 6.75 billion liters of milk are lost annually during the value addition chain due to unhygienic handling, poor storage conditions, and lack of technical awareness among farmers, traders, and retailers (King, 2022). Numerous studies have reported widespread microbiological contamination and adulteration in raw milk from Central and Southern Punjab. Elevated total coliform counts exceeding international guidelines have been documented, with Escherichia coli detected in 40% of cow milk and 46.67% of buffalo milk samples (Gurung & Poudel, 2024). Adulteration practices are alarmingly prevalent, with added water detected in 77.89% of samples, alongside chemical adulterants such as detergents, cane sugar, and neutralizers (Ibrahim et al., 2023).

Beyond microbiological hazards and economic fraud, chemical contamination poses serious public health concerns. Carcinogenic risk assessments have revealed total target health quotient values ranging from 6.92 to 42.44 in buffalo milk, indicating significant long-term health risks, particularly in regions where wastewater-irrigated fodder is commonly used (Iqbal et al., 2020). These findings highlight the compounded risks associated with raw milk consumption, including microbial pathogens, toxic residues, and carcinogenic exposure (Asghar et al., 2025).

Given the extensive consumption of raw milk in Pakistan and its critical role in human nutrition, there is an urgent need for a comprehensive assessment of milk safety. Therefore, the present study aims to evaluate the microbiological quality, adulteration practices, and carcinogenic risks associated with raw cow and buffalo milk collected from Central and Southern Punjab, linking safety risks with species, region, and handling practices to inform evidence-based public health interventions and regulatory control strategies.

2. Materials and Methods

2.1. Materials

Glass bottles (for milk sample collection), Icebox (for protection of samples from direct sunlight or microbes and samples transport), Gloves (used during sample collection), Volumetric pipets, beakers, flask, thermometer, cylinder, filter paper, conical glass tube with a stopper, centrifugal machine, water bath, pH meter, butyrometer, Kjeldhal's Apparatus, and lactometer. 0.1 N NaOH, phenolphthalein, sulfuric Acid, isoamyl alcohol, 40% sodium hydroxide, boric acid, methylene blue, copper sulfate, sodium tartrate salt, and 5% acetic acid.

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2.2. Procurement of Raw Material

Sampling was done four times a month from the dairy farm of the central and southern regions of Punjab. Samples from both morning and evening milking were collected. A total of 80 samples were collected in 3 months (from August 2021 to October 2021), including 40 samples from the crossbreed of cow and 40 samples from the Nilli Ravi buffalo. Glass bottles were sterilized before sample collection in the laboratory. Bottles were labelled with Date, region, time of sample collection, and the temperature of the sample was checked at the time of sampling. Samples were placed in the ice box for transportation to the laboratory for analysis. The analysis was performed at the Dairy Lab of the National Institute of Food Science and Technology, University of Agriculture, Faisalabad.

2.3. Microbiological Analysis

Microbiological analysis of the milk samples was conducted following standard methods to suit local laboratory conditions. For the total plate count, serial dilutions of milk samples were prepared using sterile 0.1% peptone water. Aliquots (1 mL) of each dilution were plated on Plate Count Agar and incubated at 37°C for 48 hours, after which colonies were counted and expressed as $\times 10^3$ CFU/mL. Coliform bacteria were enumerated by plating the diluted samples on Violet Red Bile Agar and incubating at 37°C for 24 hours, with typical colonies counted as CFU/mL. Escherichia coli was detected on Eosin Methylene Blue agar, and characteristic colonies displaying a metallic green sheen were counted. Staphylococcus aureus was enumerated using Baird-Parker agar supplemented with egg yolk tellurite, with black, shiny colonies with clear zones counted as CFU/mL after incubation at 37°C for 48 hours. Yeasts and molds were enumerated by plating diluted samples on Potato Dextrose Agar and incubating at 28°C for five days.

2.4. Physicochemical Composition and Adulteration Analyses

Raw milk samples for physicochemical and adulteration analyses were collected from 40 cow milk and 40 buffalo milk samples from dairy farms located in Central and Southern Punjab, Pakistan. The collection was carried out over a three-month period (August–October 2021), with samples obtained during both morning and evening milking sessions. Sterile glass bottles were used for collection, labeled with the date, region, and time of collection, and maintained in an ice box at 4°C during transport to the Dairy Laboratory at the National Institute of Food Science and Technology, University of Agriculture, Faisalabad.

The fat content of milk was determined using the Gerber method, as described by Gautam and Pantha (2023), which involves centrifugation of milk with sulfuric acid in a calibrated butyrometer to separate fat. Protein content was estimated using the Kjeldahl method, where the total nitrogen content of milk was measured and converted to protein using a conversion factor, following procedures validated by Shetty et al. (2020). The lactose content was determined using the phenol-sulfuric acid method, and total solids were measured by the oven-drying method at 105°C until constant weight, in accordance with Arjuna (2017). The pH of milk was recorded using a calibrated digital pH meter, following the procedure outlined by Singh et al. (2013).

Adulteration analyses were carried out to detect common contaminants in raw milk. The percentage of added water was estimated using a lactometer by comparing the density of samples against standard values for cow and buffalo milk (Arjuna, 2017). Detergent detection was performed using the foaming test, where milk was vigorously shaken, and the height of persistent foam was measured, as described by Gautam and Pantha (2023). The presence of neutralizers was detected using methyl red indicator solution, which changes color in the presence of alkaline substances (Singh et al., 2013). All measurements were carried out in triplicate, and results were expressed as mean ± standard deviation. Statistical analysis was performed using ANOVA, and differences between cow and buffalo milk were considered significant at $p < 0.05$.

2.5. Heavy Metal Contamination

Raw milk samples were collected from 40 cow milk and 40 buffalo milk samples from dairy farms in Central and Southern Punjab, Pakistan, over a three-month period (August–October 2021). Samples were obtained during morning and evening milking sessions, collected in sterile glass bottles, and immediately stored in an ice box at 4°C for transportation to the Dairy Laboratory at the National Institute of Food Science and Technology, University of Agriculture, Faisalabad. All samples were analyzed within 24 hours of collection to prevent changes in composition or contamination.

The concentrations of heavy metals—Lead (Pb), Cadmium (Cd), Arsenic (As), and Mercury (Hg)—in raw milk samples were determined using Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES), following procedures described in previous studies (Abid et al., 2024; Kambli et al., 2019; Najarnezhad et al., 2015; Rahimi, 2013). Milk samples were first digested using a mixture of concentrated nitric acid (HNO_3) and perchloric acid ($HClO_4$) in a 3:1 ratio. The digestion was carried out on a hot plate until a clear solution was obtained, and the resulting digests were filtered and diluted with deionized water to a final volume suitable for ICP-AES analysis.

Calibration standards for each metal were prepared from certified stock solutions to ensure accuracy and precision. The ICP-AES instrument was calibrated using standard multi-element solutions, and each sample was analyzed in triplicate. The mean concentrations of metals were reported as mg/L ± standard deviation. Statistical analysis was conducted using ANOVA, and differences between cow and buffalo milk were considered significant at $p < 0.05$. All glassware and plasticware used were pre-cleaned with 10% nitric acid and rinsed with deionized water to prevent contamination.

2.6. Carcinogenic and Health Risk Assessment

The potential non-carcinogenic and carcinogenic risks associated with heavy metal exposure through consumption of cow and buffalo milk were assessed using Total Target Hazard Quotient (THQ), Hazard Index (HI), and lifetime Cancer Risk (CR) based on the USEPA risk assessment models. The THQ for each metal (Lead, Cadmium, Arsenic, Mercury) was calculated to estimate the risk posed by chronic exposure through daily milk consumption, by comparing the estimated daily intake of each metal to its reference dose (RfD), which represents the maximum tolerable intake without appreciable risk of adverse health effects (Yu et al., 2015). The daily intake of each metal was determined by multiplying the mean concentration of the metal in milk by the average daily milk consumption and dividing by the average body weight of the consumer. THQ values below 1 indicate negligible risk, while values above 1 suggest potential non-carcinogenic health concerns.

The Hazard Index (HI) was calculated as the sum of THQs of all analyzed metals for each milk sample, reflecting the cumulative potential non-carcinogenic risk under the dose-additive assumption (Yu et al., 2015). An HI value below 1 indicates no significant risk from combined exposure, whereas a value above 1 suggests the possibility of adverse health effects from chronic exposure to multiple contaminants.

For metals with established carcinogenic potential, lifetime Cancer Risk (CR) was estimated by multiplying the chronic daily intake (CDI) of the metal by its oral slope factor (SF), following USEPA guidance. The CDI was calculated using the estimated daily intake, exposure frequency, and exposure duration over an assumed 70-year lifetime, normalized by body weight. CR values between 1×10^{-6} and 1×10^{-4} are generally considered acceptable, while values exceeding 1×10^{-4} indicate potentially high carcinogenic risk (Yu et al., 2015). All calculations were performed in triplicate, and results were expressed as mean ± standard deviation. Statistical differences between cow and buffalo milk were evaluated using ANOVA, with significance considered at $p < 0.05$.

2.7. Nutritional and Biochemical Analysis

Raw milk samples collected from 40 cow milk and 40 buffalo milk samples from Central and Southern Punjab, Pakistan, were analyzed for key nutritional and functional components. The concentrations of calcium and phosphorus in milk were determined using mid-infrared (MIR) spectrometry, a validated rapid method that provides strong predictive accuracy, with cross-validation R^2 values of 0.80 for calcium and 0.79 for phosphorus (Soyeurt et al., 2009). For validation purposes, selected samples were also analyzed using inductively coupled plasma atomic emission spectrometry (ICP-AES) for direct quantification of mineral content, and colorimetric determination of calcium was performed using the o-cresolphthalein-complexone dye method (Kaur, 2007). Vitamins A and D were quantified using the procedures described by Grace and Bernhard (1984), which involve saponification of milk fat followed by extraction and spectrophotometric determination. Omega-3 fatty acids were analyzed via capillary gas chromatography (GC) after direct transesterification of milk lipids, a method providing recovery rates of 100–102.9% and repeatability below 1.8% relative standard deviation (Golay & Dong, 2015). The casein-to-whey protein ratio was determined using portable near-infrared (NIR) spectrometry, which achieves prediction errors of ±0.06. Selected samples were also cross-validated using Fourier transform infrared (FTIR) spectrometry, with correlation coefficients of 0.976 (Riaz et al., 2026). All analyses were

performed in triplicate, and the results were reported as mean ± standard deviation. Statistical analysis to compare cow and buffalo milk was conducted using ANOVA, with significance set at $p < 0.05$.

3. Results and Discussion

3.1. Microbiological Analysis

The present study clearly demonstrates that raw buffalo milk exhibits significantly higher microbial contamination than cow milk, with both milk types failing to meet internationally accepted microbiological safety standards. The elevated microbial loads observed in both samples reflect inadequate hygienic practices during milking, handling, storage, and transportation, which remain persistent challenges in traditional dairy production systems. Quantitative analysis revealed that the total plate count of buffalo milk ($52.1 \pm 6.2 \times 10^3$ CFU/mL) was significantly higher than that of cow milk ($45.6 \pm 5.3 \times 10^3$ CFU/mL), and both values exceeded the recommended international limit of $\leqslant 20 \times 10^3$ CFU/mL. This finding indicates extensive bacterial proliferation, likely driven by delayed cooling, contaminated utensils, and unsanitary milking environments. Similarly, coliform counts were unacceptably high in both milk types, with buffalo milk (18.7 ± 4.5 CFU/mL) showing greater contamination than cow milk (12.4 ± 3.1 CFU/mL), despite zero-tolerance standards for coliform presence. The detection of coliform bacteria strongly suggests fecal contamination and highlights serious deficiencies in water quality, udder cleanliness, and handler hygiene.

The higher microbial burden in buffalo milk is consistent with previous studies. El-Leboudy et al. (2016) reported significantly greater coliform and total bacterial counts in rural buffalo milk compared to cow milk, attributing this difference to traditional husbandry and milking practices. Moreover, the richer total solids content of buffalo milk provides a more favorable substrate for microbial growth, particularly under non-refrigerated conditions. These observations are further supported by Pandey et al. (2014), who identified contaminated milking equipment, inadequate udder sanitation, and poor-quality water as the primary contributors to microbial contamination in raw milk. Although the results of the present study are statistically significant ($p < 0.05$), they are limited to a specific geographic region, which may restrict broader generalization. Nevertheless, the consistency of these findings with previously published literature strengthens their validity and underscores a widespread issue affecting raw milk safety in developing dairy sectors.

Overall, the findings emphasize an urgent need for improved hygienic interventions, including farmer education, regular sanitation of milking equipment, access to clean water, and effective cold-chain management. Implementing these measures is essential to reduce microbial contamination, enhance milk quality, and protect public health, particularly in regions where raw milk consumption remains common. In comparison with other regional and international studies, the microbial loads observed in the present study appear relatively higher, indicating more pronounced hygienic challenges in the studied areas.

Table 1: Microbiological Quality of Raw Milk

Parameter	Cow Milk (n=40)	Buffalo Milk (n=40)	Standard Limit*
Total Plate Count (×10³ CFU/mL)	45.6 ± 5.3^a	52.1 ± 6.2^b	≤20
Coliform Count (CFU/mL)	12.4 ± 3.1^a	18.7 ± 4.5^b	0
E. coli (CFU/mL)	5.2 ± 1.4^a	7.8 ± 2.0^b	0
Staphylococcus aureus (CFU/mL)	3.1 ± 0.8^a	4.2 ± 1.1^b	0
Yeast & Mold (CFU/mL)	2.8 ± 0.6^a	3.5 ± 0.9^b	≤10
Lactic Acid Bacteria (CFU/mL)	4.5 ± 1.0^a	5.8 ± 1.2^b	-

It is reported that there are comparatively lower total plate counts in raw milk samples, suggesting better handling practices in their study region. Similarly, studies conducted in controlled dairy systems have documented significantly lower coliform and pathogenic bacterial counts, highlighting the impact of improved sanitation and cold-chain management (Khalid et al., 2025). These differences emphasize that microbial contamination levels are highly dependent on farm management practices, environmental conditions, and infrastructure, and further reinforce the need for targeted interventions in informal dairy supply chains.

3.2. Physicochemical Composition and Adulteration Analyses

The present study demonstrates clear compositional superiority of buffalo milk over cow milk, while simultaneously highlighting pervasive adulteration practices that substantially compromise milk quality and safety in informal supply chains. Statistically significant differences ($p < 0.05$) were observed in key nutritional parameters, with buffalo milk exhibiting higher fat (6.8 ± 0.3%) and protein content (4.0 ± 0.2%) compared to cow milk (3.5 ± 0.2% fat; 3.2 ± 0.1% protein). Total solids followed the same trend, reaching 14.5 ± 0.4% in buffalo milk versus 12.3 ± 0.3% in cow milk, confirming the inherently richer composition of buffalo milk. These findings are consistent with earlier comparative investigations reporting higher fat and protein levels in buffalo milk than cow milk. Mahmood and Usman (2010) documented buffalo milk fat and protein contents of 7.97% and 4.36%, respectively, compared with 4.00% fat and 3.37% protein in cow milk, reinforcing the biological basis for these differences. Lactose concentration and pH values did not differ significantly between milk types and remained within acceptable physiological ranges, indicating that intrinsic milk synthesis was largely unaffected by species variation. Despite meeting compositional standards, widespread adulteration emerged as the most critical concern. Both cow and buffalo milk samples showed substantial added water—12.5 ± 2.3% and 14.8 ± 3.0%, respectively—despite zero-tolerance regulatory limits. The detection of chemical adulterants such as detergents and neutralizers in both milk types further indicates deliberate manipulation to mask spoilage, acidity, or microbial deterioration. These practices not only dilute essential nutrients but also pose serious toxicological and microbiological risks to consumers. The extent of adulteration observed aligns closely with previous reports from informal dairy markets. Khan et al. (1999) reported water dilution in all market milk samples, while Iftekhar et al. (2024) found that more than 90% of milk samples contained water adulteration exceeding 5%. Such adulteration practices exacerbate microbial growth by lowering natural antimicrobial components and increasing exposure to contaminated water sources, thereby compounding public health risks. Collectively, the findings indicate that although buffalo milk offers superior nutritional potential compared to cow milk, these benefits are significantly undermined by unhygienic handling and adulteration in informal supply chains. The coexistence of high nutritional value with unsafe handling practices presents a paradox that necessitates urgent intervention. Strengthening regulatory enforcement, improving producer education on hygienic milking and ethical practices, and implementing routine quality monitoring are essential to ensure that the nutritional advantages of milk—particularly buffalo milk—are preserved without compromising consumer safety. In comparison with findings from other studies, the compositional parameters observed in the present study fall within the reported ranges but tend to be slightly lower than those documented in controlled or commercial dairy systems, where improved feeding and management practices enhance milk quality. For example, studies conducted under improved farm conditions have reported higher fat and protein contents, reflecting the influence of nutrition and breed management. Conversely, the level of adulteration observed in the present study appears higher than that reported in more regulated markets, indicating weaker enforcement of quality control measures in informal supply chains (Asghar et al., 2025). These comparisons highlight that while the intrinsic composition of milk is largely biologically determined, external factors such as farm management, market regulation, and supply chain integrity play a crucial role in determining the final quality and safety of milk available to consumers.

Table 2: Physicochemical Composition and Adulteration.

Parameter	Cow Milk	Buffalo Milk	Standard / Reference*
Fat (%)	3.5 ± 0.2[a]	6.8 ± 0.3[b]	Cow ≥3.25, Buffalo ≥6
Protein (%)	3.2 ± 0.1[a]	4.0 ± 0.2[b]	Cow ≥3.0, Buffalo ≥3.5
Lactose (%)	4.8 ± 0.2[a]	4.6 ± 0.2[a]	4.5–5.0
Total Solids (%)	12.3 ± 0.3[a]	14.5 ± 0.4[b]	Cow ≥12.0, Buffalo ≥13
pH	6.7 ± 0.1[a]	6.6 ± 0.1[a]	6.6–6.8
Added Water (%)	12.5 ± 2.3[a]	14.8 ± 3.0[a]	0
Detergent Detection (%)	5 ± 2[a]	7 ± 3[a]	0
Neutralizer Detection (%)	8 ± 3[a]	10 ± 4[a]	0

3.3. Heavy Metal Contamination

The present findings demonstrate that buffalo milk consistently exhibits higher levels of heavy metal contamination than cow milk, with lead (Pb) and arsenic (As) exceeding permissible limits in both milk types.

This pattern indicates a potential risk of chronic exposure for consumers, particularly in populations with high milk intake. The higher concentrations observed in buffalo milk may be attributed to its greater fat and total solids content, which can facilitate the accumulation of lipophilic and protein-binding metals, thereby increasing the toxicological burden compared to cow milk. These results are supported by several regional studies that report similar contamination trends. Investigations conducted in South Asia and neighboring regions provide strong corroborative evidence. Sharma et al. (2025) reported exceedances of Pb and As in milk samples from Raipur, India, with buffalo milk showing significantly higher concentrations than cow milk. Likewise, (El-Ansary & El_Leboudy, 2015) documented that nearly 80% of analyzed milk samples in Egypt exceeded permissible limits for heavy metals, with buffalo milk containing markedly higher levels of cadmium and lead. In Pakistan, Abid et al. (2024) also observed significantly elevated lead concentrations in buffalo milk ($p < 0.05$), although mean values in some locations remained within WHO guideline limits, highlighting the importance of localized exposure assessment.

However, the literature also indicates notable geographic variability in contamination levels. Kharkwal et al. (2023) reported that concentrations of arsenic, cadmium, and lead in milk samples from Punjab, India, were largely within permissible limits, though risk assessment revealed potential carcinogenic concerns for specific population subgroups. Furthermore, a comprehensive review by (Domingo, 2021) concluded that, in many regions, exposure to heavy metals through cow milk consumption alone is unlikely to pose significant health risks. Overall, the evidence suggests that heavy metal contamination in milk is primarily region-specific rather than universally characteristic of a particular milk type. Environmental pollution, industrial activities, contaminated water sources, feed quality, and agricultural practices appear to be the dominant determinants of contamination levels. While buffalo milk shows a greater tendency toward metal accumulation, the associated health risk is largely governed by local environmental conditions. These findings underscore the need for regionally targeted monitoring programs, stricter regulation of animal feed and water quality, and routine risk assessment to safeguard public health, particularly for vulnerable consumer groups. In comparison with previously published studies, the heavy metal concentrations observed in the present study are generally higher than those reported in regions with better environmental control and regulatory oversight, but are consistent with findings from areas affected by industrial and agricultural pollution. For instance, studies conducted in less contaminated environments have reported metal concentrations within permissible limits, whereas studies from highly polluted regions have documented similar or even higher levels of contamination (Riaz et al., 2026). These variations further confirm that heavy metal accumulation in milk is strongly influenced by local environmental conditions rather than species alone. The comparatively elevated levels observed in this study, therefore, highlight the severity of environmental contamination in the studied regions and reinforce the need for stricter environmental and food safety regulations.

Table 3. Heavy Metal Contamination.

Heavy Metal	Cow Milk (mg/L)	Buffalo Milk (mg/L)	Permissible Limit*
Lead (Pb)	0.025 ± 0.004^{a}	0.038 ± 0.005^{b}	0.02
Cadmium (Cd)	0.005 ± 0.001^{a}	0.007 ± 0.002^{a}	0.01
Arsenic (As)	0.010 ± 0.002^{a}	0.014 ± 0.003^{b}	0.01
Mercury (Hg)	0.002 ± 0.001^{a}	0.003 ± 0.001^{a}	0.003

3.4. Carcinogenic and Health Risk Assessment

The present findings demonstrate that consumption of both cow and buffalo milk poses unacceptable non-carcinogenic and carcinogenic health risks, with buffalo milk consistently presenting a substantially higher risk than cow milk. The calculated Total Target Hazard Quotient (THQ) values for cow milk (7.2 ± 1.4) and buffalo milk (12.5 ± 2.1) far exceeded the safety threshold of unity, indicating a significant potential for adverse health effects arising from chronic exposure to heavy metals through milk consumption. Similarly, the Hazard Index (HI), reflecting the cumulative effect of multiple metals, reached markedly elevated levels, particularly in buffalo milk (15.0 ± 2.4), suggesting additive toxicological risks that cannot be ignored.

These results are strongly supported by existing literature (Farzand et al., 2025). A comparable risk assessment by (Yu et al., 2015) reported total THQ and HI values exceeding unity in milk products, with the highest risk observed in infants and young children due to higher milk intake relative to body weight. Likewise, (Iqbal et al., 2020) documented total target hazard quotient (TTHQ) values ranging from 6.92 to 42.44 in buffalo milk, highlighting severe non-carcinogenic risk levels associated with prolonged consumption. These findings are consistent with (Yu et al., 2015), who similarly reported elevated cancer risk indices linked to heavy metal intake from milk products, particularly for younger age groups. The repeated observation across multiple studies that children face disproportionately higher risks underscores the public health significance of these findings.

Overall, the convergence of evidence across different geographic regions, milk types, and study populations strengthens the conclusion that heavy metal contamination in milk represents a serious dietary exposure pathway. The consistently higher risk associated with buffalo milk may be attributed to its greater metal accumulation capacity, likely influenced by higher fat content and environmental exposure through feed and water. These results collectively support the urgent need for continuous monitoring of heavy metals in milk, implementation of stricter regulatory controls, and targeted risk mitigation strategies focusing on vulnerable populations, particularly infants and children.

Table 4: Carcinogenic and Health Risk Assessment

Parameter	Cow Milk	Buffalo Milk	Interpretation
Total Target Hazard Quotient (THQ)	7.2 ± 1.4^{a}	12.5 ± 2.1^{b}	>1 indicates potential risk
Hazard Index (HI)	8.5 ± 1.5^{a}	15.0 ± 2.4^{b}	>1 indicates risk
Cancer Risk (CR, lifetime)	$1.2\times10^{-3} \pm 0.3\times10^{-3a}$	$2.5\times10^{-3} \pm 0.5\times10^{-3b}$	$>10^{-4}$ is considered concerning

The present findings provide strong and specific evidence that buffalo milk possesses a nutritionally superior profile compared to cow milk, particularly with respect to essential minerals, fat-soluble vitamins, and functional lipids. Buffalo milk contained approximately 50% more calcium (180 vs. 120 mg/100 mL) and 47% more phosphorus (140 vs. 95 mg/100 mL) than cow milk, underscoring its enhanced role in supporting bone health, skeletal development, and metabolic functions. These differences are nutritionally meaningful, especially in populations where milk serves as a primary dietary source of minerals. In addition, buffalo milk exhibited markedly higher concentrations of vitamin A (52 vs. 38 µg/100 mL) and vitamin D (0.08 vs. 0.05 µg/100 mL), reflecting its higher fat content and greater capacity to retain fat-soluble micronutrients. Such enrichment is particularly relevant in regions with widespread micronutrient deficiencies, where milk contributes significantly to daily vitamin intake. The lipid quality of buffalo milk further strengthens its functional value. The higher proportion of omega-3 fatty acids (0.4% in buffalo milk compared to 0.3% in cow milk) supports a more favorable cardioprotective and anti-inflammatory profile, consistent with previous reports linking buffalo milk fat to improved health outcomes. Despite these compositional advantages, the casein-to-whey protein ratio remained comparable between the two milk types at approximately 80:20, indicating that while buffalo milk is richer in total nutrients, its fundamental protein distribution remains nutritionally balanced and similar to cow milk. However, a critical limitation emerges when these nutritional benefits are evaluated within the context of real-world consumption. Evidence from informal milk supply chains indicates that widespread adulteration, elevated microbial loads, and contamination with heavy metals substantially undermine the inherent nutritional superiority of buffalo milk. Practices such as water dilution not only reduce nutrient density but also increase susceptibility to microbial growth, while environmental and handling-related contamination introduces toxicological risks that may outweigh the compositional benefits (Riaz et al., 2025). Consequently, although buffalo milk is intrinsically more nutrient-dense than cow milk, its potential health advantages cannot be fully realized without effective quality assurance. In summary, buffalo milk demonstrates clear biochemical and nutritional superiority over cow milk; however, these benefits are conditional rather than absolute. Ensuring strict hygienic practices, controlling adulteration, and enforcing regulatory oversight across the milk value chain are essential prerequisites for translating the superior composition of buffalo milk into tangible public health gains.

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Table 5: Nutritional / Biochemical Quality of Raw Milk

Parameter	Cow Milk	Buffalo Milk	Reference / Standard
Calcium (mg/100mL)	120 ± 5[a]	180 ± 8[b]	120–150
Phosphorus (mg/100mL)	95 ± 4[a]	140 ± 6[b]	90–130
Vitamin A (µg/100mL)	38 ± 3[a]	52 ± 4[b]	30–50
Vitamin D (µg/100 mL)	0.05 ± 0.01[a]	0.08 ± 0.02[b]	0.04–0.10
Omega-3 Fatty Acids (%)	0.3 ± 0.05[a]	0.4 ± 0.06[b]	0.3–0.5
Casein: Whey Ratio	80:20[a]	82:18[a]	80:20

4. Conclusion

The present study provides a comprehensive and integrative evaluation of raw cow and buffalo milk quality in Central and Southern Punjab, Pakistan, revealing a critical paradox between nutritional richness and food safety. Buffalo milk demonstrated clear compositional and biochemical superiority over cow milk, with higher levels of fat, protein, total solids, essential minerals, fat-soluble vitamins, and omega-3 fatty acids, highlighting its substantial nutritional potential. However, these intrinsic advantages are profoundly compromised by widespread microbiological contamination, extensive adulteration practices, and elevated heavy metal residues observed in both milk types. The consistently higher microbial loads and metal concentrations in buffalo milk further exacerbate associated health risks. Health risk assessment outcomes revealed Total Target Hazard Quotient, Hazard Index, and lifetime Cancer Risk values far exceeding internationally accepted safety thresholds, indicating that chronic consumption of raw milk from the studied regions poses serious non-carcinogenic and carcinogenic risks. These risks are particularly concerning for infants, children, and populations with high milk intake. The findings clearly demonstrate that the current informal dairy supply chains fail to ensure milk safety, thereby negating the nutritional benefits of milk consumption. In conclusion, buffalo milk cannot be considered inherently healthier than cow milk under prevailing production and handling conditions. Ensuring milk safety requires urgent, coordinated interventions, including strict enforcement of food safety regulations, routine monitoring of microbiological and chemical contaminants, control of adulteration, improvement of feed and water quality, and comprehensive farmer education programs. Only through such measures can the nutritional superiority of milk—particularly buffalo milk—be translated into tangible public health benefits while minimizing health risks to consumers.

CRediT authorship contribution statement

Conceptualization: Syed Tahaa Munawar and Talha Riaz; Methodology: Ahmad Din, Muhammad Abdullah Butt, and Sawera Hayat; Investigation and Data Collection: Rabiya Riaz, Aliza Batool, Burhan Khalid, and Sadia Ansar; Formal Analysis: Hafsa Fatima and Areeba Azhar; Writing – Original Draft: Syed Tahaa Munawar and Talha Riaz; Writing – Review & Editing: Muhammad Moeid Khan and Md. Shabuddin Ahamed; Supervision: Ahmad Din. All authors have read and approved the final manuscript.

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Declaration of competing interest

The authors declared no conflict of interest.

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AI disclosure statement

Artificial intelligence tools (ChatGPT OpenAI) were used to support language editing, improve sentence clarity, and assist with structural refinement during manuscript revision. The authors reviewed and verified all content and take full responsibility for the accuracy, originality, and integrity of the work, including ensuring that all statements and citations are appropriate and scientifically sound.

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